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INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

UNEP

Environmental Health Criteria 212

Principles and Methods for Assessing Allergic Hypersensitization Associated with Exposure to Chemicals

IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS
A cooperative agreement among UNEP, ILO, FAO, WHO, UNIDO, UNITAR and OECD

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Environmental Health Criteria 212

PRINCIPLES AND METHODS FOR ASSESSING ALLERGIC HYPERSENSITIZATION ASSOCIATED WITH EXPOSURE TO CHEMICALS

Published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization, and produced within the framework of the Inter-Organization Programme for the Sound Management of Chemicals.

World Health Organization
Geneva, 1999

The **International Programme on Chemical Safety (IPCS)**, established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organisation (ILO), and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.

The **Inter-Organization Programme for the Sound Management of Chemicals (IOMC)** was established in 1995 by UNEP, ILO, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization, the United Nations Institute for Training and Research, and the Organisation for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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CONTENTS

PRINCIPLES AND METHODS FOR ASSESSING ALLERGIC HYPERSENSITIZATION ASSOCIATED WITH EXPOSURE TO CHEMICALS

NOTE TO READERS OF THE CRITERIA MONOGRAPHS

Every effort has been made to present information in the criteria monographs as accurately as possible without unduly delaying their publication. In the interest of all users of the Environmental Health Criteria monographs, readers are requested to communicate any errors that may have occurred to the Director of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda.

* * *

A detailed data profile and a legal file can be obtained from the International Register of Potentially Toxic Chemicals, Case postale 356, 1219 Châtelaine, Geneva, Switzerland (telephone no. + 41 22 – 9799111, fax no. + 41 22 – 7973460, E-mail irptc@unep.ch).

* * *

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Environmental Health Criteria

P R E A M B L E

Objectives

In 1973 the WHO Environmental Health Criteria Programme was initiated with the following objectives:

(i) to assess information on the relationship between exposure to environmental pollutants and human health, and to provide guidelines for setting exposure limits;

(ii) to identify new or potential pollutants;

(iii) to identify gaps in knowledge concerning the health effects of pollutants;

(iv) to promote the harmonization of toxicological and epidemiological methods in order to have internationally comparable results.

The first Environmental Health Criteria (EHC) monograph, on mercury, was published in 1976 and since that time an ever-increasing number of assessments of chemicals and of physical effects have been produced. In addition, many EHC monographs have been devoted to evaluating toxicological methodology, e.g., for genetic, neurotoxic, teratogenic and nephrotoxic effects. Other publications have been concerned with epidemiological guidelines, evaluation of short-term tests for carcinogens, biomarkers, effects on the elderly and so forth.

Since its inauguration the EHC Programme has widened its scope, and the importance of environmental effects, in addition to health effects, has been increasingly emphasized in the total evaluation of chemicals.

The original impetus for the Programme came from World Health Assembly resolutions and the recommendations of the 1972 UN Conference on the Human Environment. Subsequently the work became an integral part of the International Programme on Chemical Safety (IPCS), a cooperative programme of UNEP, ILO and WHO. In this manner, with the strong support of the new partners, the importance of occupational health and environmental effects was fully

recognized. The EHC monographs have become widely established, used and recognized throughout the world.

The recommendations of the 1992 UN Conference on Environment and Development and the subsequent establishment of the Intergovernmental Forum on Chemical Safety with the priorities for action in the six programme areas of Chapter 19, Agenda 21, all lend further weight to the need for EHC assessments of the risks of chemicals.

Scope

The criteria monographs are intended to provide critical reviews on the effect on human health and the environment of chemicals and of combinations of chemicals and physical and biological agents. As such, they include and review studies that are of direct relevance for the evaluation. However, they do not describe *every* study carried out. Worldwide data are used and are quoted from original studies, not from abstracts or reviews. Both published and unpublished reports are considered and it is incumbent on the authors to assess all the articles cited in the references. Preference is always given to published data. Unpublished data are only used when relevant published data are absent or when they are pivotal to the risk assessment. A detailed policy statement is available that describes the procedures used for unpublished proprietary data so that this information can be used in the evaluation without compromising its confidential nature (WHO (1990) Revised Guidelines for the Preparation of Environmental Health Criteria Monographs. PCS/90.69, Geneva, World Health Organization).

In the evaluation of human health risks, sound human data, whenever available, are preferred to animal data. Animal and *in vitro* studies provide support and are used mainly to supply evidence missing from human studies. It is mandatory that research on human subjects is conducted in full accord with ethical principles, including the provisions of the Helsinki Declaration.

The EHC monographs are intended to assist national and international authorities in making risk assessments and subsequent risk management decisions. They represent a thorough evaluation of risks and are not, in any sense, recommendations for regulation or

standard setting. These latter are the exclusive purview of national and regional governments.

Content

The layout of EHC monographs for chemicals is outlined below.

- Summary — a review of the salient facts and the risk evaluation of the chemical
- Identity — physical and chemical properties, analytical methods
- Sources of exposure
- Environmental transport, distribution and transformation
- Environmental levels and human exposure
- Kinetics and metabolism in laboratory animals and humans
- Effects on laboratory mammals and *in vitro* test systems
- Effects on humans
- Effects on other organisms in the laboratory and field
- Evaluation of human health risks and effects on the environment
- Conclusions and recommendations for protection of human health and the environment
- Further research
- Previous evaluations by international bodies, e.g., IARC, JECFA, JMPR

Selection of chemicals

Since the inception of the EHC Programme, the IPCS has organized meetings of scientists to establish lists of priority chemicals for subsequent evaluation. Such meetings have been held in: Ispra, Italy, 1980; Oxford, United Kingdom, 1984; Berlin, Germany, 1987; and North Carolina, USA, 1995. The selection of chemicals has been based on the following criteria: the existence of scientific evidence that the substance presents a hazard to human health and/or the environment; the possible use, persistence, accumulation or degradation of the substance shows that there may be significant human or environmental exposure; the size and nature of populations at risk (both human and other species) and risks for environment; international concern, i.e. the substance is of major interest to several countries; adequate data on the hazards are available.

If an EHC monograph is proposed for a chemical not on the priority list, the IPCS Secretariat consults with the Cooperating Organizations and all the Participating Institutions before embarking on the preparation of the monograph.

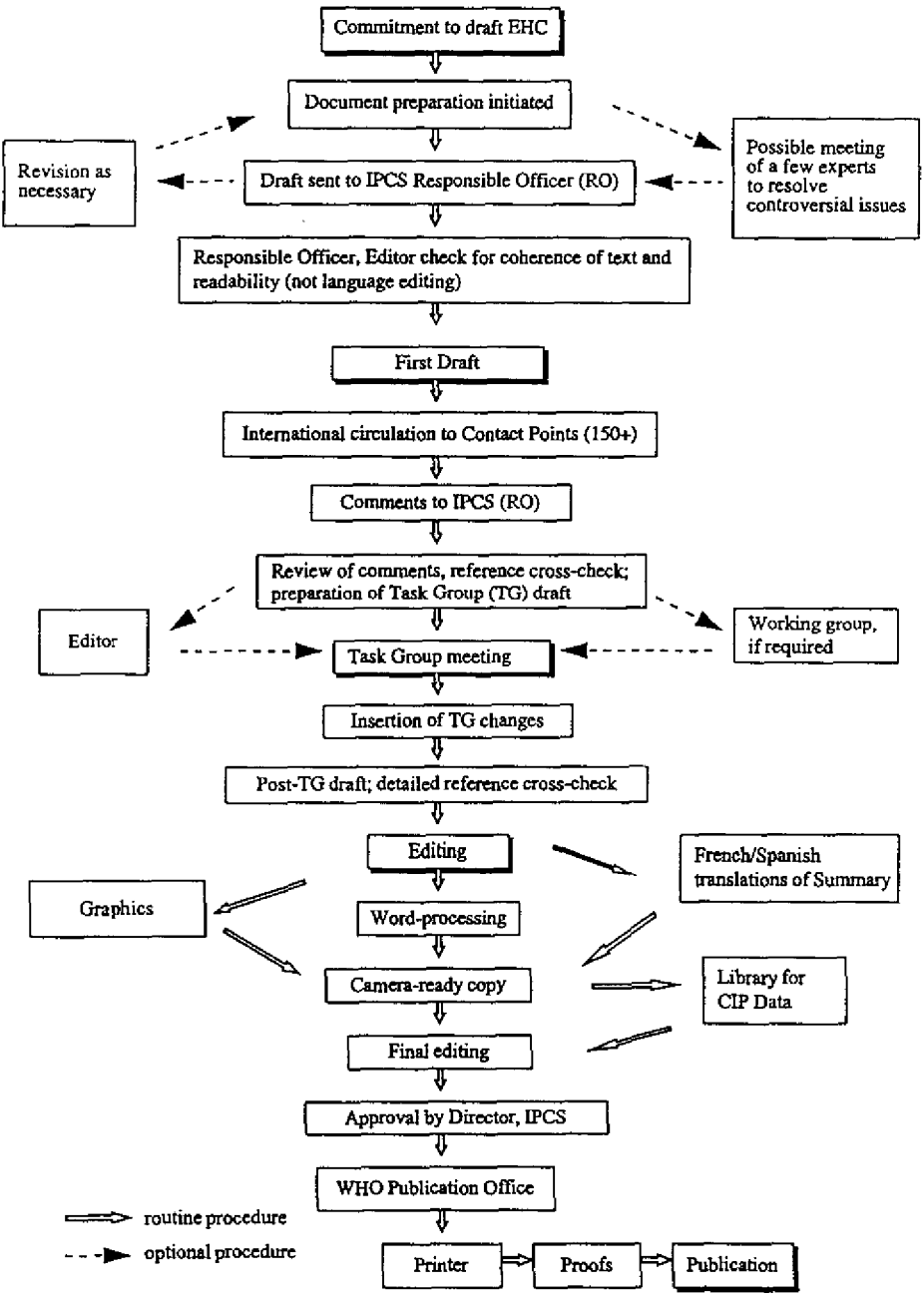
Procedures

The order of procedures that result in the publication of an EHC monograph is shown in the flow chart. A designated staff member of IPCS, responsible for the scientific quality of the document, serves as Responsible Officer (RO). The IPCS Editor is responsible for layout and language. The first draft, prepared by consultants or, more usually, staff from an IPCS Participating Institution, is based initially on data provided from the International Register of Potentially Toxic Chemicals, and reference data bases such as Medline and Toxline.

The draft document, when received by the RO, may require an initial review by a small panel of experts to determine its scientific quality and objectivity. Once the RO finds the document acceptable as a first draft, it is distributed, in its unedited form, to well over 150 EHC contact points throughout the world who are asked to comment on its completeness and accuracy and, where necessary, provide additional material. The contact points, usually designated by governments, may be Participating Institutions, IPCS Focal Points, or individual scientists known for their particular expertise. Generally some four months are allowed before the comments are considered by the RO and author(s). A second draft incorporating comments received and approved by the Director, IPCS, is then distributed to Task Group members, who carry out the peer review, at least six weeks before their meeting.

The Task Group members serve as individual scientists, not as representatives of any organization, government or industry. Their function is to evaluate the accuracy, significance and relevance of the information in the document and to assess the health and environmental risks from exposure to the chemical. A summary and recommendations for further research and improved safety aspects are also required. The composition of the Task Group is dictated by the range of expertise required for the subject of the meeting and by the need for a balanced geographical distribution.

EHC PREPARATION FLOW CHART



The three cooperating organizations of the IPCS recognize the important role played by nongovernmental organizations. Representatives from relevant national and international associations may be invited to join the Task Group as observers. While observers may provide a valuable contribution to the process, they can only speak at the invitation of the Chairperson. Observers do not participate in the final evaluation of the chemical; this is the sole responsibility of the Task Group members. When the Task Group considers it to be appropriate, it may meet *in camera*.

All individuals who as authors, consultants or advisers participate in the preparation of the EHC monograph must, in addition to serving in their personal capacity as scientists, inform the RO if at any time a conflict of interest, whether actual or potential, could be perceived in their work. They are required to sign a conflict of interest statement. Such a procedure ensures the transparency and probity of the process.

When the Task Group has completed its review and the RO is satisfied as to the scientific correctness and completeness of the document, it then goes for language editing, reference checking, and preparation of camera-ready copy. After approval by the Director, IPCS, the monograph is submitted to the WHO Office of Publications for printing. At this time a copy of the final draft is sent to the Chairperson and Rapporteur of the Task Group to check for any errors.

It is accepted that the following criteria should initiate the updating of an EHC monograph: new data are available that would substantially change the evaluation; there is public concern for health or environmental effects of the agent because of greater exposure; an appreciable time period has elapsed since the last evaluation.

All Participating Institutions are informed, through the EHC progress report, of the authors and institutions proposed for the drafting of the documents. A comprehensive file of all comments received on drafts of each EHC monograph is maintained and is available on request. The Chairpersons of Task Groups are briefed before each meeting on their role and responsibility in ensuring that these rules are followed.

WHO TASK GROUP MEETING ON PRINCIPLES AND METHODS FOR ASSESSING ALLERGIC HYPERSENSITIZATION ASSOCIATED WITH EXPOSURE TO CHEMICALS

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ENVIRONMENTAL HEALTH CRITERIA ON PRINCIPLES AND METHODS FOR ASSESSING ALLERGIC HYPERSENSITIZATION ASSOCIATED WITH EXPOSURE TO CHEMICALS

A WHO Task Group on Principles and Methods for Assessing Allergic Hypersensitization Associated with Exposure to Chemicals met at the National Institute of Public Health and the Environment, Bilthoven, Netherlands from 8 to 12 September 1997. Dr E.M. Smith, IPCS, welcomed the participants on behalf of Dr M. Mercier, Director of the IPCS, and on behalf of the three IPCS cooperating organizations (UNEP/ILO/WHO). The Group reviewed and revised the draft and made an evaluation of the risks to human health and of allergic hypersensitization associated with exposure to chemicals.

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Dr E.M. Smith and Dr P.G. Jenkins, both of the IPCS Central Unit, were responsible for the scientific aspects of the monograph and for the technical editing, respectively.

The efforts of all who helped in the preparation and finalization of the monograph are gratefully acknowledged.

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ABBREVIATIONS

APC	antigen-presenting cell
COPD	chronic obstructive pulmonary disease
DEREK	deductive estimation of risk from existing knowledge
DTH	delayed-type hypersensitivity
FcR	Fc receptor
FEV_1	forced expiratory volume in 1 second
FVC	forced vital capacity
HIV	human immunodeficiency virus
ICAM	intercellular adhesion molecule
Ig	immunoglobulin
IL	interleukin
LAK	lymphokine-activated killer
LC	Langerhans cell
LPS	lipopolysaccharide
MALTs	mucosal-associated lymphoid tissues
MDR	multiple drug resistance
NCAM	neural cell adhesion molecule
NK	natural killer
PAM	pulmonary alveolar macrophage
PDGFR	platelet-derived growth factor receptor
QSAR	quantitative structure–activity relationship
SAR	structure–activity relationship
SLE	systemic lupus erythematosus
TCPA	tetrachlorophthalic anhydride
TCR	T-cell antigen receptor
TDI	toluene diisocyanate
Th	T helper
TNF	tumour necrosis factor

PREFACE

Normal functioning of the immune system prevents serious illnesses, such as infections and tumours. Immunotoxicology represents abnormalities in the immune system produced by exposure to chemicals and drugs. One consequence of dysfunction of the immune system is partial or complete immunosuppression, resulting in reduced defences against these conditions. This is often termed "immunotoxicity" and the IPCS Environmental Health Criteria monograph 180: Principles and Methods for Assessing Direct Immunotoxicity Associated with Exposure to Chemicals (IPCS, 1996) provides an extensive review of the causes, consequences and detection of this type of disorder.

Allergy is another type of adverse effect on health produced by harmful immune responses following exposure to certain chemicals. The initial exposure results in the state of allergic sensitization, in which the immune system is primed to respond inappropriately on subsequent exposure to the same agent, and allergy is the functional disorder caused by that response. The best-known types of allergic response affect the skin, i.e., allergic contact dermatitis and atopic eczema, and the airways, i.e., asthma and allergic rhinitis, but any tissue in the body may be affected.

Allergic responses usually occur to foreign antigens, although self-antigens may sometimes be the targets of damaging immune responses. This is known as autoimmunity and may occur because the self-antigens have been modified by chemicals or because the latter have adversely affected the control mechanisms that normally prevent autoimmune reactions.

Both allergic and autoimmune disorders may be caused by the responses of the immune system to substances of low (e.g., transition metals and simple organic compounds) or high relative molecular mass (e.g., proteins, including food components). The harmful reactions may occur at the site of exposure or systemically. The genetic make-up of the individual may be one predisposing factor.

Once developed, sensitization persists, sometimes for life, and further exposure, even to a low concentration of the allergen, may result in serious disease. After the chemical nature of the substance, exposure (concentration, route, duration and frequency) is the most important factor in the development of sensitization, as increased exposure to

allergens leads to increased risk of sensitization. Allergic disorders represent major ill-health and economic loss to the public and in the workplace. There are suggestions that pollution and other environmental factors, such as lifestyle and smoking, may be involved in the rising number of affected people in both developed and developing countries.

The incidence of chemically induced autoimmune diseases is low, but they represent important adverse consequences of the use of certain medicines and, possibly, of exposure to various chemicals.

The structure and functional processes of the immune system and the mechanisms of sensitization, allergic responses and autoimmunity need to be considered in relation to the corresponding disorders and chemicals known to produce them. This consideration will include factors that affect the allergenicity of substances and the development of sensitization and autoimmunity, such as the chemical nature of allergens, special features of the causal exposures, and the physiology of affected subjects.

Allergic disorders are important causes of ill-health at work and in the community, and defining their epidemiology and the evaluation of methods to study their occurrence are crucial. Hazard identification and risk assessment are important if the incidence of allergy and autoimmune disorders is to be contained or reduced. Test methods for the prediction of some forms of sensitization and the risk of disease following a given exposure are now available.

Allergic disorders of humans have been described for many years, but the pace of advances in knowledge of the immune system means that awareness and understanding of allergy and autoimmunity and their consequences are increasing. Our understanding of allergy is developing rapidly, and hypotheses about causes and mechanisms will change as more is learnt about normal and abnormal functioning of the immune system.

Because understanding of sensitization, allergy and autoimmunity is still limited by the extent of knowledge of basic immunology there is a need for fundamental and applied research in areas of the basic mechanisms, detection and prevention of allergy.

1. THE IMMUNE SYSTEM

1.1 Introduction

The role of the immune system may be succinctly stated as the "preservation of integrity". This system is responsible for identifying what is "self" and what is "non-self". The great complexity of the mammalian system is an indication of the importance, as well as the difficulty, of this task. If the system fails to recognize as non-self an infectious entity or the neoantigens expressed by a newly arisen tumour, then the host is in danger of rapidly succumbing to the unopposed invasion. Alternatively, if some integral bodily tissue is not identified as self, then the host is in danger of turning its considerable defensive abilities against the tissue and an autoimmune disease is the result. The cost to the host of these mistakes, made in either direction, may be quite high. Therefore, an extremely complex array of organs, cells, soluble factors and interactions has evolved to regulate this system and minimize the frequency of either of the above-described errors. Recent advances in cellular and molecular biology have dramatically increased our understanding of the mammalian immune system. It is now possible to study in detail biochemical and signal transduction pathways, as well as the regulation of genes in lymphocytes, because of the novel chemical and molecular probes that have been developed. Most importantly, the identification and characterization of the cells, cell surface receptors and cytokines that participate in the immune response have enabled immunologists to produce transgenic and gene "knockout" (disrupted target gene) mice, which will allow even more in-depth study of critical elements in the immune response to antigens. Along with the increased power of experimental immunology has come the ability to study both the direct and indirect actions of drugs and environmental chemicals (i.e., xenobiotics) on immunological processes. Of particular importance are new insights regarding the interactive role of the immune system with other organ systems such as the nervous and endocrine systems. By way of mutual physical and chemical communication between these organ systems, both direct and indirect alteration of immunological function may occur through the actions of xenobiotics.

1.1.1 Evolution and function of the adaptive immune system

Even the most primitive species of animals display some form of immune system that enables identification of "non-self" and that

provides for some rudimentary host defence against environmental challenges. With the emergence of the vertebrates, however, there is seen the evolution of an adaptive immune system that has as its primary physiological responsibility protection of the organism from microbiological challenge and tumour development. The structure and function of the immune system at the anatomical, biochemical and functional levels are broadly comparable in all mammals.

Natural immunity is phylogenetically more ancient than the adaptive immune response, but nevertheless is of critical importance in providing resistance to infectious microorganisms, and the nonspecific or innate immune system acts as a first line of defence. Among the functions of the natural immune system is provision of a physicochemical barrier at external surfaces in the skin and the mucosal tissues of the gastrointestinal, reproductive and respiratory tracts, and the physical elimination of bacteria by coughing, sneezing, etc. The ability of these surfaces to renew themselves and secrete antimicrobial agents such as fatty acids and lysozyme reduces penetration by microbes. However, microbes that bypass these barriers must be dealt with by other more advanced immunological mechanisms, which can be either specific or nonspecific in nature. Cellular elements of the natural immune system include natural killer (NK) cells, mononuclear phagocytes, and eosinophil and neutrophil polymorphonuclear cells. In addition, a complex series of plasma proteins and glycoproteins together comprise the complement system, which acts together with antibody in the elimination of bacteria, but which can also be activated to provide natural immune function in the absence of, or before, a specific immune response. The adaptive immune system acts together with innate or natural immune mechanisms to provide host resistance to infectious and malignant disease.

The adaptive immune system comprises organs, tissues, cells and molecules that must act in concert to provide an integrated immune response. The three cardinal characteristics of adaptive immunity are memory, specificity and the capacity to distinguish between self and non-self. Each of these characteristics are displayed by lymphocytes: the main cellular vectors of adaptive immune responses. Immunological memory is the ability to distinguish a foreign material as a previous invader and to mount a greatly increased and lasting response to that particular antigen. This process is the product of immunocompetent cell cooperativity and allows for both amplification

of the immune response after repeated encounters with the same antigen (immunization) and tolerance to self tissues. In contrast, nonspecific or innate mechanisms do not possess individuality and do not lead to memory.

Mature lymphocytes circulate throughout the body, between and within lymphoid tissues. If a lymphocyte encounters a foreign antigen in an appropriate form under suitable conditions then the cell becomes activated and an immune response is initiated. The primary response takes place in organized lymphoid tissues. It has been estimated that in a normal adult human the immune system is capable of recognizing and responding to many millions of antigens; even antigens that have never been encountered previously, such as for instance new synthetic chemicals. This enormous repertoire is provided by the clonal diversity of lymphocytes; these cells being clonally distributed with respect to antigen specificity. Thus, each clone of mature lymphocytes differs one from another in terms of the antigenic structures that will induce activation. Antigen recognition is effected via specialized membrane receptors that have diversified among lymphocytes during development of the immune system by a process of somatic recombination of antigen receptor genes. It is the possession of these receptors by lymphocytes that confers specificity to immune responses.

Recognition of antigen by lymphocytes in primary lymphoid tissues results in rapid cellular activation and the stimulation of division and differentiation. Division provides for a selective expansion in numbers of those lymphocytes that are able to recognize and interact with the inducing antigen. Selective clonal expansion forms the basis of immunological memory. After first encounter with antigen, responsive lymphocytes have increased in number such that if the individual is exposed subsequently to the same antigenic material then an accelerated and more aggressive response will be mounted. These are the central events necessary for adaptive immunity and those that are made use of in vaccination against infectious microorganisms.

All lymphocytes involved in adaptive immune responses interact specifically with antigen, and they divide and differentiate in response to antigenic challenge. These cells may be subdivided into two main populations, T-lymphocytes and B-lymphocytes, that differ with respect to their origins and development pathways, the way in which

antigen is recognized, and the effector cells into which they ultimately differentiate. Both populations arise in the bone marrow from primitive precursors, but thereafter follow discrete developmental pathways. Cells committed to becoming T-lymphocytes (pre-T-cells) require passage through and differentiation within the thymus to achieve immunological maturity. The thymus serves also to identify and destroy most of those T lymphocytes that display membrane receptors which would permit interaction with self antigens. When they leave the thymus the mature antigen-sensitive T-lymphocytes join the recirculating pool.

Bone marrow derived B-cells also join the recirculating pool where, with T-lymphocytes, they seek antigen for which they have complementary membrane receptors. B-lymphocytes recognize antigen usually in its native form. Activation triggers B-lymphocyte differentiation and division. The end-cell of B-lymphocyte differentiation is the plasma cell that possesses the synthetic and secretory machinery to manufacture and export large amounts of antibody. The antibody secreted by an individual plasma cell is of a single specificity and matches identically the specificity of the membrane receptor on the B-lymphocyte from which the plasma cell differentiated. The purpose of antibody is essentially to form a bridge between the inducing antigen and biological mechanisms that serve to eliminate it. The interaction of antibody with antigen facilitates the activation of complement (lysis of bacteria) and phagocytosis by mononuclear phagocytes and neutrophils (intracellular killing of bacteria) and results in the clearance of pathogenic bacteria. The importance of B-lymphocytes and the antibodies that derive from their activation is protection against extracellular infection by bacteria and parasites.

The existence of T-lymphocytes was recognized for many years before the true nature of their role in adaptive immune responses was appreciated. Cell-mediated immune responses effected by T-lymphocyte participate in host defence against all types of infectious organisms, but of greatest evolutionary significance is immunity against viruses. Humoral immunity effected by antibody is of relevance only in the viraemic stage of viral infections. Viruses are obligate intracellular parasites and once inside the infected host cell are protected from antibody-mediated mechanisms.

The overall purpose of these host defence mechanisms is to provide the organism with resistance to a challenging microbial environment and to confer protection from the internal development of non-self neoplasms or tumours. When normal immune function is absent or compromised, the consequences for human health are serious. Consideration of immunosuppression and immunodeficiency illustrates the evolutionary importance of immune function.

1.1.2 *Immunosuppression, immunodeficiency and autoimmunity*

Active immune function is clearly beneficial for health, whereas the consequences of a compromised immune system are adverse health effects.

Immunodeficiency disorders can be congenital or acquired. Congenital immunodeficiency is comparatively rare, but is frequently very serious and can be fatal. Examples include a complete, or almost complete, failure of the immune system to develop due to the absence or aberrant maturation of lymphocyte or leukocyte progenitors, resulting in severe combined immunodeficiency disease or reticular dysgenesis. Without appropriate treatment these conditions are fatal, children succumbing to overwhelming infection.

Acquired immunodeficiency can be secondary to malnutrition, severe stress, treatment with immunosuppressive drugs or with cancer chemotherapeutic agents, exposure to certain environmental chemicals or infection, such as infection with the human immunodeficiency virus (HIV), the cause of acquired immunodeficiency syndrome (AIDS). In all instances immunosuppression is associated with reduced host resistance and more persistent infection, often with unusual microorganisms that are resisted well by immunocompetent individuals. Immunodeficiency is, in addition, associated with an increased incidence of malignant diseases that are known or suspected to be associated with oncogenic viruses.

The benefits that derive from active immune function do not come without a cost, however. While the adaptive immune system acts as a "friend" in providing host defence, it may also act as a "foe", being instrumental in the pathogenesis of certain diseases. The immune system can, for instance, turn on the host if the fine discrimination between self and non-self breaks down. The result is the development of autoimmune responses and autoimmune disease.

The mechanisms by which autoimmunity develops are multifactorial, complex and remain poorly understood. The majority of cases are idiopathic, although diseases such as systemic sclerosis have been associated with organic chemicals and silica.

1.1.3 Allergy and allergic diseases

Allergy may be defined as the adverse health effects resulting from hypersensitivity caused by exposure to an exogenous antigen (allergen) resulting in a marked increase in reactivity and responsiveness to that particular antigen on subsequent exposure. Allergy is not necessarily, or usually, the consequence of perturbed immune function, but the result of an immune system response to an antigen (in this case allergen) in such a way that a temporary or long-lasting disease results. The immunological processes that are involved in the development of allergic responses and allergic disease are in principle and practice no different to those that provide protective immunity and host resistance against potential pathogens.

Allergy normally develops in two phases. The first phase is induced following initial encounter of the susceptible individual with the allergen. A primary immune response is mounted that results in a state of heightened responsiveness to that particular antigen (specific sensitization). In immunological terms sensitization to an allergen does not differ from immunization to a pathogenic microorganism. Following second or subsequent exposure of the now sensitized individual to the inducing allergen a more vigorous and accelerated secondary immune response is provoked and it is at this stage that adverse health effects are normally first recognized. The aggressive secondary immune response against the allergen causes local tissue disruption and inflammation that is recognized clinically as allergic disease.

Individuals vary widely in terms of allergic responsiveness and susceptibility to allergic disease. There are a number of factors of importance here including opportunities for encounter with the inducing allergen, the route, the dose or concentration of allergen, extent and duration of exposure and genetic predisposition. The latter is incompletely understood but clearly impacts significantly upon susceptibility. Respiratory allergy (including hay fever and asthma) to protein aeroallergens is associated frequently with atopy; a genetic predisposition for increased production of IgE, the class of antibody

that causes respiratory hypersensitivity to proteins. In addition, the immunological repertoire of individuals and the ability of their immune system to recognize and respond to certain antigenic structures will also influence susceptibility.

Allergic diseases are widespread and can be caused by allergens encountered in the external environment, home or work. They range from comparatively mild inflammatory responses localized to a single site to systemic anaphylactic responses that may prove fatal. Allergic disease, as well as representing an important and widespread health problem, is also of great economic significance with respect to the cost of health care and time lost from work. It has been recognized that some forms of allergy are increasing in prevalence, compounding the health impact of these diseases. The incidence of asthma, for instance, has grown significantly in some developed countries, an increase that may be attributable to changing allergen exposure patterns, alterations in lifestyle, environmental pollution or to a combination of all of these factors.

In the context of occupational and environmental health the two most important allergic diseases caused by exposure to chemicals are allergic contact dermatitis and respiratory hypersensitivity. The former is very common and can be induced by industrial chemicals, metals and natural products. Sensitization results from dermal exposure of the susceptible individual to the inducing allergen. Allergic contact dermatitis reactions are provoked subsequently when the now sensitized individual is exposed for a second time to the inducing allergen at the same or different skin site. Many hundreds of contact allergens, varying enormously in potency, have been identified.

Although from the occupational and environmental health standpoint allergic contact dermatitis and respiratory hypersensitivity represent the most important types of allergy induced by chemicals, it should not be forgotten that exposure to xenobiotics has been implicated in other forms of allergic disease. Certain drugs are associated with systemic allergic reactions that are sometimes reminiscent of autoimmune diseases. In addition, food components and food additives are implicated in adverse reactions, which in some cases take the form of an allergic response.

1.1.4 Conclusion

An active adaptive immune system is essential for health and survival in a hostile microbiological environment. A price paid for the host resistance provided by the immune system is that some immune responses, often to benign antigens, result in the adverse health effects of allergic disease.

1.2 Physiology and components of the immune system

Immunity refers to all those physiological mechanisms/processes that enable an animal (i.e., the host) to recognize materials as foreign to "self" and to neutralize, eliminate or metabolize them, with or without injury to its own tissue. The immune system of higher animals is therefore capable of distinguishing between self materials from which they are constituted and "non-self" (i.e., those that are foreign or antigenic). It probably evolved to confer a selective advantage to organisms that could withstand colonization and microbial invasion. The immune response must decipher sometimes quite subtle differences between self and non-self, without error, to both provide protection and avoid self-attack. Accomplishment of this selective process requires the concerted action of a number of cell types. Mammals have developed a highly complex, intertwined and redundant system composed of layers of protective mechanisms to cope with more sophisticated environmental threats.

The immune system comprises both lymphoid organs and specialized cells. Erythrocytes, myeloid cells, megakaryocytes (which mature to form platelets) and lymphocytes arise from a totipotent or pluripotent stem cell in the yolk sac of the developing fetus and, later, the fetal liver. In adult mammals, the stem cells are manufactured in the bone marrow and progress via different pathways of differentiation to become mature cells that may carry out specialized functions, such as antibody production or phagocytosis (Abramson et al., 1977). The thymus and bone marrow are the primary lymphoid organs that serve to nurture the development of stem cells into mature effector cells. Mature lymphocytes traffic to the secondary lymphoid organs, the lymph nodes, spleen and mucosal-associated lymphoid tissues (MALTs), and form immune-reactive units that respond vigorously to antigens. The design of these secondary organs is such that the specialized populations of lymphocytes reside in proximity, can interact with each other, and can regulate the antigen-driven immune response required. The lymph nodes, which are situated throughout the body, filter out antigens draining from the peripheral bodily tissues.

The spleen monitors the blood and functions as a factory for red blood cell turnover. The MALTs provide a frontline defence for microbes that are ingested. Lymphocytes that reside in the spleen can, upon encountering antigen, respond *in situ* or migrate to the site of infection via the blood, colonizing a sensitized response unit in a local lymph node. The virgin stem cell is believed to receive different maturational stimuli in the microenvironment of the bone marrow, with stromal cell contact and lymphokine exposure inducing entry into one of several pathways of development. Functional lymphocytes are continuously formed from stem cells and pass from the bone marrow through the bloodstream to the lymphoid organs. The migratory pattern of the lymphocyte determines its lifespan and behaviour, as described in greater detail below for T-cells, B-cells and other immunocompetent cells.

1.2.1 T-cells

Stem cells that enter the thymus gland, formed from the third and fourth pharyngeal pouches in mammals, rapidly divide, acquire their antigen specificity and are selectively deleted if they bear any self-reactivity. The "educated" daughter cells, termed thymus-derived or T-lymphocytes, then leave the thymus and travel to other lymphoid tissues, persisting for weeks or even years. As stem cells pass through the thymic subcapsular region, cortex and medulla, they display plasma membrane-bound surface molecules that define their function. It is possible to experimentally identify and isolate subpopulations of T-lymphocytes by exploiting the differential expression of these marker glycoproteins, using alloantisera or monoclonal antibodies and immunostaining techniques. Murine T-lymphocytes possess both the Thy-1 marker and the T-cell antigen receptor (TCR)-CD3 complex, and fall into two major classes, either T-helper/inducer cells expressing CD4 or T-suppressor/cytotoxic cells, which display CD8.

Studies in inbred mice show that the T-cell antigen receptor only recognizes antigen processed and presented on major histocompatibility complex (MHC) molecules from the same thymic environment. MHC proteins are products of the immune response (Ir) genes, which are primarily responsible for tissue graft and organ transplantation rejection. In general, $CD4^+$ T-cells complex with antigen associated with MHC Class I molecules, which are only found on certain cells of the immune system, while $CD8^+$ T-cells only see antigen when associated with MHC Class I molecules, located on all

nucleated cells. T-cell selection of this type is termed positive and deletion of clones reactive to self is termed negative selection (Zinkernagel & Doherty, 1975). Upon contact with antigen, mature T-cells may either respond clonally in an antigen-specific manner and initiate an immune response, or become inactivated and eliminated in a process which is not well understood, potentially leaving the animal unable to recognize the antigen. This latter phenomenon is referred to as T-cell anergy.

The majority of lymphocytes in the peripheral blood and lymph nodes and about one half of the cells in the spleen are T-cells. Thymectomized animals or naturally occurring athymic or nude mice (because they are also hairless) and children with Di George syndrome are immunocompromised hosts that lack cell-mediated immune function and responses to T-dependent antigens (Sell, 1987). The endocrine function of the thymus has been recognized through partial recovery of T-cell function in thymectomized animals given cell-free thymic extracts, suggesting thymic hormones may, to some extent, replace thymus-driven T-cell maturation (Law et al., 1968). However, the thymic microenvironment appears necessary for proper selection and differentiation of the T-cell repertoire. Imbalances in the function of mature T-cell subpopulations may also occur clinically, as shown by HIV infection of $CD4^+$ T cells, resulting in decreased T-helper cell levels (Stahl et al., 1982; Lane & Fauci, 1985), and systemic lupus erythematosus in which lowered $CD8^+$ T-suppressor cell activity is thought to contribute to elevated antibody production and to exacerbate the autoimmune state.

1.2.1.1 *Balancing the immune response*

It is clear that in the mouse most T-cells show predominant production of two different sets of cytokines with pronounced, often mutually exclusive, effects on different features of the immune response (Romagnani, 1992a,b; Bloom et al., 1992; Mosmann & Sad, 1996). While some details of cytokine production are known to be different in the human, they are generally similar to that in the mouse. In brief, mouse Th1-cells produce IL-2, IFNγ and lymphotoxin (LT), whereas Th2-cells produce IL-4, 5,6,9,10,13, as shown in Table 1. Human Th1 and Th2 cells produce similar patterns, although the synthesis of IL-2,6,10,13 is not as tightly restricted to a single subset as in mouse T-cells. In the mouse Th1-cell (or Type I) responses result in delayed-type hypersensitivity (DTH) reactions, activation of

Table 1. Cytokine production in the mouse

Cytokine production	T-cells			Other cells					
	Th0	Th1	Th2	B-cell	Macrophage	NK-cell	Mast cell	Keratinocyte	LC
IL-1								+α	+β
IL-2	+	+							
IFNγ	+	+				+			
LT (TNFβ)	+	+							
IL-3	+	+	+				+		
GM-CSF	+	+	+					+	
TNFα	+	+	+		+		+	+	
IL-4	+		+	+			+		
IL-5	+		+						
IL-6	+		+		+			+	+
IL-10	+		+	+	+		+	+	
IL-12				+	+		+		
IL-13	+	+	+	+	+		+		

macrophages to kill phagocytosed microorganisms, and in IgG2a, rather than IgG1 and IgE, synthesis. Th2 (Type 2) responses generate IgG1- and IgE-secreting cells, and eosinophilia. Notably, Th2-derived IL-4 is an important switch factor for B cells to produce the IgG1 and IgE immunoglobulin-isotypes. Th1- and Th2-cells arise from a common lineage since they use the same T-cell receptor repertoire, and naive precursor T-cells, not yet exhibiting either of these cytokine profiles (Th0), can differentiate into both directions (see also section 2.1.5). Although cytotoxic $CD8^+$ T-cells often secrete a Th1-like cytokine pattern, there is evidence for the existence of Th2-like $CD8^+$ T (Tc2) cells in humans and mice (Croft et al., 1994; Mosmann & Sad, 1996). Type 2 cytokines such as IL-4 shift T cell differentiation away from the production of Type I cytokines, whereas the Type I cytokine IFNγ is very potent in preventing the development of Th2-cells.

Cytokines are soluble mediators synthesized by cells of the immune system that bind to specific receptors or target cells and modulate cell function in immunological reactions (Fig. 1). When starting clonal expansion after antigen stimulation, T-cells develop major cytokine profiles depending on the site of primary contact. Along mucosal surfaces predominant local IL-4 release, possibly by mast cells, basophils or locally residing T-cells, favours the development of Th2-cells (Scott, 1993; Weiner et al., 1994; Mosmann et al., 1996). In some individuals over-prone to IgE-switching, this response may be excessive, leading to mucosal allergies, such as respiratory hypersensitivity (see also chapter 4). The induction of Type 2 T-cell responses after antigen introduction along mucosal surfaces is probably further promoted by high local densities of B-cells as compared to the skin compartment. B-cells are excellent IL-10 producers, and antigen-presentation by B-cells is known to favour Th2 responses (Eynon & Parker, 1992). In addition to the archetype Type 2 cytokines, TGFβ has also been associated with Th2 functions, but preferential production by either a Th2 subset, or a distinct Th3 subset (Chen et al., 1994), is more likely to occur. As mentioned above, TGFβ plays the key role in immune suppression along mucosal surfaces, e.g., by controlling several different IFNγ-associated effector T-cell and macrophage functions (Karpus & Swanborg, 1991; Oswald et al., 1992; Khoury et al., 1992; Meade et al., 1992) and by maintaining epithelial cell layer integrity (Planchon et al., 1994). Moreover, TGFβ serves as a switch factor for IgA production. To what extent T-cells preferentially releasing TGFβ may also contribute to mucosal tolerance to IgE-inducing atopic allergens is still unclear.

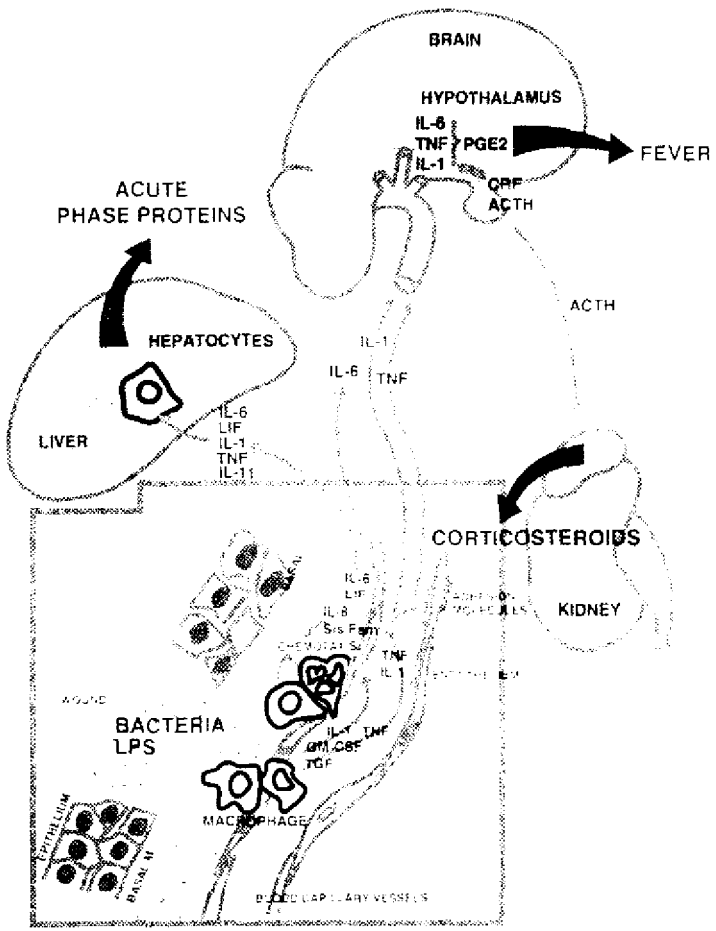

Fig. 1. Activity of various cytokines in the immune system: (a) in inflammation; (b) in immune responses; and (c) in haematopoiesis.

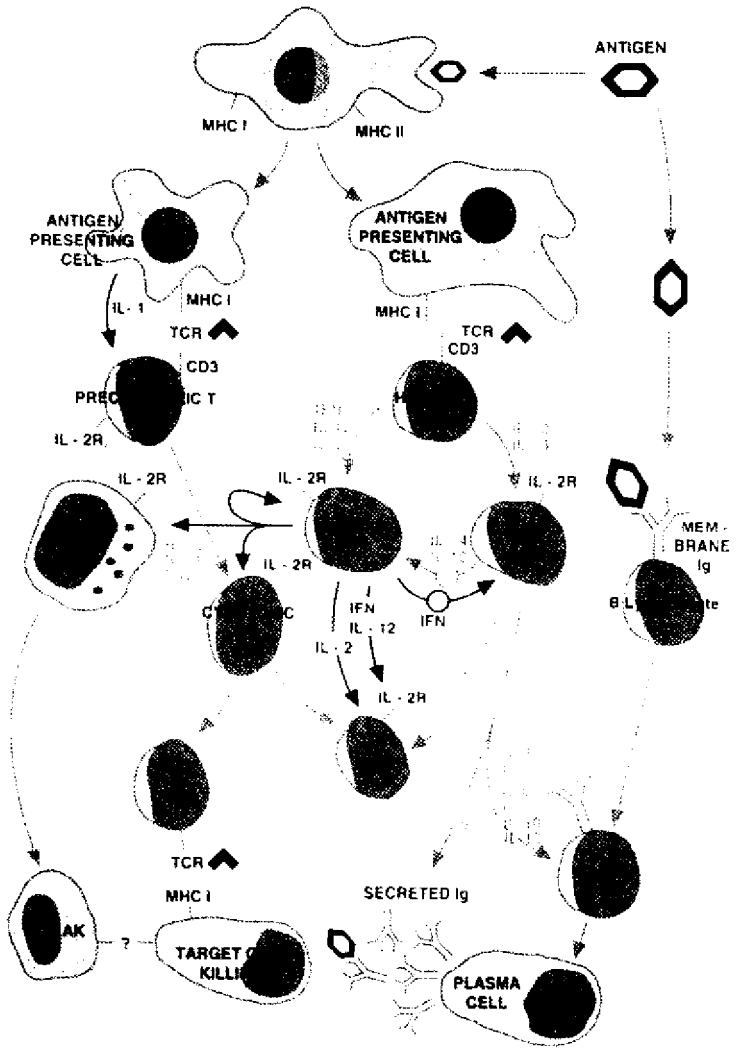
ANTIGEN
MHC I
MHC II
ANTIGEN PRESENTING CELL
ANTIGEN PRESENTING CELL
IL - 1
MHC I
MHC I
TCR
CD3
TCR
CD3
IL - 2R
IL - 2R
IL - 2R
IL - 2R
IL - 2R
MEM - BRANE Ig
IFN
IL - 12
IL - 2
IFN
IL - 2R
TCR
MHC I
SECRETED Ig
PLASMA CELL

Fig. 1b

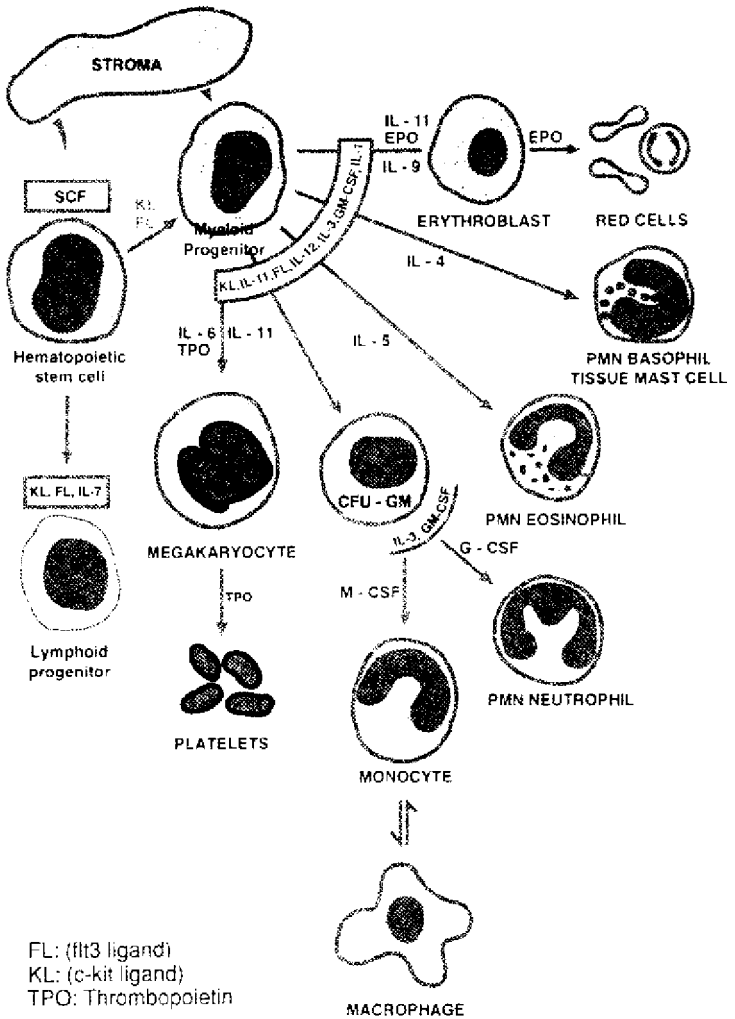
STROMA
SCF
KL
FL
Hematopoietic stem cell
KL, FL, IL-7
Lymphoid progenitor
Myeloid Progenitor
KL,IL-11,FL,IL-12,IL-3,GM-CSF,IL-1
IL - 6
TPO
IL - 11
IL - 11
EPO
IL - 9
ERYTHROBLAST
EPO
RED CELLS
IL - 4
PMN BASOPHIL
TISSUE MAST CELL
IL - 5
MEGAKARYOCYTE
TPO
PLATELETS
CFU - GM
IL-3, GM-CSF
G - CSF
M - CSF
PMN EOSINOPHIL
PMN NEUTROPHIL
MONOCYTE
MACROPHAGE
FL: (flt3 ligand)
KL: (c-kit ligand)
TPO: Thrombopoietin

Fig. 1c

In sharp contrast, along the skin route local release of IL-12 from, for instance, macrophages and NK-cells stimulates the production of IFNγ by T cells and facilitates predominant development of Th1 cells. Exposure of the skin to exogenous antigenic substances, including contact allergens, therefore preferentially induces specific Type 1, pro-inflammatory T-cell responses.

1.2.2 B-cells

In contrast to T-lymphocyte maturation, the development of lymphocytes capable of synthesizing and secreting antibody (immunoglobulin) molecules in mammals is thought to occur in several sites, including the bone marrow, spleen and MALTs. Because these cells were first characterized in birds, which, unlike mammals, possess a unique lymphoid organ, the bursa of Fabricius, and because the precursors of these cells are formed in the bone marrow, these cells have been termed B-lymphocytes. B-cells tend to reside for long periods of time in the secondary lymphoid organs and form the lymphoid follicles and germinal centres. Following activation by antigen or antigen-activated T-helper cells (Noelle et al., 1990) and lymphokines, B-cells proliferate and terminally differentiate to antibody-producing plasma cells, which turn over rapidly and are replenished by newly differentiated cells.

Like the T-cell antigen receptor (TCR)-CD3 complex, B-cells express surface antigen-combining receptor molecules which are of identical specificity to the immunoglobulins they synthesize and secrete. The diversity of the natural world has necessitated a complex series of molecular events in B-cell development designed to produce a spectrum of immunoglobulins capable of protecting the organism. B-cell maturation is marked by immunoglobulin gene rearrangements, recombinations and somatic mutations, so that a relatively small number of genes may efficiently produce a large number of antibody specificities.

B-lymphocytes synthesize immunoglobulins of five different types: IgM, IgG, IgA, IgD, and IgE. These proteins are composed of two separate types of polypeptide chains joined by disulfide linkages, termed the heavy and light chains because of differences in their relative molecular masses (the heavy chains are about twice as large) (see Fig. 2). Light chains are derived from either κ or λ genes and combine with the five different heavy chains μ, γ, α, δ and ϵ (i.e., for

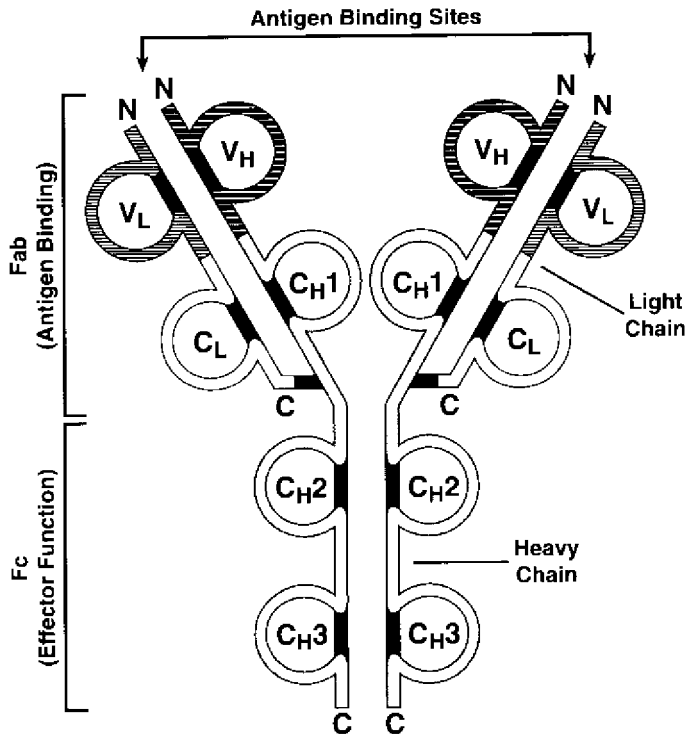

Fig. 2. Basic structure of immunoglobulins.

All immunoglobulin molecules consist of a basic unit of four polypeptide chains, two identical heavy chains and two identical light chains linked together by disulfide bonds. The class and subclass are determined by the specific type of heavy chain used in the molecule (i.e., γ1-4, μ, α, δ). Each chain contains globular regions called domains, which are stabilized by disulfide bridges. The first domain at the N-terminal ends of both the heavy and light chains is composed of regions of highly variable amino acid sequences (hypervariable regions), which form the antigen binding site, and less variable stretches (framework regions). The Fc portion of the molecule, which consists of 2 or 3 constant domains (depending on the isotype) from each heavy chain, mediates effector functions — complement activation and binding to Fc receptors — specific to each isotype and subclass (adapted from Janeway et al., 1997; Roitt et al., 1998).

the five different types of immunoglobulin identified above). Enzymatic digestion of immunoglobulin molecules yields fragments which indicate arrangement in a Y-shaped structure, consisting of two arms containing the antibody-combining sites for antigen, Fab fragments, and a tail region (Fc) which is important for effector functions and regulation of antibody responses. Surface immunoglobulin is predominantly of the IgM and IgD types on naive B cells and secreted immunoglobulin may be either IgM, IgG of four subclasses (1 to 4), IgA, or IgE. IgM is primarily secreted early, in what is termed the primary antibody response to antigen, with IgG constituting the later, secondary response. Lymphokines such as IL-4 and TGFβ induce heavy chain class switching in B-cell antibody responses, leading to the production of either IgGl and IgE, or IgA, respectively (Coffman et al., 1986; Coffman et al., 1989). The nature of the antigen encountered portends these lymphokine-mediated events. IgA-secreting B-cells are predominant in the MALTs, while IgE is of central importance in allergic reactions.

In addition to surface immunoglobulin, B-cells display receptors for Fc regions of immunoglobulin molecules, MHC Class II molecules, receptors for complement proteins, and the CD40 molecule which plays an essential role in the contact between B- and T-cells. B-cells appear to be comprised of two separate lineages, those that do and those that do not express the surface marker CD5 (E32). $CD5^+$ B-cells comprise a small percentage of the splenic B-cell population, are more prevalent in the peritoneal cavity of mice, and appear to be long-lived, activated cells that differ from conventional B-cells in their activational characteristics and capacity for self-renewal.

1.2.3 Macrophages

Stem cells also give rise to mononuclear phagocytes of the myeloid series, of which the macrophage is the primary cell type. Immature macrophages leave the bone marrow and are found in the lymphoid organs, the liver, lungs, gastrointestinal tract, central nervous system, serous cavities, bone, synovium and skin, and differentiate within these sites. Macrophages are attracted to microbes by the gradient of foreign molecules emanating from them, a process called chemotaxis. Upon contact, the macrophage can engulf the microbe, process and present the derived antigen via its MHC molecules to T cells, and secrete cytokines (e.g., IL-1, TNFα, IL-12), degradative enzymes, complement components, reactive oxygen intermediates and

coagulation factors. Macrophages readily infiltrate tumours and provide one mechanism of host defence against malignancies.

1.2.4 Antigen-presenting cells

If an antigen penetrates the tissues it will be processed by antigen-presenting cells (APCs) and transported to the draining lymph nodes. Antigens that are encountered in the upper respiratory tract or intestine are trapped by local mucosal-associated lymphoid tissues, whereas antigens in the blood provoke a reaction in the spleen. Macrophages in the liver will filter blood-borne antigens and degrade them without producing an immune response, since they are not strategically placed with respect to lymphoid tissue. Classically, it has always been recognized that antigens draining into lymphoid tissue are taken up by macrophages. They are then partially, if not completely, broken down in the lysosomes; some may escape from the cell in a soluble form to be taken up by other APCs and a fraction may reappear at the surface either as a large fragment or as a processed peptide associated with MHC Class II major histocompatibility molecules. Although resting resident macrophages do not express MHC Class II, antigens are usually encountered in the context of a microbial infectious agent which can induce the expression of MHC Class II by its adjuvant-like properties expressed through molecules such as bacterial lipopolysaccharide (LPS). There is general agreement that the APC must bear antigen on its surface for effective activation of lymphocytes and ample evidence that antigen-pulsed macrophages can stimulate specific T- and B-cells both *in vitro* and when injected back *in vivo*. Some antigens, such as polymeric carbohydrates like ficoll, cannot be degraded because the macrophages lack the enzymes required; in these instances, specialized macrophages in the marginal zone of the spleen or the lymph node subcapsular sinus, trap and present the antigen to B-cells directly, apparently without any processing or intervention from T-cells. Notwithstanding this impressive account of the macrophage in antigen presentation, there is one function where it is seemingly deficient, namely, the priming of naive lymphocytes. Animals that have been depleted of macrophages by selective uptake of liposomes containing the drug dichloromethylene diphosphonate are as good as control animals with intact macrophages in responding to T-dependent antigens. It must be concluded that cells other than macrophages prime T-helper cells and it is generally accepted that these belong to the group of dendritic cells.

Dendritic cells are large, motile, weakly phagocytic, "professional" APCs that usually have several elongated pseudopodia. Dendritic cells comprise about 2% of the cells in the secondary lymphoid organs. They are localized strategically in the T-cell areas of the lymph node (interdigitating dendritic cells). Interdigitating cells express large amounts of MHC Class II molecules, and this expression plays a pivotal role in the presentation and induction of certain kinds of immune cells (such as Th 1) and the presentation of antigen to $CD4^+$ T-cells. Active follicular dendritic cells, although not derived from haematopoietic stem cells, express high levels of CD23 (an IgE Fc receptor) and C3 receptors, which allows them to trap antigen-antibody complexes and present them to memory B-cells. Normal skin contains a population of dendritic cells called Langerhans cells that change their morphology to become interdigitating dendritic cells within the T-cell areas of lymph nodes. Langerhans cells give the immune system information regarding foreign substances that breach the skin. Langerhans cells pick up skin-sensitizing antigens (e.g., antigens of the poison ivy plant) and migrate to the draining lymph nodes. Langerhans cells are important in the delayed-type hypersensitivity response known as contact dermatitis.

The need for physical linkage of hapten and carrier strongly suggests that T-helper cells must recognize the carrier determinants on the responding B-cell in order to provide the relevant accessory stimulatory signals. However, since T-cells only recognize processed membrane-bound antigen in association with MHC molecules, the T-helper cells cannot recognize native antigen bound simply to the Ig-receptors of the B-cell. Primed B-cells can present antigen to T-helper cells; in fact, they work at much lower antigen concentrations than conventional presenting cells because they can focus antigen through their surface receptors. They must therefore be capable of processing the antigen and the current view is that antigen bound to surface Ig is internalized in endosomes, which then fuse with vesicles containing MHC Class II molecules with their invariant chain. Processing of the protein antigen then occurs and the resulting antigenic peptide is then recycled to the surface in association with the Class II molecules where it is available for recognition by specific T-helper cells.

1.2.4.1 *Co-stimulatory molecules in T-cell activation*

Binding of the antigen/MHC-complex to the T-cell receptor (Fig. 3) and co-receptors like CD4 and CD8 is not sufficient to

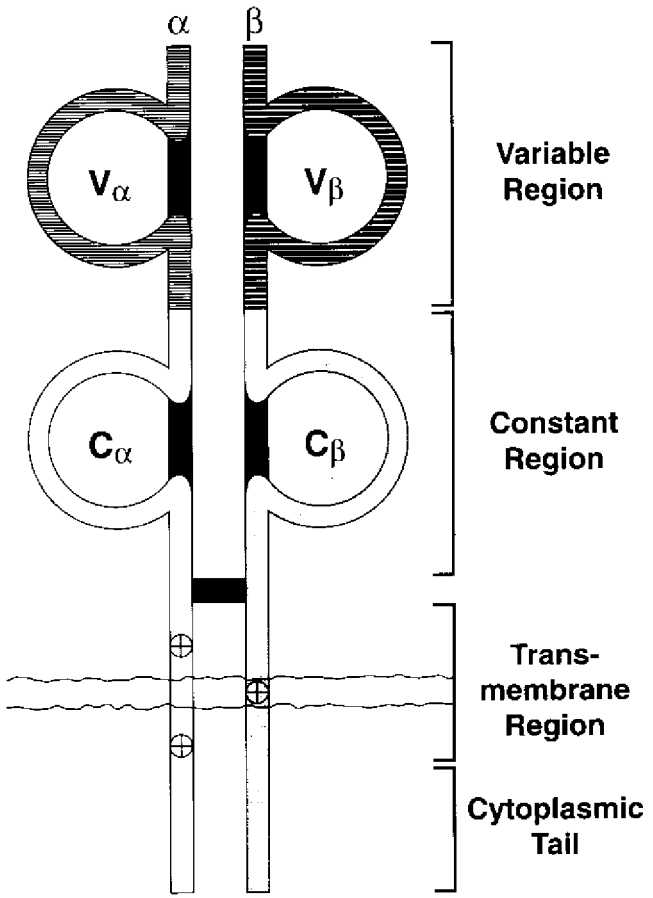

Fig. 3. The T-cell receptor.

The T-cell receptor is a heterodimeric molecule composed of two transmembrane glycoprotein chains linked together by a disulfide bond in a structure that is highly similar to the Fab portion of the immunoglobulin molecule. The external structures of each chain consist of a constant and a variable domain, which form the antigen binding sites (adapted from Janeway et al., 1997; Roitt et al., 1998).

stimulate naive T-lymphocytes to proliferate and differentiate into effector T-cells. For antigen-specific clonal expansion and differentiation, a second, co-stimulatory signal is required. The same cell that presents the specific antigen to the T-cell receptor must deliver this co-stimulatory signal. The best-characterized co-stimulatory moleculeson APCs are the so-called B7 molecules, B7.1 (CD80) and B7.2 (CD 86). Their receptor on T-cells is CD28; all three molecules mentioned are members of the so-called immunoglobulin superfamily. B7.2 is present on resting APCs, whereas B7.1 is expressed predominantly on activated cells. It has been suggested that B7.2 is of particular importance in the allergic immune response and represents a potential therapeutic target (Robinson, 1998). However, clear functional differences between B7.1 and B7.2 have not been defined (Lenshow et al., 1996; Chambers & Allison, 1997).

On naive T-cells, CD28 is the only receptor for B7 molecules. Activated T-cells, in contrast, also express another receptor for B7 called CTLA-4, which closely resembles CD28 but delivers a negative signal to the T-cells (Chambers & Allison, 1997). Thus, binding of B7 to CTLA-4 will contribute to limiting or down-regulating the proliferative response and T-cell production of IL-2.

Because of the requirement for co-stimulatory signals to obtain productive antigenic stimulation of T-cells, only so-called professional APCs, that is cells that are able to deliver proper co-stimulation, can initiate a T-cell-dependent immune response. If antigen binds to the T-cell receptor in the absence of proper co-stimulation, the T-cell will not be activated but may instead become refractory to activation, a state called anergy. In addition to the co-stimulatory B7 molecules, a professional APC must also express adhesion molecules like ICAM-1, ICAM-2 and LFA-3 and be able to process antigen. There is evidence that different types of APCs differ with regard to their co- stimulatory properties.

1.2.5 Adhesion molecules

Adhesive interactions of leukocytes with other immune cells or with non-immune cells are central to the successful functioning of the immune system. Such cell-cell interactions are mediated by different types of accessory molecules which stabilize attachment, for instance between T-cells and APCs, and which may provide (co-)stimulatory signals upon triggering of the antigen receptor. These molecules are

also regularly used as identification markers for distinct leukocytes subclasses or for their activational state (Schleimer & Bochner, 1998). Three families of such cell surface molecules have been categorized:

(i) The immunoglobulin-gene superfamily includes the antigen-specific receptors of B- and T-cells as well as the CD4 and CD8 molecules and their respective ligands MHC Class II and I; the adhesion molecules CD2, CD54, CD58 and CD102 also belong to this group.

(ii) The integrin family accounts for antigen-independent adhesion between cells; their ligands are found on other leukocytes, on endothelial cells and in the extracellular matrix; some representative members of this family are CD11a/CD18, CD11b/CD18, CD11c/CD18 (referring to the α/β chains, respectively) and the so-called very late activation (VLA-) molecules on T-lymphocytes, which facilitate the migration of these cells to peripheral inflammatory sites.

(iii) The third family, the selectins, can be expressed on leukocytes (L-selectin) and endothelium (E-selectin). These molecules play a role in the directed migration of lymphocytes (for instance naive lymphocytes bind preferentially to the high endothelial cells in the lymph nodes), neutrophils and macrophages.

Table 2 shows the molecules facilitating the cellular contact between APC and T-cells, and adhesion molecules playing a role in the migration of leukocytes are shown in Table 3. Fig. 4 illustrates antigen presentation and cell-cell contact.

1.2.6 *Fc receptors*

Fc receptors (FcR) are cell surface glycoproteins interacting specifically with the Fc domains of different isotypes of immunoglobulins (Ravetch, 1994, 1997; Gergely & Sarmay, 1996; Deo et al., 1997; Vivier & Daeron 1997). FcRs are widely distributed on cells of the immune system and mediate different effector responses. In addition, they play an important role in the initiation of immunocomplex-triggered inflammation and regulate the antibody production of B-cells. Immunoglobulin-binding receptors, including the high affinity receptor for IgE (FcϵRI) on mast cells and basophils, the high

Table 2. Adhesion and (co-)stimulatory molecules mediating antigen presentation to T-cells (modified from Janeway et al., 1997)

	Adhesion molecules expressed on antigen-presenting cell (APC)	Ligand expressed on T-cell
Initial contact between APC and T-cell	CD58 (LFA-3)	CD2
	CD54(ICAM-1) }	CD11a/CD18 (LFA-1)
	CD102 (ICAM-2) }	
	CD11a/CD18 (LFA-1)	CD50 (ICAM-3)
Antigen presentation and T-cell activation	antigenic peptide in MHC context	TCR/CD3
	MHC-Class II	CD4
	MHC-Class I	CD8
	CD80 (B7.1) }	{ CD28
	CD86 (B7.2) }	{ CTLA-4

Table 3. Adhesion molecules mediating leukocyte migration (from Janeway et al., 1997)

	Adhesion molecules expressed on leukocyte	Ligand on endothelium or extracellular matrix
Migration of naive T-cells into lymphoid tissue	CD62L (L-selectin)	{ CD34 { GlyCAM-1 { MadCAM-1 (Mucosae)
Migration of memory T-cells into peripheral tissue	CD11a/CD18(LFA-1)	{ CD54 (ICAM-1) { CD102 (ICAM-2)
	Cutaneous lymphocyte antigen (CLA)	CD62E (E-selectin)
	CD49d/CD29 (VLA-4) CD49d/CD29 (VLA-5)	CD106 (VCAM-1) fibronectin
Migration of neutrophil and macrophages into peripheral tissue	sialyl-Lewis ^{x} moiety	{ CD62E (E-selectin) { CD62P (P-selectin)
	CD11a/CD18 (LFA-1)	{ CD54 (ICAM-1) { CD102 (ICAM-2)
	CD11b/CD18 (MAC-1)	CD54 (ICAM-1)

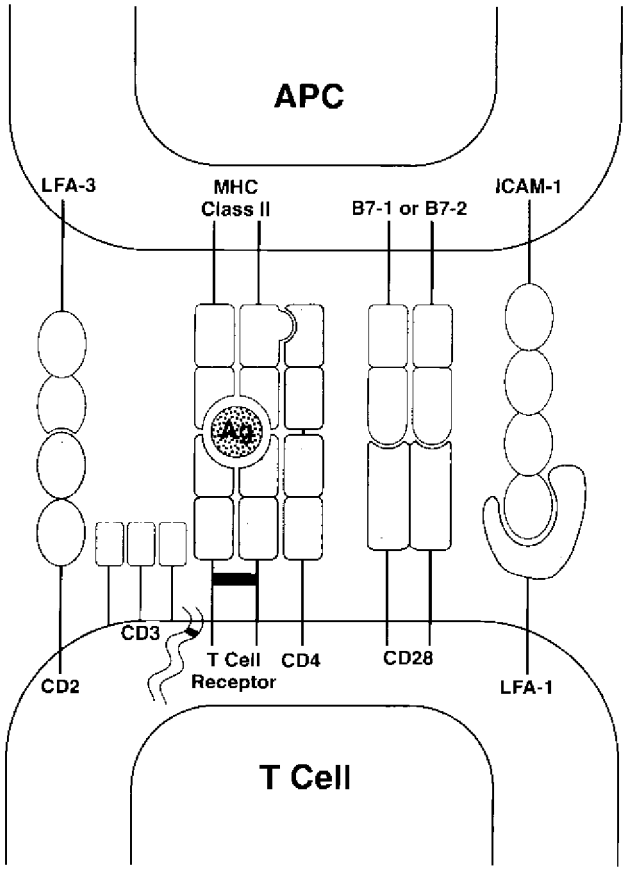

Fig. 4. Antigen presentation.

Effective antigen presentation and subsequent T-cell activation requires interactions between multiple cell surface molecules. The initial interaction between T-cells and antigen-presenting cells (APCs) is via low affinity interactions between lymphocyte function-associated antigen-1 (LFA-1) on the T-cell and intercellular adhesion molecules (ICAMs) on the APC. The interaction of CD2 on the T-cell with LFA-3 on the APC also facilitates communication between the two cell types. The T-cell receptor recognizes antigenic peptides presented via MHC molecules to provide the first signal for activation. In general, T-cells must receive a second signal in order to proliferate and differentiate. This second signal is delivered via the co-stimulatory molecules B7-1 or B7.2 on the APC, which bind to the CD28 molecule on the T-cell. CD4 or CD8 molecules enhance binding of MHC molecules and also act as signal transducers (adapted from Janeway et al., 1997; Roitt et al., 1998).

and low affinity receptors for IgG (FcγRI, FcγRII and FcγRIII) and the high affinity receptor for IgA, belong to the immunoglobulin supergene family. The low affinity FcεRII (CD32) is a lectin-like molecule (Table 4).

The ligand binding chains (α) of all FcγRs contain extracellular parts comprising Ig-domains (FcγRI has three, the others two). The high affinity IgE-binding receptor (FcεRI) is a tetrameric molecule containing one α, one β and two γ chains. The IgE-binding site is located on the extracellular part of the α chain. The β chain has four transmembrane loops while the dimeric γ chains possess very long cytoplasmic tails.

FcγRI, FcγRIII and FcεRI belong to the family of multisubunit immune recognition receptors (MIRRs), which are characterized by a complex hetero-oligomeric structure in which ligand binding and signal transducing functions are segregated into distinct receptor substructures (Table 5).

1.2.7 Polymorphonuclear leukocytes

Polymorphonuclear leukocytes (PMNs) are myeloid phagocytic cells important for the inflammatory responses of both specific and nonspecific immunity. Polymorphonuclear leukocytes are also called granulocytes because they contain granules composed of digestive enzymes and bactericidal substances. The granulocyte progenitor can develop into cells called either neutrophils, basophils/mast cells or eosinophils, names which refer to the variable dye staining patterns of their cytoplasm. These cells are also chemotactic and are attracted by lymphokines released from lymphocytes in areas of infection. Like macrophages, polymorphonuclear leukocytes participate in antibody-dependent cell-mediated cytotoxicity (ADCC) reactions, in which coating (opsonization) of microbial surfaces by specific antibody enhances their recognition by cytotoxic or phagocytic leukocytes.

1.2.8 Cytotoxic lymphocytes

Cytotoxic lymphocytes are defined by their capacity to recognize and kill target cells. These cells fall into at least two different populations, a) those that require recognition of MHC Class I

Table 4. Cellular distribution and binding properties of Fcγ receptors

Class	CD	Relative molecular mass	Affinity (K_a)	Expression[a]	Ig-binding[b]
FcγRI	CD64	72 000	10^8-10^9 M^{-1}	Mo, M	hu, 3>1>4>>2
FcγRII	CD32	40 000	<10^7 M^{-1}	Mo, N, Ba, Eo, Langerhans cell, B-cell	hu, 3>1>>2,4 mu, 2b>>2a
FcγRIII	CD16	50 000–80 000		Thr, endothelial cells of the placenta	
FcγIIIa			$3x10^7$ M^{-1}	Mo, M, LGL/NK, T-cell	hu, 1=3>>2,4 mu, 1=3>>2,4
FcγIIIb			<10^7 M^{-1}	N	

[a] Mo = monocyte, M = macrophage, N = neutrophil granulocyte, Ba = basophil granulocyte, Eo = eosinophil granulocyte, Thr = platelet, LGL = large granular lymphocyte, NK = natural killer cell
[b] hu = human, mu = murine

Table 5. Multisubunit immune recognition receptors (MIRRs) family

Receptor	Ligand-binding subunit	Signal transducing subunit
BCR (B-cell antigen receptor)	mIg	Igα (CD79a) Igβ (CD79b)
TCR (T-cell antigen receptor)	α-β or γ-δ	CD3γ, δ and ε ζ-ζ or ζ-η
FceRI	α-chain	β and γ chain
FcγRIIIa	α-chain	FceRIγ-chain or TCR ζ-chain
FcγRI	α-chain	FceRIγ-chain

molecules for their activation, namely $CD8^+$ T-cells, and b) those that are silenced by recognition of these molecules, namely natural killer (NK) cells, previously named "null cells" or large granular lymphocytes (LGL). Cytotoxic $CD8^+$ T-cells constitute the major population of cytotoxic T lymphocytes (CTL) and are crucial for the defence against intracellular, in particular viral, pathogens. Peptides derived from such pathogens are processed into the endogenous pathway of antigen presentation and exposed on the outer cell membrane by Class I molecules. This complex is recognized by the T-cell receptor, after which CTL-target cell binding is further stabilized by CD8-Class I interaction. In contrast, NK cell-target cell recognition is largely non-specific, but involves receptors recognizing disturbed surface carbohydrates and an Fc receptor for IgG that can facilitate antibody-dependent cell-mediated cytotoxicity (ADCC). NK cells are unique in bearing distinct receptors which, when bound to MHC Class I molecules, deliver signals interfering with their cytolytic activity.

For both types of cytotoxic lymphocytes the actual killing process involves two major mechanisms, i.e., release of a membrane pore-forming protein named perforin from granules, leading to osmotic lysis of target cells, and release of lymphotoxin which activates enzymes in the target cell to cleave DNA in the nucleus. The latter process is also known as apoptosis. Most cytotoxic lymphocytes also express a member of the tumour necrosis factor (TNF) superfamily, i.e., Fas-ligand, mediating a third lytic mechanism for target cells

expressing the Fas antigen. The killing capacity of cytotoxic lymphocytes is greatly enhanced by distinct cytokines, in particular IL-2 and IL-12. Microscopically this is reflected by the appearance of more prominent granules, e.g., in the so-called lymphokine-activated killer (LAK) cells. Both major cytotoxic lymphocyte populations are crucial to various phases of viral attack, but are not prominent in causing allergic disorders. Nevertheless, contact allergens may directly associate with surface-bound Class I molecules or enter the cytoplasm of, for instance, Langerhans cells and associate with peptides presented along the endogenous route of antigen presentation. In this way, $CD8^+$ T-cells may become involved in allergic contact dermatitis reactions.

1.2.9 Mast cells

Mast cells are derived from precursors in the bone marrow that migrate to specific tissue sites to mature. While they are found throughout the body, they are most prominent in the skin, the upper and lower respiratory tract, and the gastrointestinal tract (Tharp, 1990). In most organs mast cells tend to be concentrated around the small blood vessels, the lymphatics, the nerves and the glandular tissue (Tharp, 1990). These cells contain numerous cytoplasmic granules that are enclosed by a bilayered membrane. There appear to be two different populations of mast cells in humans, based on the presence or absence of certain proteolytic enzymes, notably tryptase and chymase (Tharp, 1990). Mast cells found in the skin and connective tissue have both enzymes, while those in the alveoli, bronchial and bronchiolar regions, and mucosa of the small bowel contain only tryptase (Irani et al., 1986). However, both types of cells are triggered in the same manner.

Mast cells may be activated by antigen-specific IgE bound to high affinity receptors (Fc RI), antigen-specific IgE bound to low affinity IgG receptors (FcϵRII/III), or through complement receptors. Following activation, most cells release preformed mediators such as histamines and generate newly formed mediators such as TNFα and leukotriene C4 (LTC4) (Van Loveren et al., 1997). Both mast cells and basophils arise from CD34 pluripotent stem cells. At what point the cell lineages diverge is unknown, but mature mast cells depend on the local production of C-kit ligand (stem cell factor) for their survival. Basophils will not survive in the presence of stem cell factors but do respond to IL-3.

1.2.10 Basophils

Basophils represent approximately 1% of the white blood cells in peripheral blood. They have a half-life of about 3 days. They respond to chemotactic stimulation and tend to accumulate in inflammatory reactions. Basophils have high affinity IgE receptors as do mast cells. Cross-linking of surface-bound IgE by a multivalent specific allergen causes changes in the cell membrane and signal transduction that result in the release of mediators from the cytoplasmic granules. These preformed mediators include histamine, many other potent mediators, and proteolytic enzymes (Tharp, 1990; Goust, 1993; Janeway et al., 1997). Release of these substances from mast cells and basophils is responsible for the early phase symptoms seen in allergic reactions, which occur within 30 to 60 min after exposure to the allergen. IL-4 synthesis and release occurs hours later. Release of these basophil-derived mediators is believed to contribute to the late phase allergic response. The clinical manifestations due to release of both preformed and newly synthesized mediators from mast cells and basophils vary from a localized skin reaction to a systemic response known as anaphylaxis. Symptoms depend on variables such as route of exposure, dosage and frequency of exposure (Marsh & Norman, 1988).

1.2.11 Eosinophils

Eosinophils represent 2–5% of the leukocytes. Polymorphonuclear eosinophils resemble polymorphonuclear neutrophils, with the difference that they contain large red granulations (eosin staining) and refringent crystals, which may also be traced in the expectorates of asthmatic patients (Charcot-Leyden crystals). Eosinophil counts are increased, especially in allergic reactions, but they also act as a defence against certain parasites, in chronic inflammatory phenomena, and perhaps also in the defence against cancer. Like neutrophils, they do not return to the bone marrow from which they originate, but are eliminated via mucosal surfaces.

In the biphasic pattern of certain asthma attacks (an acute phase followed, about 6 h later, by a late phase), eosinophils attracted to the inflammatory zone during the late phase cause extensive destruction of the bronchial mucosa. This is similar to the destruction by eosinophils of certain parasites like schistosomes, responsible for schistosomiasis.

1.2.12 Complement components

Protective immunity requires the interaction of the immune cell types described above with secreted proteins found in the blood and lymph. In addition to antibody and lymphokines, the complement proteins represent a series of important protective substances (Table 6). More than 20 of these proteins participate in reactions that mediate lysis of foreign cells. Complement-mediated lysis of bacterial cells, for example, can take place through two routes, the classical pathway, which is catalysed by complexes of antibody molecules, or the alternative pathway, which can be activated by the antigen alone and by some immunoglobulins (Fig. 5). This results in deposition of a membrane attack complex of complement proteins on the surface of the microbial cell, leading to lysis. This process occurs as a cascade of enzymatic cleavage reactions, yielding both the lytic structure and production of biologically active components that induce migration of lymphocytes and an inflammatory response.

1.2.13 Immunoglobulins

Table 7 summarizes the human immunoglobulin isotypes and their concentrations in serum.

1.2.13.1 IgG

IgG represents 75–80% of the total Ig in humans. IgG2 and IgG4 cross the placental barrier. Thus, at birth, a baby temporarily carries IgG of its mother, which lasts for 4–6 months.

IgG intervenes in infections by means of opsonization and it can neutralize toxins. IgG appears especially following a secondary immune response, i.e., after a second encounter with antigen. The secretion of IgG is modulated by collaboration between B- and T-lymphocytes. IgG is strongly opsonizing for macrophages and polymorphonuclear cells possessing receptors for the Fc portion of IgG.

Antigenic analysis of IgG myelomas revealed further variation and showed that they could be grouped into four isotypic subclasses now termed IgG1, IgG2, IgG3 and IgG4. The differences all lie in the heavy chains, which have been labelled $\gamma 1$, $\gamma 2$, $\gamma 3$ and $\gamma 4$,

Table 6. Principal components of the complement system

Protein	Relative molecular mass	Concentration in serum (μg/ml)	Characterization and function
Early components			
Classical pathway			
C1q	410 000	70	consists of a collagen-like and a globular part; binds to the Fc part of Ig
C1r	85 000	50	serine protease; activates C1s
C1s	85 000	50	serine protease; activates C4-C2
C4	210 000	300	C4b binds to C2b
C2	110 000	25	serine protease; catalytical part of C4bC2b[a]
Lectin pathway			
MBL (Mannose-binding lectin)	410 000	1	consists of a collagen-like and a carbohydrate part
MASP1 (Mannose-binding lectin associated serine protease)	85 000	5	serine protease; activates MASP2
MASP2	85 000	5	serine protease; activates C4
Alternative pathway			
Factor-D	25 000	1	serine protease; activates factor-B
Factor-B	93.000	200	serine protease; as the component of C3bBb[a] convertase activates C3
Properdin	220 000	25	stabilizes the C3bBb[a] convertase

Table 6 (contd).

Protein	Relative molecular mass	Concentration in serum (µg/ml)	Characterization and function
Common component of the various pathways			
C3	190 000	1300	together with C3b, interacting with C4b2b[a] and C3bBb[a] forms C5-convertase; fragment C3a is one of the anaphylatoxins
Terminal components			
C5	190 000	70	fragment C5b binds C6; fragment C5a is one of the anaphylatoxins
C6	120 000	60	binds C7
C7	110 000	55	binds C8
C8	150 000	55	binds C9
C9	70 000	60	its polymerized form is the MAC (membrane attack complex)

[a] The MBL-MASP complex (which is structurally similar to the C1 complex) activates the complement system. The carbohydrate-binding domain of MBL binds to the carbohydrate components of various microorganisms and the MASP cleaves C4.

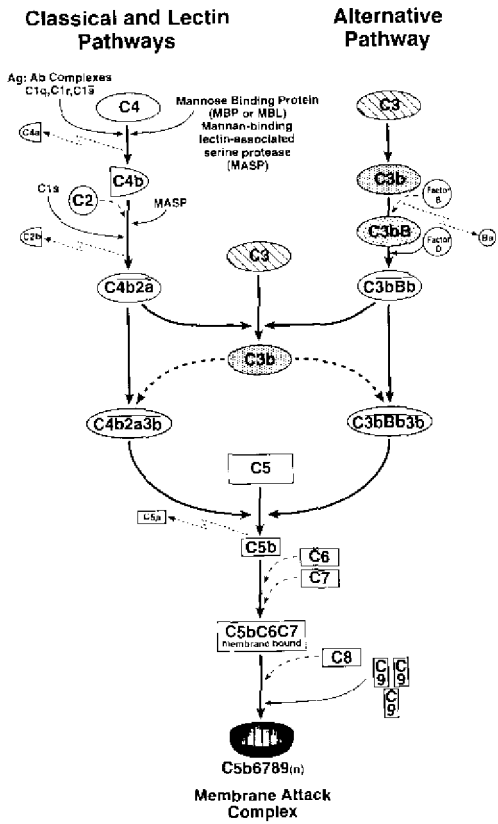

Fig. 5. Complement pathways.

There are two main pathways via which the effector functions of complement can be activated. The classical pathway is triggered by antigen:antibody complexes, while the alternative pathway may be triggered by a number of substances such as bacterial lipopolysaccharide (LPS) and cell wall components from various pathogens. The binding of serum lectins (such as mannose-binding protein) to pathogens can also initiate complement activation via the components of the classical pathway. Both pathways ultimately generate products that activate C3 and C5. The activation of C5 triggers the subsequent assembly of complement components C5b–C9 into the membrane attack complex, a hydrophobic "pore" that destroys the membrane integrity of the pathogen. In addition to its function in inducing cell lysis, the complement cascade produces fragments of complement components with specific biological functions, such as the chemotactic properties of C5a. A bar above the components indicates an enzymatically active complex of the components under the bar. Dashed arrows indicate contribution or release of a component. Solid arrows indicate an activation reaction (adapted from Janeway et al., 1997; Roitt et al., 1998).

Table 7. Human immunoglobulin isotypes

Class	Subclass	H-chain	Relative molecular mass	Concentration in serum (mg/ml)
IgA	IgA1	α1	150 000, 300 000, 400 000[a]	3.0
	IgA2	α2	150 000, 300 000, 400 000[a]	0.5
IgD	-	δ	180 000	trace
IgE	-	ε	190 000	trace
IgG	IgG1	γ1	150 000	9.0
	IgG2	γ2	150 000	3.0
	IgG3	γ3	150 000	1.0
	IgG4	γ4	150 000	0.5
IgM	-	μ	950 000[b]	1.5

[a] monomeric, dimeric, trimeric
[b] pentameric

respectively. These heavy chains show considerable homology and have certain structures in common with each other — those which react with specific anti-antisera — but each has one or more additional structures characteristic of its own subclass arising from differences in primary amino acid composition and in interchain disulfide bridging. These give rise to differences in biological behaviour (Table 8).

1.2.13.2 IgA

IgA represents 15-20% of the human serum immunoglobulin pool, where it occurs as a monomer of the regular immunoglobulin four-chain unit, in contrast to secretory IgA, which mainly occurs in dimeric form. The J chain which joins 2 IgA monomers facilitates the transfer of the secretory component through cells. IgA is the predominant immunoglobulin in seromucous secretions such as saliva, colostrum, milk, and tracheobronchial and genitourinary secretions. Dimer secretory IgA (sIgA), which may be of either of two subclasses (IgA1 or IgA2), but is mainly IgA2, is normally associated with yet another protein, known as the secretory component. The bound

Table 8. The properties of human Ig isotypes

	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgD	IgE
Complement activation, classical pathway	++	+	+++	-	+++	-	-	-	-
Complement activation, alternative pathway	-	-	-	-	-	+	-	-	-
Placental transfer		+		+	-	-	-	-	-
Binding to macrophages and other phagocytic cells	+	-	+	-	-	-	-	-	+
High affinity binding to mast cells and basophils	-	-	-	-	-	-	-	-	+++

secretory component facilitates the transport of sIgA through the epithelial cell layer(s) into the secretions and protects the antibody-dimer against subsequent proteolytic attack. IgA2 predominates in secretions since many microorganisms in the respiratory and gastrointestinal tracts release proteases that cleave IgA1, but not IgA2. Next to IgA, varying levels of IgE may be produced by locally residing plasma cells, but the primary site of action of this antibody isotype is in the sub-epithelial mucosal layers, e.g., in sensitizing locally intruding protozoan parasites and worms for subsequent cytolytic attack, notably by eosinophils. The secretory IgA (IgA-s) does not opsonize. It fixes antigen via its variable part and forms unabsorbed complexes. By capturing antigens, it prevents bacteria and viruses from adhering to the mucous membrane, thereby preventing their penetration into the organism.

The Fc fragment of IgA does not play any role, probably because it is obstructed by the secretory component.

IgA deficiency is encountered in one of 700 individuals, causing in such patients more frequent respiratory or gastrointestinal infections. In case of IgA deficiency, IgM can take over. In severe cases, there may be a simultaneous deficiency of both IgA and IgM.

1.2.13.3 *IgM*

IgM represents about 10% of immunoglobulins. IgM antibodies are pentamers (5 units), the monomeric units being fixed by a J chain. They are also known as macroglobulins or heavy globulins. IgM is the first to appear in an immune response, and is the predominant antibody isotype in the early phase of humoral immunity. As it has a short life span, its presence points out to a recent infection (e.g., in toxoplasmosis). Owing to its polyvalent structure, IgM can easily produce agglutination and readily fixes complement. Because of its large volume, it remains localized principally in blood. It does not cross the placental barrier and is the first molecule to meet a viral or microbial intruder in a blood vessel.

IgM antibodies tend to be of relatively low affinity as measured against single determinants (haptens) but, because of their high valency, they bind with considerable avidity to antigens with multiple epitopes. For the same reason, these antibodies are extremely efficient agglutinating and cytolytic agents and, since they appear early in the

response to infection and are largely confined to the bloodstream, it is likely that they play a role of particular importance in cases of bacteraemia. The isohaemagglutinins (anti-A, anti-B) and many of the "natural" antibodies to microorganisms are usually IgM; antibodies to the typhoid O antigen (endotoxin) and the WR antibodies in syphilis are also found in this class. IgM appears to precede other isotypes in the phylogeny of the immune response in vertebrates.

Monomeric IgM (i.e., a single four-peptide unit), with a hydrophobic sequence in the C-terminal end of the heavy chain to anchor the molecule in the cell membrane, is the major antibody receptor used by B-lymphocytes to recognize antigen.

1.2.13.4 IgD

This class was recognized through the discovery of a myeloma protein that did not have the antigenic specificity of A or M, although it reacted with antibodies to immunoglobulin light chains and had the basic four-peptide structure. The hinge region is particularly extended and, although protected to some degree by carbohydrate, it may be this feature that makes IgD, among the different immunoglobulin classes, uniquely susceptible to proteolytic degradation, and accounts for its short half-life in plasma (2.8 days). It has been demonstrated that nearly all the IgD is present, together with IgM, on the surface of a proportion of B-lymphocytes where it seems likely that they may operate as mutually interacting antigen receptors for the control of lymphocyte activation and suppression. The greater susceptibility of IgD to proteolysis on combination with antigen could well be implicated in such a function.

1.2.13.5 IgE

The plasma level of IgE in normal individuals is low (Table 7). The IgE level is commonly increased in patients suffering from Type I allergies. It is a cytophilic Ig, i.e., it fixes to the surface of certain cells, especially mast cells and basophils. It does not fix complement. IgE occurs predominantly in perivascular tissues where mast cells are localized. IgEs are responsible for Type I allergic reactions. The binding of IgE with an antigen specific to this IgE on the mast cell membrane provokes the release of mediators from mast cell granules (degranulation)(see section 1.2.9).

IgE plays a major role in allergy, but it also appears to intervene in the defence against parasites and perhaps also against cancer cells. A high IgE level in apparently healthy babies has been suggested as an accurate indicator of later allergic disorders (see chapter 5).

IgE levels are particularly elevated in atopic eczema and in intestinal parasitoses. Similarly elevated levels are also found in certain myelomas and in disorders involving a long- or short-term deficiency in T-lymphocytes, such as measles, infectious mononucleosis, Hodgkin's disease, and dysglobulinaemia. Specific IgE plays an important role in Type I allergies.

1.3 Immunotoxicology

Immunotoxicology may be defined as the scientific discipline concerned with the adverse effects resulting from the interaction of the immune system with xenobiotics. It includes the consequences of an action (i.e., either suppression or enhancement) by a substance (or its metabolite) on the immune system, as well as the immunological response to such a substance (IPCS, 1996). A major focus of immunotoxicology is the detection and evaluation of undesired effects of substances by means of appropriate experiments. The prime concern is to assess the importance of these interactions in regard to human health. Toxic responses may occur when the immune system is the target of chemical insults, resulting in altered immune function; this in turn can result in decreased resistance to infection, certain forms of neoplasia, or immune dysregulation or stimulation which exacerbates allergy or autoimmunity. Alternatively, toxicity may arise when the immune system responds to the antigenic specificity of the chemical as part of a specific immune response (i.e., allergy or autoimmunity). Certain drugs induce autoimmunity. The differentiation between direct toxicity and toxicity due to an immune response to a compound is, to a certain extent, artificial. Some compounds can exert a direct toxic action on the immune system as well as altering the immune response. Heavy metals like lead and mercury, for instance, manifest immunosuppressive activity, hypersensitivity and autoimmunity.

1.4 Immunosuppression/immunodeficiency

1.4.1 *Biological basis of immunosuppression/immunodeficiency*

The occurrence of acquired immunodeficiency states was recognized sporadically in scattered individuals during the 1960s and 1970s. In the late 1970s and early 1980s, a new syndrome that spread rapidly through certain groups was identified as a generalized type of acquired immunodeficiency syndrome (AIDS). This disorder was found to be due to a specific retrovirus that infects and destroys T helper (Th) cells in humans (Fauci et al., 1991). These helper lymphocytes have been identified in experimental studies as the key cells in the recognition of antigen. Decrease in numbers of Th-cells leads to impaired immune responses to a variety of infectious agents as well as the occurrence of certain types of neoplasms. AIDS appears to result from declining numbers of Th-cells with persistence of residual populations of $CD8^+$. Progression of AIDS is associated with progressive loss of the Th-cells and an increased frequency of infections by bacterial, fungal, viral and parasitic agents.

Other types of acquired immunodeficiency conditions have been recognized and defined in the past two decades. Many have been related to specific immunosuppressive drugs, chemotherapeutic agents and certain chemicals (IPCS, 1996). The immunosuppressive effects of xenobiotics in humans due to environmental exposure, when compared to genetically determined immunodeficiency defects, do not reveal the same degree of severity and persistence in the xenobiotic-related immune defects as seen in the genetic disorders.

The dynamic nature of the immune system renders it especially vulnerable to toxic influence. Reactions of lymphoid cells are associated with gene amplification, transcription and translation. Compounds that affect the processes of cell proliferation and differentiation are especially immunotoxic. This applies in particular to the rapidly dividing haematopoietic cells of the bone marrow and thymocytes. Thus, the disappearance of lymphoid cells from bone marrow, blood and tissue, and thymus weight may be the first and most obvious signs of toxicity. Thymocytes are very susceptible to the action of toxic compounds (Schuurman et al., 1992). It should be noted that thymocyte depletion, suggestive of toxicity towards this cell population, may actually be an indirect effect in cases where the cell microenvironment is damaged and unable to support thymocyte

growth. The susceptibility of thymocytes to toxicity is related to the fragile composition of these cells, especially cortical thymocytes, and to the sensitive interactions between thymocytes and their micro-environment. For instance, thymocytes are programmed to enter apoptosis when activated during the physiological process of selection. The main function of the thymus is T-cell (repertoire) generation during fetal and early postnatal life. Its susceptibility to toxic compounds and the subsequent effects on the cell-mediated immune system are most prominent during this period of life. The skin, respiratory tract and gastrointestinal tract together form an enormous surface that is in close contact with the outside world, and they are potentially exposed to a vast magnitude of microbial agents and potential toxicants. For the respiratory tract, this is illustrated by human data on the immunopathogenesis of lung diseases including asthma, fibrosis and pulmonary infections. Examples of inhaled pollutants that may induce these diseases are oxidant gases and particulates such as silica, asbestos and coal dust.

The skin is an important target in immunotoxicology, as, for instance, when there is contact with chemical allergens (Kimber & Cumberbatch, 1992a,b) and UV-B irradiation (Goettsch et al., 1993). The skin can respond to many xenobiotics by a specific immune response (contact hypersensitivity) or by a non-specific inflammatory response (contact irritancy); both responses are associated with the induction of pro-inflammatory cytokines.

Drugs provide examples illustrating susceptibility to immunotoxic effects. A number of cytostatic drugs are immunosuppressant. In clinical medicine, cytostatic drugs used in cancer therapy often produce bone marrow depression as a major side effect with increased risk for infections as the result.

1.4.2 Consequences of immunosuppression/immunodeficiency

The major consequence of immunodeficiency or impaired immune responsiveness is failure of protection of the host by antibody or effector cells directed against specific target antigens. Antibody and effector cells are essential for a protective effect against infectious and toxic agents that can cause destructive tissue injury and disseminated infections (Buckley, 1992). An impaired immune response also limits the response to protective vaccines that normally build adequate levels of cellular and antibody protection against infectious agents. Selective

impairment of immune responsiveness in some instances may also lead to hypersensitivity states due to dysregulation. This effect could also result in autoimmune disease by promoting recognition of self-antigens, and hyperresponsiveness with increased antibody and effector cell production (Bigazzi, 1988; Broughton & Thrasher, 1988; Chandor, 1988). Increased potential for the development of neoplasia and disseminated malignancies, especially those of the lymphocytic tissues, may occur with impaired immune surveillance (Radl et al., 1985; Byers et al., 1988).

The duration of immunodeficiency states might be transient or long-lasting, depending on the severity and site of the specific xenobiotic effect (Bekesi et al., 1987; Broughton & Thrasher, 1988). The immune impairment that results from continued specific drug therapy with immunosuppressive agents or human immunodeficiency virus (HIV) infection are the only examples of long-lasting acquired immunodeficiency in humans (Jenkins et al., 1988; Fauci et al., 1991). Indeed, studies that have reported acquired deficiency of immune function as a result of xenobiotics or radiation have shown the marked capacity for self-restoring activity of the immune system, so that once an offending agent has been cleared from the body the various cellular components return to a normal state (Kishimoto & Hirano, 1984).

1.5 Immunological tolerance

Immunological tolerance refers to a state of non-responsiveness that is specific for a particular antigen, and is induced by prior exposure to that antigen. Tolerance can be induced to non-self antigens, but the most important aspect of tolerance is self-tolerance, which prevents the body from mounting an immune attack against itself. The potential for attacking the body's own cells arises because the immune system randomly generates a great diversity of antigen-specific receptors, some of which will be self-reactive. Cells bearing these receptors must be eliminated, either functionally or physically.

1.5.1 T-cell tolerance to self-antigens

The thymus is central to the development of T-cells. Within the thymus, T-cells develop from precursors that have not undergone rearrangement of their T-cell antigen receptor (TCR) genes. In the thymus, T-cells acquire the "education" that ensures that they respond

to antigens only in the context of molecules encoded by self major histocompatibility complex (MHC) molecules. It is likely that self-reactive T-cells are also dealt with and eliminated in the thymus.

The high proliferative rate of thymocytes is paralleled by a massive rate of cell death: the vast majority of T-cells, at the double positive ($CD4^+$ $CD8^+$) stage, die within the thymus. Among the factors that account for this are aberrant T-cell antigen receptor (TCR) rearrangement, negative selection, and failure to be positively selected. Positive selection occurs when T-cells, with some degree of binding avidity for polymorphic regions of major histocompatibility complex (MHC) molecules, are selected for survival. The MHC molecules are encountered on thymic cortical epithelial cells, and binding is presumed to protect the cells from programmed cell death. This positive selection process ensures that the mature T-cell only recognizes antigen (peptides) when associated with self-MHC molecules, and so will be self-MHC restricted. Negative selection, on the other hand, eliminates self-reactive T-cells, discarding those clones of T-cells that are specifically reactive to self-antigens present intrathymically.

The timing and precise localization of negative selection depends on a variety of factors, including the accessibility of developing T-cells to self-antigen, the combined avidity of the T-cell receptor and accessory molecules, CD8 or CD4, for the self-MHC-self-peptide complex, and the identity of the deleting cells. Elimination of self-reactive cells is clearly a function of the thymic dendritic cells or macrophages which are rich in MHC Class I and II molecules and situated predominantly at the corticomedullary junction. Some medullary or cortical epithelial cells may also impose negative selection. Other cells involved in deletion may be the thymocytes themselves. Specialized "veto" cells bearing self epitopes would impart a negative signal, killing the self-reactive clone. Under physiological conditions, veto signals occur when a T-cell with T-cell receptors for self antigens binds to a veto cell. The veto cell is a specialized T-cell expressing self epitopes. For the veto effect to occur, the T-cell antigen receptor (TCR) has to bind to self antigen in association with MHC Class I on the veto cell, while the CD8 of the veto cell binds to MHC Class I on the T-cell. Once binding has occurred, the T-cell is killed.

1.5.2 B-cell tolerance to self antigens

Production of high-affinity autoantibodies is T-cell dependent. For this reason, and since the threshold of tolerance for T-cells is lower than that for B-cells, the simplest explanation for non-self-reactivity by B-cells is a lack of T-cell help. Nevertheless, circumstances exist in which B-cells need to develop tolerance directly. For example, there may be cross-reactive antigens on microorganisms, which include both foreign T-cell-reactive epitopes and other epitopes resembling self epitopes and capable of stimulating B-cells (molecular mimicry). Such antigens could result in a vigorous antibody response to self antigens. Furthermore, in contrast to T-cell receptors, the immunoglobulin receptors on mature, antigenically-stimulated B-cells can undergo hypermutation and may acquire anti-self reactivities at this late stage. Tolerance must thus be imposed on B-cells, both during their development and after antigenic stimulation in secondary lymphoid tissues.

The fate of self-reactive B-cells has been determined using transgenic technology. The transgenic models showed that induction of tolerance by self-antigens could lead to one of several end results. The outcome depends on the affinity of the B-cell antigen receptor and on the nature of the antigen it encounters, whether an integral membrane protein, such as an MHC Class I molecule, or a soluble and largely monomeric protein present in the circulation.

When B-cells encounter cell-membrane-associated self-antigens capable of cross-linking Ig receptors on the B-cells with high avidity, the B-cells are eliminated from lymphoid tissues. This type of tolerance occurs whether the self-antigens are expressed on cells in the bone marrow or elsewhere. In either case, the bone marrow contains residual self-reactive B-cells, suggesting that immature B-cells are less readily deleted than immature T-cells during the early stages of differentiation.

If self-reactive B-cells are exposed to soluble antigen that is largely monomeric (not capable of cross-linking receptors), then the cells are not deleted from secondary lymphoid tissues, where they can be found in normal numbers, but are rendered anergic. This effect only occurs when the antigen is above a critical concentration threshold. Anergy is associated with down-regulation of the membrane IgM receptor. The maturation of the self-reactive B-cells is also arrested in

the follicular mantle zone and there is a striking reduction in marginal zone B-cells with high levels of surface IgM. No evidence for the activity of T-cells or of anti-idiotypic B-cells was found in these transgenic models.

1.5.3 Tolerance to non-self antigens

1.5.3.1 Scope

Exposure to environmental and occupational allergens mainly takes place along the skin and the mucosal surfaces lining the gastrointestinal tract and the airways. Since no nutrients have to pass the skin, skin barrier function simply focuses on exclusion of exogenous molecules. Any macromolecule bypassing the skin epithelial barrier is a potential health threat, and is subjected to pro-inflammatory responses aimed at the most rapid destruction and/or killing of the exogenous material. In sharp contrast, mucosal surfaces along the gastrointestinal tract and the airways face a liquid or moist environment which may contain valuable nutrient molecules, next to a plethora of potentially toxic substances, including microorganisms. Subtly balanced defence mechanisms have evolved, therefore, along these mucosal surfaces to exclude microorganisms, and to facilitate the entry of smaller nutrient molecules, such as oligopeptides.

As a consequence, mucosal contacts with potential allergens may, depending on the conditions, lead to either tolerance or sensitization. The molecular and cell-biological characterization of cytokines and adhesion molecules has led to better understanding of the mechanisms involved in oral tolerance. There are primary, non-immunological factors determining mucosal defence against exogenous toxic pressures, including the roles of transmembrane transporter molecules and TGFβ in epithelial barrier function, as well as alveolar macrophages and secretory IgA. The dichotomy between Th1- and Th2-type immune responses in skin and mucosa, and the supplementary role of TGFβ are important.

1.5.3.2 Mucosal defence against exogenous toxic pressures

Distinct molecular mechanisms provide primary protection of mucosal tissues against toxic pressure from exogenous toxic agents. If these mechanisms fail, exogenous compounds penetrate the mucosa, reach mucosal immunocytes, and induce undue immune reactivity. This leads to local release of immunopharmacological mediators, such

as leukotrienes, further enhancing entry of xenobiotics by opening the tight junctions. Studies, primarily aiming at elucidating mechanisms of cytostatic drug-resistance in tumour cells, have shown the existence of different molecular pumps mediating transmembrane transport of potentially toxic molecules. Localization of these molecules on the outer plasmacellular membrane contributes to the efflux of exogenous toxic substrates from the cell interior to the extracellular space, and localization on vesicle membranes contributes to their loading into exocytotic vesicles, thus facilitating their removal. While over-expression of these molecules on tumour cells contributes to resistance to a vast array of cytostatic drugs (multidrug resistance: MDR), the presence of such molecular pumps on epithelial cells lining mucosal surfaces is thought to mediate a primary barrier function to exogenous toxic pressure.

MDR-related proteins are abundantly present in various normal tissues (Flens et al., 1996; Izquierdo et al., 1996). There, MDR-related proteins represent physiological mechanisms of cellular resistance to potentially toxic compounds. In normal tissues high levels of these proteins can be observed on the luminal membranes of epithelial cells lining mucosal surfaces chronically exposed to xenobiotic agents, such as the respiratory epithelia in the trachea and bronchi within the lung, and colonic epithelial cells. In the gut they are thought to prevent too high intracellular concentrations of potentially toxic molecules showing some degree of lipophilicity (van der Valk et al., 1990; Weinstein et al., 1991). No regulatory mechanisms have yet been defined determining to what extent MDR molecules are expressed in mucosal lining cells. It is also still unknown whether chronic inflammatory processes in the gastrointestinal tract and airways might develop after failure of detoxifying mechanisms similar to those mediating drug-resistance in tumour cells.

Mucosal epithelial barrier function is not only dependent on the capacity of individual cells to resist uptake and passage of potentially toxic molecules, but also on the integrity of the epithelial cell layer(s). Important roles in maintaining this integrity are played by two cytokines, IFNγ and TGFβ (Planchon et al., 1996). Of substances released by lymphocytes, including those that reside in the mucosa, only IFNγ has been reported to have a potent effect in reducing the barrier function of epithelial monolayers *in vitro* (Madara & Stafford, 1989; Adams et al., 1993). TGFβ was found to enhance the integrity of epithelium for normal homoeostasis (Derynck et al., 1988; Graycar

et al., 1989; Planchon et al., 1994). TGFβ stimulates the synthesis of extracellular matrix proteins (collagen, fibronectin) by up-regulating their gene expression (Ignotz & Massague, 1986) and alters the expression of integrins that act as receptors for these proteins, thereby enhancing the cell's ability to bind them (Heine et al., 1989). IFNγ and TGFβ antagonism is most clearly revealed by the striking ability of TGFβ1 to reduce the capacity of IFNγ to disrupt epithelial barrier function (Planchon et al., 1996).

Another critical factor in the prevention of the potential harmful entry of excessively large doses of antigens or microorganisms into the mucosal tissues is the presence of IgA in the mucosal secretions. IgA is highly efficient in complexing luminal antigenic molecules and particles, thus reducing their chance of sneaking through the epithelial barrier, and facilitates their uptake and degradation by luminal phagocytes, e.g., pulmonary alveolar macrophages (PAMs). The fact that TGFβ is an important factor in switching B-cell immunoglobulin synthesis to IgA production supports the critical role of this cytokine in maintaining homoeostasis within the mucosal tissues.

The maintenance of homoeostasis in the lungs requires particular protection against environmental antigens. Chronic inflammatory immune responses would be detrimental for these delicate tissues involved in gas exchange. Highly active macrophages are present within the alveolar spaces able to digest and eradicate exogenous antigens and microorganisms, thus preventing these from even reaching the epithelial barrier. Activation of PAMs is reflected by their production of nitric oxide synthetase, leading to the local release of nitric oxide, known as an effector molecule in macrophage-mediated antimicrobial responses (Nussler & Billiar, 1993). Since nitric oxide release is not a constitutive property of resident PAMs, effective scavenging function requires a milieu of activating cytokines, such as IFNγ, and the often synergistic cytokines IL-2 and TNFα. On the other hand, under steady state conditions, pro-inflammatory processes are tightly controlled by lymphocytostatic signals generated by the same resident PAMs. The mechanism(s) by which PAMs mediate immune suppression, e.g., of T-cell proliferation, has been the subject of much debate, and proposed mediators include prostaglandins (Monick et al., 1987; Fireman et al., 1988), TGFβ (Roth & Golub, 1993) and interleukin-1 receptor-antagonist (Moore et al., 1992). TGFβ has been identified as a most critical mediator in suppressing local pro-inflammatory responses by its unique activity in

antagonizing IFNγ-induced macrophage activation (Bilyk & Holt, 1995).

1.5.3.3 *Induction of oral tolerance*

Chase (1946) confirmed that oral feeding of antigen could result in a state of specific immunological unresponsiveness. Feeding contact allergens to guinea-pigs made the animals refractory to subsequent sensitization via the skin. Handling of antigen by the gut is important in terms of both general and secretory immunity. The induction of immunological unresponsiveness in humans by oral ingestion of potential allergens was supported by the observation that South American Indians ate poison ivy leaves in an attempt to prevent contact sensitivity reactions to the plant (Dakin, 1982).

Systemic unresponsiveness after antigen feeding has been described for a large variety of T-cell-dependent antigens, of which the protein ovalbumin has been most extensively studied (reviewed in Mowat, 1987). In addition, proteins such as bovine serum albumin (Silverman et al., 1982; Domen et al., 1987), particulate (erythrocyte-bound) antigens (Kagnoff, 1982; MacDonald, 1983; Mattingly, 1984), inactivated viruses and bacteria (Stokes et al., 1979) and autoimmune-related antigens (Thompson & Staines, 1990), as well as contact allergens, have been shown to induce oral tolerance (Asherson et al., 1977; Newby et al., 1980; Gautam et al., 1985). Generally, T-cell-mediated delayed-type hypersensitivity responses and IgE production are the types of immune responses to which tolerance develops most readily. Persistent tolerance can be induced with relatively low antigen doses (proteins: Heppel & Kilshaw, 1982; Jarrett & Hall, 1984; contact allergens: Asherson et al., 1977; Polak, 1980; van Hoogstraten et al., 1992; Hariya et al., 1994). In sharp contrast, local (secretory) IgA responses are generally unaffected (Challacombe, 1983; Fuller et al., 1990). The apparent ability of the intestinal immune system to prevent allergic hypersensitivity to soluble, non-replicating antigens seems to be an important factor in preventing enteropathies (Mowat, 1984, 1987; Mowat et al., 1986; Challacombe & Tomasi, 1987). In contrast to potentially harmful, pro-inflammatory DTH and IgE responses, the secretory IgA response seems favourable. This immunoglobulin does not fix complement, nor does it cause allergic reactions, whereas its release may rather prevent enteropathies by inhibition of the entry of potentially damaging molecules. Abrogation of oral tolerance to, for instance, ovalbumin was found to lead to hypersensitivity responses

in the intestinal mucosa and gut-associated lymphoid tissues, resembling those observed in food-sensitive enteropathies, e.g., coeliac disease. Indeed, IgE and DTH responses are most frequently associated with clinical food hypersensitivity.

1.5.3.4 Factors determining the development of oral tolerance

Several factors can play a role in the development of mucosal tolerance, notably the nature of the antigens and the genetic background, age and immune status of the individual. With regard to the nature of the antigens, available experimental and clinical evidence indicates that the ability of antigens to sensitize along the skin route parallels the ability to induce tolerance upon mucosal exposure. Thus, feeding of chemicals such as dinitrochlorobenze (DNCB) and picryl chloride, which are strong sensitizers when first applied to the skin, rapidly induces tolerance. Also nickel, which is amongst the top ten of clinical contact sensitizing agents, is an effective tolerance inducer in both experimental animals and humans (van Hoogstraten, 1991, 1992, 1993). However, when the mucosal epithelial barrier fails to prevent antigen passage, in particular the entry of live viruses or bacteria, this may lead to priming for pro-inflammatory immune responses rather than to the induction of tolerance. The fact that such microorganisms are strong inducers of local IL-12 and IFNγ release suggests that these cytokines could play a role as antagonists for tolerance induction. Indeed, adequate vaccination via the oral route can be achieved with live, attenuated strains of microorganisms, e.g., with poliomyelitis vaccine (Stites & Terr, 1991).

Essentially similar requirements for skin-sensitizing and mucosal-tolerizing capacities of chemical allergens are also evident from the apparent lack of major genetic influences on either of these phenomena in outbred animals or humans. No or minimal genetic restrictions have been found for the risk of developing contact allergies to, for instance, nickel, nor for the induction of oral tolerance to the same allergens. It would appear that the same T-cell-receptor repertoire is being addressed under both conditions but that, depending on the site of first encounter with the allergen, sensitization or tolerance may ensue. On the other hand, inbred mouse strains can show strong differences in their ability to develop tolerance after protein feeding (Stokes et al., 1983 a,b; Tomasi et al., 1983; Lamont et al., 1988). Noticeably, certain mouse strains that are prone to

autoimmune diseases fail to develop oral tolerance to some proteins (Carr et al., 1985).

With regard to age, it was demonstrated in mice that ovalbumin did not induce tolerance for either DTH or antibody responses during the early postnatal period (1–2 days old), suggesting an increased risk of allergic sensitization during infancy. The lack of tolerance development in neonatal mice may be due to immaturity of the intestinal immune system at birth in this species. The ability to develop tolerance starts around day 4, but a transient defect in tolerance induction occurs around the time of weaning (Strobel & Ferguson, 1984; Hananan, 1990). Interestingly, clinical food hypersensitivities in human infants often develop around the time of weaning. This may be directly related to the physiological and dietary changes associated with weaning, when large numbers of new antigens are introduced. At the other end of the time scale, in ageing individuals reduced abilities to develop new hypersensitivities and tolerance are observed.

1.5.3.5 *Orally induced flare-up reactions and desensitization*

Considering the immune status of individuals, strong and long-lasting oral tolerance can only be achieved in naive individuals, i.e., those who have not been previously exposed to the antigen via the skin. In mice, a single feed of ovalbumin was reported to suppress fully subsequent systemic immune responses, and this state of tolerance persisted for up to two years. In contrast, in primed animals tolerance is hard to induce but partial and transient unresponsiveness (desensitization) may eventually develop after prolonged feeding of the antigen. Similar results have been obtained in guinea-pig studies with various different chemical allergens, including dinitrochlorobenzene (Polak, 1980), nickel (van Hoogstraten, 1994) and amlexanol (Hariya et al., 1994). Unfortunately, essentially similar results have been obtained in early clinical trials aiming at the treatment of autoimmune diseases, e.g., rheumatoid arthritis and multiple sclerosis, by oral administration of relevant auto-antigens (Weiner et al., 1994). Another problem with oral tolerance induction in previously sensitized individuals arises owing to the tendency of former inflammatory sites to re-inflame (flare-up reaction). Local flare-up reactions confirm a previous sensitization process, and are probably due to allergen-specific effector T-cells, which can persist for periods up to several months at former inflammatory sites (Scheper et al., 1983).

Two distinct features of immunocyte maturation may explain the seemingly insurmountable differences between immunological responses in naive and primed individuals, involving changes in expression patterns of cellular adhesion/homing molecules, and lymphocyte maturation features. First, a qualitative distinction exists between naive (difficult to stimulate/afferently acting) cells and effector/memory cells (easy to stimulate/efferently acting). In contrast to naive lymphocytes, which only are activated by allergen (modified-self constituents) if presented by professional dendritic (e.g., Langerhans) cells, their progeny, known as effector/memory lymphocytes, can also be stimulated by other cell types presenting allergen-modified MHC Class II molecules, e.g., monocytes, endothelial cells and B-cells. Effector/memory cells display increased numbers of intercellular adhesion molecules (ICAMs), allowing for more promiscuous cellular interactions. Amongst these, the most prominent ICAMs are the CD28 and LFA-1 molecules, with B7-1/2 and ICAM-1 as their respective ligands on APCs. Also, priming of T-cells leads to the loss of homing receptors, such as L-selectin, which facilitate interactions with high endothelial venules in peripheral lymph nodes. Apparently, after sensitization T-cells are less capable of recirculating through the lymphoid organs, but gain in ability to migrate into the peripheral tissues. Indeed, interactions with endothelia within inflamed skin are facilitated by the enhanced expression of ICAMs like the cutaneous lymphocyte-associated antigen CLA. Thus, effector/memory T-cells largely distribute over the peripheral tissues where conditions may be insufficient to convey effective tolerogenic signals. The second problem in inducing tolerance in previously primed individuals relates directly to the actual mechanism(s) of oral tolerance.

1.5.3.6 *Mechanisms of tolerance*

As discussed above, a preliminary factor contributing to immunological non-responsiveness and/or lack of hypersensitivity reactions at mucosal surfaces is the epithelial barrier function, preventing entry of potentially harmful allergens. Obviously, from an immunological point of view, this is a null-event and does not have implications for subsequent encounters with the same allergen. Also as discussed above, TGFβ, a cytokine locally produced by epithelial cells and immunocytes, plays a pivotal role in maintaining epithelial barrier integrity. Importantly, the same cytokine also has broad non-specific immunosuppressive functions, for example, antagonizing

phagocytic effector cell functions of pulmonary alveolar macrophages. Similarly, other immunosuppressive cytokines may be locally released from epithelial cells and may act in concert with TGFβ to down-regulate immune effector functions, such as epithelial cell-derived P15E-related factors which show sequence homology with retroviral envelope proteins (Oostendorp et al., 1993).

In contrast, specific immunological tolerance depends on decreased responsiveness of specific B- or T-cells, or release of immunosuppressive mediators from these cells after specific challenge. Exposure to high doses of antigens may induce clonal deletion or anergy of specific B- or T-cells by induction of apoptosis or antigen-receptor down-regulation (Jones et al., 1990; Schönrich et al., 1991; Ohashi et al., 1991; Melamed & Friedman, 1993).

Generally, ligation of the T-cell antigen receptor (TCR) in the absence of appropriate co-stimulatory signals results in T-cell non-responsiveness, not only in Th1- but also in Th2- cells. Human $CD4^+$ Th2-clones specific for the house dust mite allergen *Der p* I can be rendered non-responsive to subsequent *Der p* I challenges by incubating them with *Der p* I-derived peptides, representing the relevant minimal T-cell activation-inducing epitopes, in the absence of professional APC (Yssel et al., 1992). The anergized Th2-cells also failed to produce cytokines (including IL-4 and IL-13) and failed to provide help for B-cell IgE synthesis. The mechanisms underlying this T-cell unresponsiveness have not yet been determined. Although these cells cannot be activated through their T-cell antigen receptor (TCR), they proliferate well in response to IL-2, or following activation by Ca^{++} ionophore and the phorbol ester 12-O-tetradecanoylphorbol 13-acetate (TPA), suggesting that TCR activation or signalling pathways immediately downstream of the TCR are disturbed.

Interestingly, the anergized Th2 cells expressed normal levels of CD40 ligand, but their lack of help for B-cell IgE synthesis could not be restored by exogenous IL-4 or IL-13, suggesting that in addition to CD40L-CD40 interactions, other molecules are required for initiating productive T- and B-cell interactions resulting in Ig isotype production. It is likely that these molecules are down-regulated in anergic T-cells. Peptide-induced Th2 cell tolerance and inhibition of T-cell help for IgE synthesis may provide the basis for successful immunotherapy in allergy. This anergy-based type of tolerance is generally short-lived, since (functionally) deleted lymphocytes are

gradually replenished by newly arising clones in the bone marrow and thymus and, in experimental animal models, cannot be transferred to naive recipients, since these still contain a fully functional repertoire, compensating for any missing clones. On the other hand, mucosal contacts of naive individuals with relatively low amounts of antigens, such as can be the case with environmental or occupational exposure to chemical sensitizers, frequently induces a long-lasting state of specific tolerance. Transfer of lymphoid cells, in particular T-cells, from orally tolerized animals to syngeneic naive recipients prevents their capacity to subsequently mount immune responses to the same allergen, revealing the existence of so-called regulatory or suppressor T-cells (Polak et al., 1980; van Hoogstraten et al., 1992, 1994; Weiner et al., 1994).

Although "professional" suppressor T-cells may not exist (Bloom et al., 1992; Arnon & Teitelbaum, 1993), available data support the possible development of specific regulatory T-cells that suppress distinct immune functions. Depending on the experimental models, such regulatory T-cells can belong to either or both the $CD4^+$ or $CD8^+$ subsets (Bloom et al., 1992). Regulatory T-cells may exert their suppressive actions through different pathways, including the shedding of TCR-α chains or hapten-binding TCR, through anti-idiotypic reactivities, or through IL-2/cytokine consumption from the milieu (Bloom et al., 1992; Fairchild et al., 1993; Kuchroo et al., 1995). There is evidence that regulatory T-cells most often exert their role, after antigen-specific activation, by releasing distinct cytokines antagonizing specific effector T-cell functions (see section 1.2.1).

When starting clonal expansion after antigen-stimulation, T-cells develop major cytokine profiles depending on the site of primary contact (see section 1.2.1.1). For potential mechanism(s) of oral tolerance T-cell subsets producing mutually suppressive cytokines can be regarded as suppressor, or, better, as regulatory cells, depending on the functions tested. Considering overt inflammatory reactions as being most harmful to the individual and the primary cause of mucosal hypersensitivities, Th2-cells and putative TGFβ producing Th3-cells are the most obvious candidates to mediate oral tolerance to proteins and chemical allergens.

1.5.3.7 Conclusions

Although the phenomenon of oral tolerance has been known for over a century the research on cellular resistance molecules, T-cell cytokine patterns and cellular adhesion molecules has opened promising avenues for further research on mechanisms and therapeutic options. Clearly, the skin-versus-mucosa routing hypothesis discussed above leaves many questions unanswered, such as the question of why some chemicals may elicit strong Th2 responses and IgE antibody production even when applied to the skin, without apparent reduction of delayed allergic reactivity (Dearman et al., 1991). The preliminary understanding of regulatory mechanisms in allergic contact dermatitis has not yet led to further therapeutic progress. So far, no methods of permanent desensitization have been devised. Nevertheless, the way in which T-cells specifically recognize distinct allergens, as well as how these and other inflammatory cells interact to generate inflammation, is beginning to be understood. Defined cellular interaction molecules and mediators provide promising targets for anti-inflammatory drugs. Obviously, drugs found to be effective in preventing severe T-cell-mediated conditions, e.g., rejection of a vital organ graft, should be carefully evaluated before their use in allergic skin disease is considered.

2. HYPERSENSITIVITY AND AUTOIMMUNITY – OVERVIEW OF MECHANISMS

Numerous environmental chemicals have the ability to produce a hypersensitivity response. Although hypersensitivity diseases are common, affecting millions of people, the incidence associated with environmental pollutants or occupational exposure is largely unknown. The characteristic that distinguishes allergic responses from immune mechanisms involved in host defence is the nature of the reaction, which often leads to tissue damage. Chemically induced hypersensitivities usually fall into two responses distinguished not only mechanistically but temporally: (1) immediate hypersensitivity, which is mediated by immunoglobulin, most commonly IgE, and is manifested within minutes of exposure to an allergen, and (2) delayed-type hypersensitivity (DTH), a cell-mediated response that occurs within 24–48 h. The type of immediate hypersensitivity response elicited (anaphylactic, cytotoxic, Arthus or immune complex) depends on the interaction of a sensitizing antigen or structurally related compound with antibody. Delayed-type hypersensitivity responses are characterized by T-lymphocytes bearing antigen-specific receptors which, on contact with macrophage-associated antigen, respond by secreting cytokines that mediate the delayed-type hypersensitivity response. Almost any organ can be targeted by hypersensitivity reactions, including the gastrointestinal tract, blood elements and vessels, joints, kidneys, central nervous system and thyroid, although the skin and lung, respectively, are the most common targets.

Various risk factors are involved in producing allergic sensitization and influencing its severity. For instance, in the case of aeroallergens, exposure can play a role in the primary sensitization, in the development of symptomatic allergic disease, and in the frequency and severity of acute symptomatic episodes. Other risk factors include genetic predisposition, and age at the time of the primary exposure.

Exposure to enzymes (mainly proteases) used in detergents have also been associated with respiratory sensitization and symptoms. Though sensitization is due to more than one factor, magnitude of exposure has been demonstrated as a critical factor in the control of primary sensitization to enzyme-containing detergents (Sarlo et al., 1997).

Environmental factors have been suggested to contribute to the prevalence of allergic diseases by modulating the allergen load required for the sensitization as well as for the exacerbation and intensity of allergic symptoms (Ollier & Davies, 1994).

2.1 Classification of immune reactions

Gell & Coombs (1963) classified immune reactions into four basic types. Since then knowledge of immune reactions has increased and the frequent overlaps between the different types must be stressed. This classification is still very useful but the physiopathological reality is frequently more complex.

The four major types of hypersensitivity according to Gell & Coombs (1963) are:

Type I anaphylactic, immediate reaction
Type II cytotoxic reaction
Type III immune complex reaction
Type IV delayed or cell-mediated reaction

Sometimes a fifth type of hypersensitivity is added, i.e., Type V stimulatory hypersensitivity (Roitt et al., 1998). In addition, certain allergic diseases can be expressions of two or more types of hypersensitivity.

The sections below review the mechanistic basis for phenomena and diseases associated with each type of hypersensitivity.

2.1.1 *Type I hypersensitivity*

The distinguishing feature of Type I hypersensitivity is the short time lag, usually seconds to minutes, between exposure to antigen and the onset of clinical symptoms. The key reactant in Type I or immediate sensitivity reactions is IgE (see Fig. 6). Antigens that trigger formation of IgE are called atopic antigens, or allergens (Marsh & Norman, 1988). Atopy refers to an inherited tendency to respond to naturally occurring inhaled and ingested allergens with continual production of IgE (Terr, 1994a). Patients who exhibit allergic or immediate hypersensitivity reactions typically produce antigen-specific IgE in response to a small concentration of antigen (Atkinson

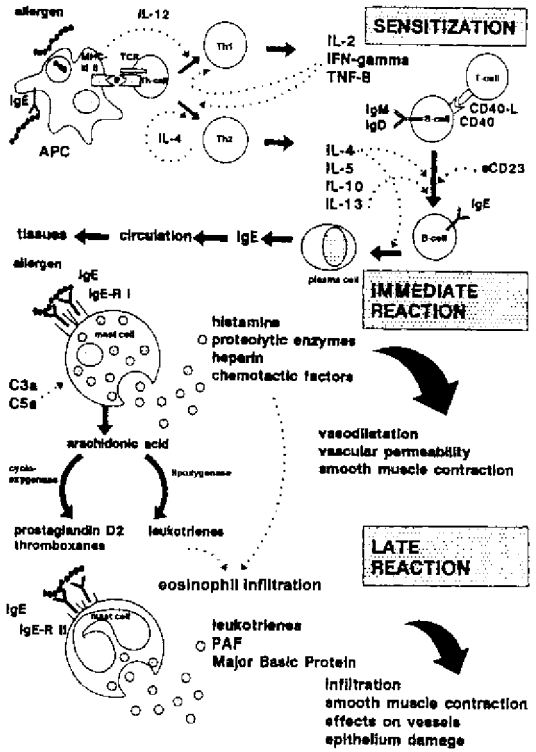

Fig. 6. The mechanism of Type 1 hypersensitivity reactions

& Platts-Mills, 1988). IgE levels appear to depend on the interaction of both genetic and environmental factors.

Prausnitz & Kustner (1921) showed that a serum factor was responsible for Type I reactions. This type of reaction is known as passive cutaneous anaphylaxis. It occurs when serum is transferred from an allergic individual to a non-allergic individual, and then the second individual is challenged with specific antigen. This experiment was conducted in 1921 but it was not until 1966 that the serum factor responsible, namely IgE, was identified (Ishizaka & Ishizaka, 1966).

IgE is primarily synthesized in the lymphoid tissue of the respiratory and gastrointestinal tracts. The regulation of IgE production appears to be a function of T-cells. Th2 cytokines, in particular IL-4 and IL-13 are essential for IgE synthesis, i.e., for the final differentiation and isotype switch of the IgE-producing B-cells, committing particular B-cells to IgE production (Goust, 1993). IL-2, IL-5 and IL-6 also play a role, probably as sequential growth and differentiation factors that select for IgE synthesis (Tharp, 1990).

Once an individual has become sensitized, the IgE produced spreads throughout the body and binds in the peripheral tissues to mast cells and basophils via the high affinity receptor for IgE (FcϵRI). Upon contact with the allergen, the IgE molecules will be cross-linked and the cells will release their granules supplying the tissue with histamine, proteolytic enzymes, heparin and chemotactic factors for eosinophils, neutrophils and monocytes. These mediators induce vasodilatation, increased vascular permeability and smooth muscle contraction and lead to an "immediate reaction", which becomes clinically manifest within 20 min as a typical "wheal and flare" in the skin or as bronchoconstriction in the respiratory tract.

At the same time, from the cell membrane new mediators, such as prostaglandin-D2, thromboxanes and leukotrienes are being generated. Together with the now attracted and activated eosinophils (which produce platelet activating factor and major basic protein), these mediators cause further infiltration, smooth muscle contraction, mucosal oedema and damage of the epithelial cells, resulting in the so called "late phase" reaction (12–24 h after challenge). Like the immediate reaction the late phase responses can be observed both in the skin and in the respiratory tract.

While actual antibody synthesis is regulated by the action of cytokines, the tendency to respond to specific allergens appears to be linked to inheritance of certain MHC genes. Various HLA class II antigens seem to be associated with a high response to individual allergens (Goust, 1993). As an example, individuals who possess the HLA antigens B7 and DR2 are more likely to respond to a specific ragweed antigen (Goust, 1993). The nature of this association is unclear at this time.

2.1.1.1 Anaphylaxis

Anaphylaxis is the most severe type of allergic response, as it involves multiple organs and may be fatal. Anaphylactic reactions are typically triggered by glycoproteins or large polypeptides. Smaller molecules, such as penicillin, are haptens that may become immunogenic by combining with host cells or proteins. Typical agents that induce anaphylaxis include venom from insects in the *Hymenoptera* family, drugs such as penicillin, and foods such as seafood or egg albumin (Widmann, 1989).

Allergic reaction to allergens (e.g., in food, venom) that result in systemic anaphylaxis are, in the vast majority of instances, believed to be mediated by allergen-specific IgE bound to high affinity IgE receptors (FcεRI) on the surfaces of basophils and mast cells. As described earlier, the subsequent activation of basophils/mast cells results in the release (e.g., histamines) and generation (e.g., leukotrienes) of potent chemical mediators of anaphylaxis.

2.1.2 Type II hypersensitivity

Type II hypersensitivity reactions are caused by IgG and IgM antibodies directed towards cell surface antigens. These antigens may be altered self-antigens or heteroantigens. Such antibodies, bound to the cell membrane, can activate inflammatory phagocytes by Fc receptor triggering. These phagocytes will then try to kill or to inactivate their target as they would kill a microorganism. If they are unable to phagocytose the whole cell, they will cause cell damage by secreting oxygen radicals and by generating inflammatory mediators such as arachidonic acid metabolites (prostaglandins and leukotrienes) from their cell membrane.

Moreover, cell-bound antibodies activate the complement system. The presence of C3b on the cell membrane, in addition to the immunoglobulin, facilitates phagocytosis, whereas the further complement cascade will induce membrane perforation and cell lysis. Together, these reactions result in destruction of antibody-coated cells and thus in cytopenia or in considerable tissue damage.

Not only granulocytes and macrophages are able to kill antibody-coated cells. Specialized large granular non-B, non-T-lymphoid cells, called natural killer (NK) cells, also bear Fc receptors (CD16) and are

capable of killing antibody-coated target cells. NK cell-mediated killing is achieved by the release of cytoplasmic granules containing perforin and granzymes. This process is called antibody-dependent cell-mediated cytotoxicity (ADCC) and, although not yet recognized at the time of Gell & Coombs (1963), it should strictly be considered as a Type II effector mechanism. ADCC reactions have been well established *in vitro* to tumour antigens and viral proteins, but their precise role in host defence and hypersensitivity reactions is still not completely understood.

2.1.3 Type III hypersensitivity — immune complex reaction

Type III hypersensitivity reactions are similar to Type II reactions in that IgG or IgM is involved and that destruction is complement-mediated. However, in the case of Type III diseases, the antigen is soluble. When soluble antigen combines with antibody, complexes are formed that precipitate out of the serum. These complexes deposit in the tissues and bind complement, causing damage to the particular tissue. Deposition of antigen-antibody complexes is influenced by the relative concentration of both components. If a large excess of antigen is present, sites on antibody molecules become filled before cross-links can be formed. In antibody excess, a lattice cannot be formed due to the relative sparsity of antigenic determinant sites. The small complexes that result in either of the above cases remain suspended or may pass directly into the urine. Precipitating complexes, on the other hand, occur in mild antigen excess, and these are the ones most likely to deposit in the tissues. Sites where this typically occurs include the glomerular basement membrane, vascular endothelium, joint linings, and pulmonary alveolar membranes (Roitt et al., 1998).

Complement binds to these complexes in the tissues, causing the release of mediators that increase vasodilation and vasopermeability, attract macrophages and neutrophils, and enhance binding of phagocytic cells by means of C3b deposited in the tissues. If the target cells are large and cannot be engulfed for phagocytosis to take place, granule and lysosomal contents are released by a process known as exocytosis (Roitt et al., 1998). This results in the damage to host tissue that is typified by Type III reactions.

2.1.3.1 *Arthus reaction*

The classic example of a Type III reaction is the Arthus reaction, a local necrotic lesion resulting from a local antigen-antibody reaction produced by intradermal injection of an antigen into a previously sensitized animal. This reaction is characterized by erythema and oedema, peaks within 3 to 8 h, and is followed by a haemorrhagic necrotic lesion that ulcerates. The inflammatory response is due to antigen-antibody combination and subsequent formation of immune complexes that deposit in small dermal blood vessels. Complement is fixed, attracting neutrophils and causing aggregation of platelets. Activation of complement is, in fact, essential for the Arthus reaction, as the C3a and C5a generated activated mast cells to release permeability factors, with the consequent localization of immune complexes along the endothelial cell basement membrane (Terr, 1994b). The Arthus reaction is rare in humans.

2.1.4 *Type IV — delayed-type hypersensitivity*

Type IV reactions were originally described by Gell & Coombs (1963) as those skin reactions which take more than 12 h to develop after antigen application. The classical Type IV reaction is the tuberculin reaction, which reaches its maximum 24–72 h after the intradermal injection of mycobacterial extracts. This delayed type skin reaction to intradermally injected protein is characterized by a pronounced induration reflecting a dense mononuclear cell infiltrate.

Since it became clear that antigen-specific T-cells are responsible for these reactions, the term Type IV reactivity has been used not only in relation to delayed-type hypersensitivity (DTH) reactions in the skin, but also to T-cell-mediated inflammatory reactions in other tissues. In addition, other T-cell-mediated reactions, such as those to infectious agents or tumour antigens, which are rather protective than hypersensitive, are regularly described as Type IV reactions.

Although $CD8^+$ T-cells have been shown in some experimental animal models to transfer DTH, generally $CD4^+$ T-cells are held responsible for DTH responsiveness. The majority of antigen specific T-cells cloned from DTH reaction sites are of the $CD4^+$ subset. Increased frequencies of antigen-specific $CD4^+$ T-cells can also be detected in the circulation of sensitized individuals. Such memory T-cells show enhanced expression of adhesion molecules, which

facilitates their recirculation through the peripheral tissues. So, whereas priming of naive T-cells takes place in the lymph nodes draining the area of antigen contact, the secondary DTH response of memory T-cells rather takes place in the peripheral tissues at the site of antigen contact.

Here they may encounter the antigens for which they were originally sensitized. The T-cells do not recognize the whole antigen or conformational epitopes as antibodies do, but they recognize small peptides derived from these antigens after processing by antigen-presenting cells (APC). MHC class II molecules bind these peptides already within the intracellular vesicles and present them subsequently on the APC membrane to helper T-cells (Fig. 5). If the memory T-cells recognize the peptide in its MHC class II context, the cells become activated and produce a characteristic set of cytokines.

In the DTH reaction that now develops, predominantly mononuclear cells are attracted from the circulation and contribute to the local inflammatory reaction. An essential chemokine found to play a role in the early accumulation of leukocytes at the DTH reaction site is IL-8 (Larsen et al., 1995), whereas RANTES (Regulated in Activation Normal T-cells Expressed and Secreted), produced by endothelial cells, was shown to attract preferentially macrophages and $CD4^+$ T-cells to the DTH reaction (Marfaing-Koka et al., 1995). In addition to a number of different chemokines, IFNγ, TNFα and LT (lymphotoxin) are produced in the DTH reaction (Tsicopoulos et al., 1992). These are typical Th1 effector cytokines, which are either directly cytotoxic for pathogens or indirectly by activating the macrophage bactericidal mechanism. Together, the cytokine cascade during this secondary response shows an extreme amplification power, as illustrated by experimental studies in which measurable oedema could be triggered by only one specific T-cell (Marchal et al., 1982). Therefore, DTH reactions are mediated by Th1 cells, the most prominent cytokines being IL-2, LT and IFNγ. Indeed, at the site of DTH reactions these cytokines can be detected (Tsicopoulos et al., 1992). It should be realized, however, that the immune response is always the resultant of a Th1-Th2 balance and that this delicate balance can be influenced by several external factors, such as drugs, hormones, infections and altered antigen exposure. Chronic antigen stimulation, for instance, may induce a shift away from Th1, DTH-associated immunity towards a Th2 response (Kitagaki et al., 1995; Mosmann & Sad, 1996). Th2 cytokines, such as IL-4, IL-5 and IL-10,

rather help to induce antibody responses, particularly IgE. In chronic infectious disease indeed high levels of antibodies can be detected while DTH reactivity is waning (*Mycobacteria*, *Trichophyton*). In the human system, a Th1 to Th2 shift correlating with a clinical conversion from disease resistance to susceptibility and disease progression has been shown in *Leishmania*, *Candida*, *Mycobacteria* and HIV infection (Mosmann & Sad, 1996).

2.1.4.1 Mechanisms of allergic contact dermatitis

a) Sensitization

In allergic contact dermatitis, Type IV reactivity is raised against small, chemically reactive environmental agents that enter the body via the skin. In the skin, epidermal dendritic Langerhans cells (LC), bearing large numbers of class II molecules (HLA-DR, -DP and -DQ) on their cell membrane, are the primary allergen-presenting cells. They form a contiguous network in which agents penetrating the skin are efficiently trapped. Langerhans cells stem from the bone marrow, but their continuous presence in the epidermis is at least partly maintained by local proliferation (Czernielewski & Demarchez, 1987; Breathnach, 1988).

Upon penetration through the epidermis, contact allergens readily bind to a plethora of skin constituents. Whereas most allergens bind spontaneously, some need metabolic conversion (Anderson et al., 1995) or photoinduced activation before they bind. The latter allergens are called contact photoallergens (White, 1992).

Only those allergens that modify the Langerhans cell MHC Class II molecules can eventually sensitize T-cells; this occurs either by direct binding to the MHC Class II molecules and to peptides within their grooves or by uptake and processing of haptenized proteins followed by presentation of the derived peptides in the MHC Class II molecules of the antigen-presenting cells. It has been shown that in individuals allergic to nickel some nickel-specific T-cell clones recognize unprocessed nickel, bound to the MHC Class II molecules of fixed antigen-presenting cells, whereas other nickel-specific T-cell clones are dependent on viable antigen-presenting cells for processing, most likely of preformed nickel-protein conjugates (Moulon et al., 1995).

In a similar way, MHC Class I peptides may become modified by the allergen, triggering class I restricted, CD8-positive T-cell clones. Notably the size of the allergic metal ions is much smaller than the peptides to which they bind in the groove. In some instances different metallic allergens modify the MHC Class II peptides in a very similar way. T-cell clones then show complete cross reactivity between the metals, for instance between nickel and palladium, or between nickel and copper (Pistoor et al., 1995). Clinical signs of cross reactivity of nickel with other related metals, such as cobalt, however, most probably result from concomitant sensitization by exposure to metal alloys.

The majority of chemically reactive allergen, however, binds covalently to distinct amino acids, thus forming haptenized proteins. Usually, haptens, like picryl- or penicilloyl- are much larger than metal allergens, and hapten-specific T-cell responses, i.e., independent of the carrier protein, can be observed.

Upon exposure of the skin to chemical contact sensitizing agents, cytokine production in the epidermis by both keratinocytes and Langerhans cells is immediately up-regulated, thereby initiating the process of Langerhans cell maturation and migration.

The first cytokine to be up-regulated, within 15 min after allergen application, is IL1β, produced by Langerhans cells. This up-regulation was found to be allergen-specific, just like the subsequent production of IL-1α, and of the chemokines IP-10 (IFNγ-inducible protein 10) and MIP-2 (macrophage inflammatory protein-2) by keratinocytes, and could not be detected upon irritant application. TNFα up-regulation in keratinocytes, on the other hand, appeared to be a less allergen-specific event. The most relevant cytokines for Langerhans cell maturation and egress are GM-CSF, IL-1 and TNFα, while IL-10, which is also produced by keratinocytes, but at a later stage, may serve as a down-regulatory molecule for Langerhans cell maturation. (Heufler et al., 1988; Kimber & Cumberbatch, 1992a,b; Enk & Katz, 1995). Interestingly, IL-1 and TNFα were found to down-regulate the membrane expression on Langerhans cells of E-cadherin, a molecule that mediates Langerhans-cell-keratinocyte adhesion. Thus, Langerhans cells with allergen-modified MHC Class II molecules leave the epidermis and migrate via the dermis and lymphatics to the draining lymph nodes, where they settle within the paracortical areas. Indeed increased numbers of dendritic cells appear in the regional

lymph nodes around 24 h after hapten application (Kimber et al., 1990). Whereas resident epidermal Langerhans cells are still relatively inefficient antigen-presenting cells, once they have arrived in the lymph nodes they have matured into fully active antigen-presenting dendritic cells and are capable of stimulating even naive unprimed T-cells. Naive cells express, in contrast to memory cells, low levels of cellular adhesion molecules (CAM) and therefore require optimally functioning antigen-presenting cells for stimulation. Matured Langerhans cells or dendritic cells (DCs) have an increased expression of MHC Class II, ICAM-1 and B7 molecules, allowing for optimal T-cell triggering (Steinman et al., 1995); in addition the intricate structure of the paracortical area offers an appropriate environment for this sensitization process to take place. Naive T-cells, again in contrast to memory cells, recirculate preferentially through the peripheral lymphoid organs, rather than through the tissues, due to the expression of distinct adhesion molecules (L-selectin) that recognize the high endothelial venules in the lymph nodes. The probability of hitting unprimed specific T-cells is thus increased.

When successful triggering and subsequent proliferation of allergen-specific T-cells have taken place, the lymphocyte progeny will leave the lymph nodes to join the recirculating pool of lymphocytes. The frequency of specific cells in the circulation can thus be increased from around 1:100 000 to 1:1000-10 000 and the individual has now become "sensitized".

b) Elicitation of allergic contact dermatitis

Upon re-exposure to contact sensitizing agents, specific recirculating memory T-cells present in the skin immediately recognize the allergen modified MHC Class II molecules on the Langerhans cell membranes. The probability that the allergen is indeed found by specific memory T-cells is largely increased by the expression of organ-specific interaction molecules on the T-cell surface. The cutaneous lymphocyte-associated antigen (CLA), recognized by the monoclonal antibody HECA-452, is present on a small subpopulation (approximately 16%) of peripheral blood T-cells which preferentially recirculates via the skin. Here the endothelial adhesion molecule E-selectin acts as a vascular addressin for the skin-homing memory T-cells (Picker et al., 1993; Bos & Kapsenberg, 1993). As described in the section on Type IV – delayed-type hypersensitivity (section 2.1.4), mainly CD4-positive allergen-specific

cells thus enter the skin. Since memory cells have relatively low stimulation thresholds, they can be triggered by less efficient antigen-presenting cells, like the local resident Langerhans cells. The T-cells will now initiate a Th1-type cytokine cascade, which eventually leads after 24–72 h to the typical delayed-type contact allergic reaction. Because the reaction takes place in superficial layers of the skin, erythema and blistering are characteristic features, in contrast to the tuberculin DTH where induration is most pronounced.

The challenge reaction in allergic contact dermatitis resolves spontaneously within one week. It is therefore commonly used as a primary diagnostic test in allergic contact dermatitis. To this end, low non-toxic dosages of allergen are generally applied onto the skin under an occlusive patch to allow for maximal skin penetration. The main drawbacks of such an *in vivo* skin test procedure are the potential sensitization and boosting by such an intense allergen contact. Indeed it was shown experimentally in guinea-pigs that even one epicutaneous application of allergen could direct the immune response towards (still subclinical) sensitization, as shown by a failure of subsequent tolerance induction (Van Hoogstraten et al., 1994). Also clinically, occasional sensitization by epicutaneous skin tests can be observed. For this reason much effort has been put in the development of *in vitro* diagnostic procedures in allergic contact dermatitis (Von Blomberg et al., 1990).

Up to now, *in vitro* assays in allergic contact dermatitis have been successful for relatively non-toxic water-soluble allergens, such as metal salts. For other allergens, occasionally positive results are obtained by pre-pulsing antigen-presenting cells or proteins or by using special solvents. So, despite the fact that most of our knowledge of the pathogenesis of human allergic contact dermatitis is due to *in vitro* experiments with blood from allergic patients, for routine assessment of allergic contact dermatitis these assays are still too complicated.

Repeated contact with low dosages of allergen, as typically occurs for most contact allergens, may lead to continuous triggering of Type IV reactivity in the skin and thus to an allergic contact dermatitis (Scheper & Von Blomberg, 1992). The dermatitis only disappears when the allergen is entirely eliminated from the environment.

Even if the reaction is clinically healed, allergen-specific T-cells may persist in the skin for up to several months. Thus, locally increased allergen-specific hyperreactivity, either detectable through accelerated "retest" reactivity (peaking at 6–8 h) or flare-up reactivity after allergen entry from the circulation, may be observed for several months at former allergic contact dermatitis reaction sites (Scheper et al., 1983; Yamashita et al., 1989). The presence of specific T-cells at former eczematous sites can thus be maintained by low dosages of inhaled or ingested allergen, in the absence of allergenic skin contacts.

Repeated contact with relatively high dosages of allergen, on the other hand, may result in a local desensitization. The initial erythematous reaction gradually decreases. Such a local hyporesponsiveness of the skin, which is known as "hardening" in occupational contact dermatitis, is largely reversed after a period of allergen restrain. However, also systemically, DTH reactivity decreases upon repeated allergen application. This decrease in DTH is associated with increased antibody responses and a shift towards immediate-type hypersensitivity, reflecting a shift from Th1 to Th2 reactivity (Boerrigter & Scheper, 1987; Kitagaki et al., 1995).

It appears, therefore, that although exposure of the skin to exogenous antigens generally results in Th1 responses, the microenvironment in chronically inflamed tissues, rather than the site of allergen exposure or the nature of the allergen, determines the type of immune reaction.

2.1.4.2 *T-cell responses in chemically induced pulmonary diseases*

Asthma is a chronic pulmonary inflammatory disease associated with bronchial hyperreactivity. In the majority of asthma cases a clear association exists with atopic IgE-mediated hypersensitivity, involving relatively large protein allergens. Here T-cells dominate in the late phase and the chronic reaction. The pivotal role of T-cells in chronic asthma is stressed by the finding of activated T-cells in the bronchial mucosa and the effectiveness of T-cell immunosuppressive drugs. In particular, the number of activated CD4-positive cells was found to correlate with the numbers of eosinophils in the bronchoalveolar lavage (BAL) and with disease severity (Walker et al., 1991). The T-cells, present in the bronchial mucosae and in the lavage fluid, were shown to produce predominantly Th2 cytokines, in particular IL-5, a cytokine known to activate eosinophils (Corrigan & Kay, 1992).

Therefore, although T-cell-mediated immunity is clearly playing a role, Type IV reactivity, mediated by Th1 cells, does not seem to be involved in this type of asthma.

Of particular interest is the hypersensitivity pneumonitis induced by environmental small chemical allergens. Such allergens are known to cause DTH when applied to the skin. Occasionally these allergens induce, in addition or as first manifestation, asthmatic disease upon inhalation. It could be questioned whether these allergens, in contrast to the atopic protein allergens, would induce Type IV reactivity.

Experimentally it has been shown in mice that Type IV hypersensitivity to small chemical allergens, such as picryl chloride, can indeed induce lung disease upon intranasal application (Garssen et al., 1991, 1994).

Contact allergens that have been reported to induce asthma include formaldehyde, platinum salts, nickel, cobalt and chromium (Nordman et al., 1985; Estlander et al., 1993; Cirla, 1994; Park et al., 1994; Merget et al., 1996). In a number of cases this asthmatic disease could be associated with the presence of circulatory IgG to the causative allergen and positive bronchial provocation tests.

Trimellitic anhydride, phthalic anhydride and toluene diisocyanate are reactive chemicals behaving primarily as respiratory allergens, causing asthmatic disease and pulmonary irritation. The immune reactions leading to asthmatic disease are quite variable; the role of clear-cut Type IV reactivity is uncertain.

2.1.5 *Type V stimulatory hypersensitivity*

Stimulatory hypersensitivity occurs when antibodies binding to a cell surface molecule cause inappropriate stimulation of the cell. Normal feedback inhibition will then fail. An example is Graves' disease (exophthalmic goitre), in which autoantibodies to the thyroid-stimulating hormone receptor on thyroid cells stimulate the production of excessive amounts of thyroid hormone, resulting in disease.

2.2 Regulation of hypersensitivity

In 1986, the existence of two $CD4^+$ Th-cell subsets was discovered in mice, and they were designated Th1 and Th2. Their

identification has greatly improved understanding of the regulation of immune effector functions, not least on Type I and Type IV hypersensitivity responses. These Th subsets are defined by the patterns of cytokines that they produce, which leads to strikingly different T-cell functions (Table 9). Broadly speaking, Th2-cells are more efficient B-cell helpers, especially in the production of IgE antibody, whereas Th1-cells mediate DTH reactions. In addition, they cross-regulate by producing mutually antagonistic cytokines. Their specific function and characteristics in rodents and humans have not yet been clearly established (Muraille & Leo, 1998).

Table 9. Characteristics of Th1- and Th2-associated immunity[a] *in vivo* (modified from Röcken et al., 1996)

Characteristics	Th1	Th2
IFNγ	high	variable, frequently low
IL-2	high	variable, frequently low
IL-4	low/negative	high
major mode of action	DTH reactions (cellular immunity) complement-binding antibodies and IgE	eosinophil-associated cytotoxicity non-complement-binding antibodies and IgE
protective effects	against intracellular microorganisms and tumours	against extracellular parasites
harmful effects	contact hypersensitivity tissue-specific autoimmunity allergic encephalitis juvenile diabetes rheumatoid arthritis thyroiditis uveitis	atopic diseases immunoglobulin-mediated autoimmunity bullous autoimmune diseases sclerosing diseases ?

[a] Th1 and Th2 immunity characterizes T-cell populations, not single T-cells

In addition to Th1- and Th2-cells, additional cytokine production phenotypes of $CD4^+$ cells exist. They are, however, characterized less thoroughly. Most resting T-cells mainly produce IL-2 on first contact with antigen, and differentiate within a few days into cells producing multiple cytokines, such as IL-4 and IFNγ. In addition to Th1- and Th2-cells, the existence of undefined precursor cells has been suggested. These precursor cells (IL-2 producing) are the virgin Th cells, producing only or predominantly IL-2, and Th0 cells are in the process of differentiation, producing cytokines of both Th1 type (such

as IL-2 and IFNγ) and Th2 type (such as IL-4, IL-5 and IL-10). The pathways of differentiation from the precursor cells are, however, unclear. In addition, it is unknown whether there is a single common precursor cell or whether precursor cells are already committed to a particular cytokine pattern before exposure to antigen (the cytokine production profiles of Th-cell subsets in the mouse are shown in Table 1). In conclusion, it is believed that Th1- and Th2-cells represent the most differentiated populations of the $CD4^+$ phenotype that develop following prolonged exposure to antigen or following stimulation by potent immunogens.

At least two mechanisms can influence the selective differentiation of Th-cell subsets. Firstly, the cytokines that are present during differentiation, in particular IFNγ, IL-4 and IL-12, may greatly influence the type of Th that will be generated. IFγ augments development of Th-type responses and IL-4 promotes differentiation of Th-cells (Romagnani, 1992a). Secondly, the type of APC is thought to influence the characteristics of immune responses. Upon activation, Th2 cells express p39 on their surface, which interacts with CD40 on the surface of B-cells. The interactions of p39 with CD40 and of T-cell antigen receptor (TCR) with antigen and MHC Class II together lead to production of IL-4, IL-5 and IL-6 by Th2 cells, stimulating B-cells to antibody production. Th1 cells, on the other hand, may interact with macrophages. A pair of cell surface molecules analogous to p39/CD40 have not as yet been identified. However, the interaction of Th1 cells with macrophages leads to IFNγ production by Th1 cells, stimulating macrophages to produce monokines.

The difference in APCs, macrophage versus B-cell, that preferentially activates Th1 or Th2 suggests differences in antigen requirements for activation, e.g., large particulate antigens requiring phagocytosis for Th1 and low antigen concentration for Th2. Whereas moderate concentrations of antigen preferentially activate Th1, extremely high concentrations are believed to inhibit Th1 and select for Th2 responses (Pfeiffer et al., 1991).

If Th1 and Th2 clones are stimulated by immobilized anti-CD3 (in the absence of APC), both types produce their respective cytokine pattern. The proliferative responses are, however, very different. Whereas Th2 clones exhibit good proliferative responses, Th1 not only fail to do so, but are even rendered incapable of proliferating in response to exogenously added IL-2 (Williams & Unanue, 1990;

Williams et al., 1990). These Th1 clones are in a state of anergy or tolerance (Schwartz & Weiss, 1990).

IFNγ inhibits the proliferation of Th2 responding to either IL-2 or IL-4, but does not inhibit Th1. IL-10 inhibits the synthesis of cytokines by Th1 cells, and, although growth factor requirement is not affected, the reduction in IL-2 synthesis can lead to decreased proliferation. It has been shown in *in vitro* human systems that IL-10 can suppress the antigen-presenting capacity of monocytes and dendritic cells by down-regulation of MHC Class II. IL-10 had no effect on the antigen-presenting capacity of B-cells or down-regulation of their MHC Class II. These results suggest a mechanism for the general observation that macrophages/dendritic cells preferentially stimulate Th1, whereas B-cells preferentially stimulate Th2.

IL-2 is a T-cell growth factor (TCGF) that mediates autocrine proliferation of Th1, whereas the TCGF IL-4 mediates autocrine proliferation of Th2. Interestingly, it has been shown that IL-4 is the major TCGF produced by T-cells from lymphoid organs that drain mucosal tissues, whereas IL-2 is the major TCGF produced by T-cells from other lymphoid organs (Daynes et al., 1990b). Involvement of dehydroepiandosterone in this site/tissue-specific control on lymphokine production was suggested (Daynes et al., 1990a). Dihydrotestosterone and 1,2,5-dihydroxyvitamin D3 also change the cytokine production pattern of T-cells.

In humans, a predominant fraction of $CD4^+$ T-cell clones was found to produce IL-2, IL-4 and IFNγ, although the quantities varied considerably. Bearing in mind the findings in mice (see above), it was thought that unrestricted profiles are mainly a property of T-cells that are not yet committed to a certain differentiation pathway. Consequently, functional heterogeneity of $CD4^+$ cells should most likely be found in chronically stimulated responders. Kapsenberg et al. (1991) studied two categories of patients, those with nickel hypersensitivity, an example of Type IV hypersensitivity, and those with house dust mite (*Dermatophagoides pteronysinnus* (Dp)) hypersensitivity, an example of Type I hypersensitivity.

Most house dust mite-specific T-cell clones from peripheral blood (Wierenga et al., 1990) and lesional skin biopsies of house dust mite-allergic patients show a Th2-like production profile. House dust mite-specific clones from atopic patients induce IgE production (see also

below). It was shown that this production is dependent on a high IL-4/IFNγ ratio, and is not dependent on the origin of B-cells. Only IgE specific to house dust mite (and not, for instance, IgE specific to tetanus toxoid or *Candida albicans*) was elevated in atopic house dust mite-allergic patients.

The majority of allergen-specific human T-cell clones produce IL-4 and IL-5, but not IFNγ. Virtually all T-cell clones specific for bacterial components, which were derived from the same patients, was found to produce large amounts of IL-2 and IFNγ, and few produced IL-4 and/or IL-5 (Wierenga et al., 1990; Parronchi et al., 1991). In a subsequent study, antigen-specific T-cell clones were derived for the bacterial antigen purified protein derivate (PPD) from *Mycobacterium tuberculosis* and for the helminth antigen *Toxicara canis* excretory-secretory (TES). Most PPD-specific clones produced IL-2 and IFNγ, but not IL-4 and IL-5, whereas most TES-specific clones produced IL-4 and IL-5, but not IL-2 and IFNγ. This study shows that in the course of natural immunization certain infectious agents preferentially expand T-cell subsets. PPD expands Th1, parallelling the (Th1-mediated) tuberculin DTH, whereas TES expands Th2, parallelling the (Th2-mediated) parasite infection.

In a large series of human T-cell clones, all Th1 clones were found to lyse EBV-transformed autologous B-cells pulsed with the specific antigen, and the decrease of Ig production correlated with the lytic activity of Th1 clones against autologous antigen-presenting B-cell targets (Romagnani, 1991). This suggests an important mechanism for down-regulation of antibody responses *in vivo*.

Almost all nickel-specific T-cell clones produce TNFα, GM-CSF, IL-2 and high levels of IFNγ, but low or undetectable levels of IL-4 and IL-5, thus resembling Th1 cells. Nickel induces DTH in the skin of allergic patients. Since IFNγ is an important mediator for DTH, IFNγ may be essential to DTH. However, no clear difference in cytokine production profile between allergic patients and control individuals was found.

2.2.1 *Regulation of IgE synthesis by IL-4 and IFNγ*

Atopy is associated with enhanced serum titres of allergen-specific IgE. The production of IgE is heightened and sustained by B-cells in atopic patients. IL-2 secreted by Th cells is necessary for the

production of all isotypes of immunoglobulins (Kapsenberg et al., 1991). Activated B-cells are induced by IL-4 to undergo immunoglobulin heavy-chain rearrangements to the ϵ-constant region, resulting in synthesis of IgE (Coffman et al., 1986). So far, IL-4 can mediate this isotype switch, which is blocked very efficiently by IFNγ (Romagnani, 1991). IFNγ induces switching to γ2a (Coffman et al., 1986). IL-4 and IFNγ are produced by Th2 and Th1 cells, respectively; a response that involves mainly Th2 cells should produce a large amount of IgE, whereas responses involving mainly Th1 cells, such as DTH reactions, should be non-permissive for IgE production. *In vivo* experiments have confirmed these predictions. IL-4-deficient mice lack IgE and IgG1 responses (Kuhn et al., 1991), whereas transgenic mice constitutively producing IL-4 show elevated serum IgE levels. Injection of mice with anti-IgD antibodies results in a strong stimulation of both B- and T-cell populations, leading to polyclonal antibody production and very high IgE levels. Anti-IL-4 antibodies dramatically reduce IgE levels after anti-IgD immunization, whereas anti-IFN-γ antibodies elevate IgE levels even further. Similarly, administration of IFN-γ results in considerable inhibition of the IgE response. Because the anti-IgD immunization leads to a response that involves high levels of Th2 cytokines, all of these results are consistent with the effects of IL-4 and IFN-γ on IgE synthesis as defined by *in vitro* model systems. Similar correlations between Th2-like responses and high IgE production are seen during several parasite infections.

2.2.2 Eosinophilia and IL-5

Many parasitic infections induce high levels of circulating eosinophils. Because IL-5 has been implicated as a major growth and differentiation factor for eosinophils the association of IgE and eosinophilia may be explained by the association of IL-4 and IL-5 as products of Th2-cells (Gulbenkian et al., 1992). Supporting evidence has been provided by experiments *in vivo*, in which administration of anti-IL-5 during a strong anti-parasitic immune response completely abrogated eosinophilia (Coffman et al., 1989), and from studies of transgenic mice that express high levels of IL-5. The major abnormality in these animals is the presence of extremely high levels of eosinophils in the blood and various lymphoid organs. Patients with filaria-induced eosinophilia exhibit a significantly greater frequency of IL-5-producing T-cells than uninfected individuals.

2.2.3 The relationship between Th2 cells and type I hypersensitivity

In mice, in addition to enhancing IgE production via IL-4, Th2 cells also influence other features of allergic reactions. Firstly, IL-3, IL-4 and IL-10 are mast cell growth factors that act in synergy, at least *in vitro*, and secondly, IL-5 induces the proliferation and differentiation of eosinophils *in vitro* and *in vivo* (Coffman, 1989; Sanderson, 1990). In addition, IL-3 and IL-4 have been shown to enhance the secretory function of murine mast cells. So, Th2-cell activation not only increases the level of IgE synthesized, but also potentially increases the number of IgE-binding cells that will degranulate in response to allergen challenge.

Mast cells and basophils produce IL-4. It has been hypothesized that IL-4 produced by these cells induces the development of Th2 cells, and that these cells in turn produce IL-4. In addition, mast cells are an important source of IL-5.

2.2.4 IL-12 drives the immune response towards Th1

The pivotal role of the cytokine IL-12 in the differentiation of Th-cells towards Th1 is evident from both *in vitro* and *in vivo* studies (Scott, 1993). IL-12 is produced by T-cells, B-cells, macrophages and dendritic cells and stimulates the production of IFNγ from T-cells and NK-cells. IL-12 enhances Th1-cell expansion in cell lines from atopic patients (Manetti et al., 1993). The presence of IL-12 during primary stimulation of naive CD4+ cells skews the response in the direction of Th1 differentiation. These data suggest that IL-12 may be the IL-4 equivalent for the differentiation of Th1-cells. IL-10 has been shown to inhibit lymphocyte IFN-γ production by suppressing IL-12 synthesis in accessory cells. A variety of pathogens that are associated with Th1 development have been shown to induce IL-12 production (Scott, 1993).

2.2.5 IL-13, an interleukin-4-like cytokine

Information on cytokine IL-13 is based on limited information about its activities *in vitro*. As it shares biological activities with IL-4, these activities will, however, be briefly discussed. IL-13 is produced by activated T-cells. The activities *in vitro* of IL-13 are similar to those of IL-4, with two major exceptions. Firstly, IL-13 does not act on T-cells and secondly, IL-13 does not act on murine B-cells (Zurawski & de Vries, 1994). Similarly to IL-4 and IL-10, IL-13 inhibits the

production by LPS-stimulated monocytes of proinflammatory cytokines, chemokines and haematopoietic growth factors. In contrast to IL-10, however, IL-13 up-regulates the antigen-presenting capacity of monocytes. Similarly to IL-4, IL-13 inhibits transcription of IFNγ and both α- and β-chains of IL-12. Thus, IL-13 may (like IL-4) suppress the development of Th1- cells through down-regulation of IFNγ and IL-12 production by monocytes, favouring the generation of Th2 cells. Also in the mouse, IL-13 inhibits production of proinflammatory cytokines and expression of IL-12 α- and β-chain mRNA. Murine IL-13 does not affect macrophage antigen-presenting capacity. Similarly to IL-4, IL-13 acts on human B-cells in inducing class switch to production of IgG4 and IgE and inducing CD23 surface expression (Punnonen et al., 1993; Punnonen & de Vries, 1994). Following activation of T-cells, IL-13 is produced earlier and for much longer periods than IL-4 (Yssel et al., 1994). Thus, IL-13 may play an important role in the regulation of enhanced IgE synthesis in allergic patients. In contrast to IL-4, murine and human IL-13 do not induce IgE synthesis in murine B-cells. Importantly, this may restrict the use of mice as an animal model for allergy.

In summary, IL-13 may favour development of Th2-cells, consistent with the induction of IgG4 and IgE synthesis. Determination of the actual role of IL-13 requires more information on the biological effects *in vivo*.

2.3 Autoimmune reactions

A wide spectrum of human and animal diseases appears to be wholly or partially attributable to autoimmune reactions. Despite the extensive growth of information relating to the mechanisms of self-tolerance (see section 1.5), the understanding of the mechanisms leading to pathogenic autoimmunity is still fragmentary and incomplete (Theofilopoulos, 1995a).

Important issues that need to be resolved in this context concern: (i) the nature of the inciting antigens (self, neo-self, foreign); (ii) the definition of the criteria by which a disease can be termed autoimmune; (iii) the principles that govern the spectrum and extent of an autoimmune response; (iv) the mechanisms by which spontaneous remissions and exacerbations of autoimmune diseases occur; (v) the nature of environmental factors that initiate/precipitate autoimmune reactions; (vi) the structural and other characteristics that

differentiate pathogenic from non-pathogenic autoantibodies and T-cells; and (vii) the identity of the genes that predispose or accelerate autoimmunity, as well as their mechanism of action (Theofilopoulos, 1995a).

The most urgent of these questions concerns the nature of the inciting antigen. Although autoimmune disorders are often defined and diagnosed by the presence of autoantibodies (Osterland, 1994), it should be noted that (a) autoantibodies may indeed be the actual pathogenic agents of disease (e.g., autoimmune haemolytic anaemia, pemphigus, and myasthenia gravis; see sections 2.6.3, 2.6.5 and 2.6.6), (b) they may arise as a consequence of another disease process (e.g., organ-specific autoantibodies due to tissue damage to those organs), or (c) they may merely mark, like footprints, the presence of the etiological agent while not themselves causing disease (Naparstek & Plotz, 1993; Theofilopoulos, 1995a). The latter possibility is complicated by the fact that determinants recognized by the autoantibody and the prerequisite Th-cell may reside on different molecules within a supramolecular complex (Theofilopoulos, 1995a). For example, for many years, it was believed that native nDNA itself was the immunogen for anti-nDNA antibodies, but efforts to induce such autoantibodies by immunization with nDNA have generally been unsuccessful. It has been suggested that for anti-nDNA antibody induction, the scenario may involve intermolecular help, via the binding of nucleosomes or other protein-DNA complexes to anti-DNA idiotype-displaying B-cells, followed by processing of the protein and presentation to the corresponding Th-cells (Theofilopoulos, 1995a). In this connection it is of interest that in systemic autoimmune diseases autoantibodies frequently appear to be directed against the entire set of polypeptides associated with discrete supramolecular cellular entities, such as the nucleosome particle or the nucleocytoplasmic ribonucleoprotein particles (see Table 10).

It has become clear that T-cells are primary players in the initiation and perpetuation of spontaneous (Theofilopoulos & Dixon, 1985; Singer & Theofilopoulos, 1990) as well as induced systemic autoimmune disorders (Druet, 1989; Goldman et al., 1991). Many immune responses seem to be functionally dominated either by Th1 or Th2 cytokines. Therefore, the Th1-Th2 balance during immune reactions *in vivo* significantly determines the outcome of immunopathological processes (Röcken et al., 1996). Whereas organ-specific autoimmune disease are predominantly mediated by IFNγ-producing

Table 10. Examples of autoantigens in organ-specific and systemic autoimmune diseases[a]

Organ/cell/nucleus	Target antigens	Diagnosis
Organ-specific autoimmune diseases		
Pancreatic islet cells	glutamic acid decarboxylase 65 glutamic acid decarboxylase 67 tyrosine phosphatase IA-2 tyrosine phosphatase IA-2b	insulin-dependent diabetes mellitus
Adrenal cortex	21-hydroxylase	Addison's disease
Leydig cells, testes, granulosa theca	cytochrome side-chain cleavage enzyme	hypogonadism
Ovary	17a-hydroxylase	hypogonadism
Gastric parietal cell	H^+/K^+-ATPase intrinsic factor	pernicious anaemia
Thyroid epithelium	thyroid peroxidase thyroglobulin thyroid-stimulating hormone (TSH) TSH-receptor triiodothyronine thyroxine	autoimmune thyroid diseases
Hepatocyte	CYP 2D6 (LKM-1) halothane-induced hepatitis	chronic active hepatitis
Melanocyte	tyrosinase	vitiligo
Parathyroid	calcium-sensing receptor	autoimmune parathyroidism
Systemic autoimmune diseases		
Native DNA	DNA backbone	systemic lupus erythematosus (SLE)-renal
ss-DNA	nucleotides	SLE and other connective tissue diseases

Table 10 (contd).

Nucleoprotein	DNA histone	SLE – central nervous system, renal –
	histone 1 H1, 2A, 2B, 3, 4	drug-induced SLE
	histone 2 H3	connective tissue disease
Sm	SnRNP	SLE
Nuclear RNP	non Sm SnRNP	mixed connective tissues disease, SLE
Ribosomal RNP	phosphoproteins	SLE
Scl-70	topoisomerase 1	scleroderma
Centromere	kinetochore	CREST[b], Raynaud's syndrome
SS-A (Ro)	RNP	SLE-cutaneous, photosensitivity
SS-B (La)	RNA-pol protein	Sicca syndrome, SLE, neonatal lupus
Cardiolipin	phospholipid	SLE – thrombosis, cytopenia
PM-1	protein complex	myositis, scleroderma
Jo-1	histidyl tRNA synthesis	myositis
Mi-2		dermatomyositis
PCNA	cyclin	SLE
Ku	protein on terminal chromosome	SLE
	nucleolar	RNA-pol 1, RNA
	fibrillaren	scleroderma, drug-induced connective tissue disease
Nuclear membrane	laminins	scleroderma, SLE

[a] modified from Osterland (1994) and Song et al. (1996a); responses encompass both Th1 and Th2 responses and involve both Th1 (IFNα) and Th2 (IL-4)

[b] CREST (calcinosis, Raynaud's phenomenon, oesophageal involvement, sclerodactyly and telangiectasia)

Th1-cells, IL-4-producing Th2-cells are involved in immunoglobulin-mediated autoimmune diseases such as systemic lupus erythematosus (SLE) (Goldman et al., 1991; Röcken et al., 1996) (Table 9).

The major non-mutually exclusive etiological concepts of autoimmune disorders have been reviewed (Theofilopoulos, 1995a,b) and are summarized in Table 11.

Table 11. Possible mechanisms of autoimmune reactions (modified from Theofilopoulos, 1995a,b)

Release of anatomically sequestered antigens
The "cryptic self" hypothesis
The self-ignorance hypothesis
The molecular mimicry hypothesis
The "modified self" hypothesis
Immunoregulatory disturbances
Errors in central or peripheral tolerance
Polyclonal activators

2.4 Possible mechanisms of autoimmune reactions

2.4.1 *Release of anatomically sequestered antigens*

In general, antigens associated with peripheral tissues, especially those sequestered behind anatomic barriers, may not come into contact with the developing T-cell repertoire, and, therefore, tolerance may be unnecessary for such antigens. Induction of organ-specific autoimmune disease following contact with antigens of such so-called "immunologically privileged" sites has been well documented, as exemplified by the development of ophthalmia following eye injury and orchitis following vasectomy.

Data have also clearly established that antigens associated with peripheral tissues can cause tolerance, and therefore loss of susceptibility to tissue-specific autoimmune diseases, when experimentally introduced into the thymus. Intrathymic injection of pancreatic islet cells can prevent autoimmune diabetes in the

BioBreeding (BB) rat (Posselt et al., 1992) and the non-obese diabetic (NOD) mouse (Gerling et al., 1992). Tissue trauma alone may not be sufficient to elicit a conventional self-directed immunological response. Tissue-trophic pathogens, such as viruses, may be important in inducing the initial damage that results not only in availability of previously sequestered antigens but also in the production of co-stimulatory factors necessary for the immune response.

2.4.2 *The "cryptic self" hypothesis*

A corollary hypothesis for the mechanism of induction of pathogenic autoimmune responses addresses molecular, rather than anatomic, sequestration and relates to the presence of cryptic self-determinants. Each self-protein presents only a small minority of dominant determinants, which are involved in negative selection during thymic maturation and development of tolerance of the organism to them. Because of many constraints to peptide presentation, only a few peptide stretches of a given protein antigen are presented to the T-cell repertoire, namely those that have the highest affinity to the MHC-binding site and are present at a sufficient concentration. These peptides are the so-called dominant antigenic determinants. It is important to realize that, because antigen-presenting cells cannot distinguish "self" and "non-self" proteins, foreign and "self" peptides are presented indiscriminately (Bloksma et al., 1995). The subsequent immune responses, however, are diametrically opposed to each other. Whereas foreign peptide sequences, in general, induce "stimulatory" T-cell responses, the dominantly presented "self" sequences induce "inhibitory" T-cell responses through peptide-specific thymic cell deletion during development of the T-cell repertoire and/or induction of specific tolerance or anergy in the established peripheral T-cell repertoire. The poorly displayed majority of subdominant/cryptic determinants, constituting the "cryptic self", do not induce tolerance and, therefore, a large cohort of potentially "self"-reactive T-cells exists. The presentation of cryptic "self" peptides, however, can be up-regulated under certain conditions (Lehmann et al., 1993). Evidence for the role of cryptic determinants in the pathogenesis of autoimmunity has been provided in the non-obese diabetic mouse (NOD) model (Kaufman et al., 1993; Tisch et al., 1993), but the exact mechanisms of these immune responses are not fully known. One suggestion is that pathogens such as viruses may provide the initial stimulus through increased presentation of the subdominant determinant, either by molecular mimicry (see below)

and/or by interferon-induced up-regulation of gene-expression, including genes for antigen-presenting MHC molecules (Theofilopoulos, 1995a).

Processing of chemically altered "self" proteins may result in the presentation of cryptic, thus potentially T-cell-activating, self-peptides by creation of new binding sites with high affinity to MHC molecules or modification/preventing of the physiological intracellular protein degradation (Bloksma et al., 1995). Expression of "cryptic self" peptides of nucleolar proteins appears to be a decisive step in the pathogenesis of $HgCl_2$-induced formation of anti-nucleolar autoantibodies in mice (Kubicka-Muranyi et al., 1996).

2.4.3 The self-ignorance hypothesis

Evidence suggests that mature resting T-cells specific for extrathymic antigens presented by non-professional antigen-presenting cells (other than dendritic cells and macrophages) are induced to undergo anergy because of the absence of appropriate "second signals" or "co-stimulatory" factors (Theofilopoulos, 1995a). An alternative possibility is that there is no induction of anergy, but that the mature T-cells are unable to receive appropriate signals and/or help. This would result in T-cells simply ignoring such antigens and remaining quiescent. It follows that, if adequate antigen presentation and co-stimulation occurs through professional antigen-presenting cells, then these self-reactive but quiescent cells may be activated and cause tissue damage (Theofilopoulos, 1995a).

2.4.4 The molecular mimicry hypothesis

Molecular mimicry is defined by homology in a linear amino acid sequence between "self" molecules and foreign molecules. The above theories of cryptic or ignored "self" are compatible with the molecular mimicry hypothesis of autoimmunity, particularly as it pertains to infectious agents. Closely related or identical peptides are often found in unrelated proteins. Thus, many peptide fragments of infectious agents are homologous with host proteins. Among microbial antigens implicated in autoimmunity induced by molecular mimicry, heat shock proteins (hsp), found in virtually all life forms, have received prime attention (Minowada & Welch, 1995). Comparisons of the amino acid sequence of hsp60 with the entire database of known human sequences revealed that 86 human peptides have similar regions to hsp60 and, of

these, 19 are known disease-associated autoantigens (Jones et al., 1993). However, the importance of mimicry to the pathogenesis of spontaneous autoimmune disease is uncertain, as it is unclear why immunological responses to hsp, which are expressed in every cell, could lead to organ-specific autoimmune diseases.

2.4.5 The "modified self" hypothesis

This theory suggests that autoimmunity may arise as a result of an immune response against modified "self" determinants ("neo-self" determinants), which may be particularly relevant for chemical-induced autoimmune responses. Drugs, their metabolites or other haptenic chemicals may bind to "self" determinants. A number of possibilities should be considered.

2.4.5.1 Hapten-induced antibody responses to "modified self"

In such reactions the hapten conjugates to "self" and forms an integral component of the determinant that is recognized by the antibody. In this mechanism hapten-specific T-cells provide cognate help to the B-cell that is then induced to synthesize antibodies which recognize the hapten-modified but not the native form of the "self" protein. Therefore, these reactions against a particular hapten are not truly autoimmune in nature. Penicillin, quinidine, halothane (Gut et al., 1995), and tienilic acid are good examples of compounds that can induce antibody responses to hapten-modified "self".

2.4.5.2 Hapten-induced autoantibodies that recognize "self" proteins

In their native form these can be considered true autoimmune responses, since the determinant that is recognized by antibody does not incorporate a drug-derived determinant. However, the determinant that is recognized by the Th-cells that promote the B-cell response may be drug-derived. The following theories have been put forward:

a) Drugs might break tolerance by binding to "self" macromolecules, thereby creating new determinants that could be recognized by T-cells. T-cells recognizing this new determinant would clonally expand and go on to provide help for B cells that recognize adjacent autoantigens on the same drug "self" conjugate. These in turn would clonally expand and differentiate into autoantibody-producing plasma cells (Fig. 7). In this way the normal process of suppression that

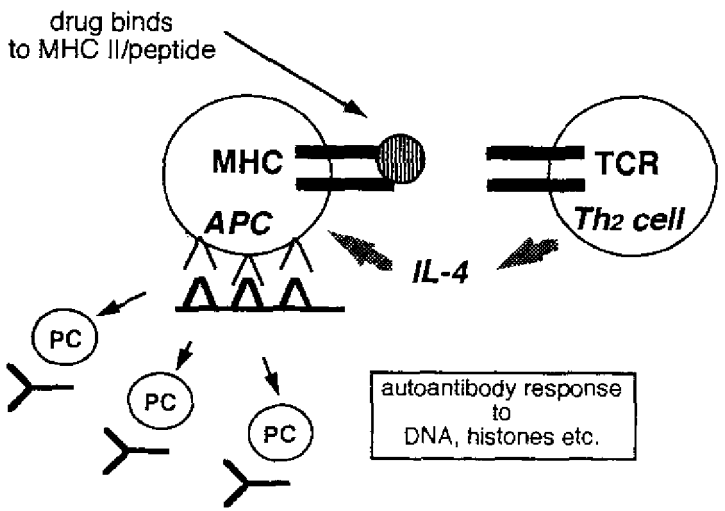

Fig. 7. Mechanisms of autoimmunity: drug-modified MHC II. The formation of reactive metabolites in monocytes (antigen-presenting cells (ARC) and neutophils induces immune dysregulation, and activated autoreactive B-cells (plasma cells (PC)) produce autoantibodies to a variety of "self" antigens (adapted from Kammüller, 1996).

operates through either clonal or functional deletion of Th-cells is effectively bypassed (Allison, 1989). A considerable body of experimental evidence, largely from work with mice, supports this concept. Administration of arsenilic acid-conjugated autologous thyroglobulin or dinitrophenylated autologous immunoglobulin to mice has been found to lead to breakdown of tolerance and elicitation of autoantibodies to these self-proteins (Weigle, 1965; Iverson, 1970). Furthermore, mice sensitized to *p*-aminobenzoic acid (PAB) and then administered PAB-conjugated isologous red blood cells developed a T-cell-dependent antibody response to their own red blood cells, with consequent haemolytic anaemia (Yamashita et al., 1976).

b) It has been postulated that a drug or metabolite might interact chemically with "self"-MHC molecules on antigen-presenting cells (macrophages or B-cells) in such a way that they appear as "non-self" to T-cells. These T-cells, following clonal expansion, would then provide help indiscriminately to all B-cells carrying the drug-modified

"self"-MHC molecule. Assuming that the drug modifies MHC molecules without regard for the antigen specificity of the B-cell, the resulting cognate T-/B-cell interaction would lead to polyclonal B-cell activation and induction of synthesis of antibodies of multiple, including "anti-self", specificities (Gleichmann et al., 1984, 1989). This mechanism would be analogous to a graft-versus-host (GVH) reaction. Indeed, experiments with mercuric chloride (Pelletier et al., 1994), D-penicillamine (Tournade et al., 1990) and gold salts (Schuhmann et al., 1990) in Brown Norway rats or particular strains of mice led to immune disregulatory changes (elevated immunoglobulin levels, particularly IgE, induction of autoantibodies to the glomerular basement membrane, DNA, IgG, collagen and nuclear and nucleolar proteins) resembling those seen in graft-versus-host (GVH) disease (Gleichmann et al., 1984, 1989; Goldman et al., 1991; Bloksma et al., 1995). The elevations in IgE, IgG1 and IL-4 in mercury chloride-treated susceptible mice and rats implicate the Th2 subset in this response (Goldman et al., 1991).

In order to become antigenic to T-cells, haptens need to bind to carrier proteins and it has been discussed whether or not T-cells may require covalent modification of MHC molecules for hapten recognition. Several studies investigating trinitrophenol- and gold-hapten formation have pointed to a major role of hapten-modified MHC-associated peptides as T-cell-antigenic structures (Martin & Weltzien, 1994; Sinigaglia, 1994; Weltzien et al., 1996).

c) Another theory of drug-induced autoimmunity suggests that certain drugs or chemicals might induce, or protect from suppression, populations of T-cells that recognize unmodified "self" MHC. This would be analogous to graft-versus-host reactions, but the difference with the aforementioned mechanism would be that the chemical's effect is targeted at the T-cell rather than the B-cell. In the Brown Norway (BN) rat model of autoimmunity induced by D-penicillamine, gold and mercuric chloride, autoreactive T-cells that recognize unmodified "self" MHC Class II molecules on normal B-cells have been reported, rather than T-cells that recognize chemically modified "self" (Pelletier et al., 1994). This supports the concept that several compounds might induce autoreactivity by modifying T-cells rather than B-cells.

2.4.6 Immunoregulatory disturbances

2.4.6.1 Errors in central or peripheral tolerance

Errors in central or peripheral tolerance at the T- or B-cell level have also been suggested as causes for autoimmunity.

The association between development of immunodeficiency, benign or neoplastic lymphoproliferation and autoimmune diseases, particularly in the context of thymic abnormalities, is well known (Fudenberg, 1966). It has been observed upon immunosuppressive treatment, among others with cyclophosphamide and cyclosporin A. The reversibility of lymphoproliferative lesions upon withdrawal of the immunosuppressive drug therapy suggests a causal relationship (Starzl et al., 1984). Studies in rodents have provided more solid evidence of the relationship between the development of autoimmune disease and induced disturbance of thymic function (Sakaguchi & Sakaguchi, 1989, 1990; Barrett et al., 1995). Notably, cyclosporin A, which is successfully used in the prevention of transplant rejection and treatment of various autoimmune diseases in humans, has been shown to interfere with the deletion of T-cells recognizing autoantigens in the thymic medulla and to cause organ-specific and systemic autoimmune disease under specific conditions. This occurs when cyclosporin A is given to neonates (Sakaguchi & Sakaguchi, 1989), but not to older animals (Hess & Fischer, 1989), and to bone marrow transplant recipients that received a high dose of irradiation prior to transplantation (Glazier et al., 1983; Hess & Fischer, 1989). The development of autoimmune disease under these conditions has been attributed to the absence of an established regulatory peripheral T-cell repertoire. Because cyclosporin A may interfere at different levels of immunological tolerance, autoreactive T-cells leaving the thymus as a consequence of cyclosporin A treatment may not be functionally inactivated in the periphery (Prud'Homme et al., 1991). However, a study using the bone marrow transplant model in different mouse strains suggested the involvement of other mechanisms, because effects of cyclosporin-A on T-cell deletion did not correlate with development of autoimmune effects (Bryson et al., 1991). The study suggested a polyfactorial etiology of cyclosporin-A-induced autoimmune disease and may explain why autoimmune side-effects have been observed only rarely in cyclosporin A-treated human bone marrow transplant patients (Jones et al., 1989).

Patients with primary immunodefiency, especially various B-cell deficiencies, are known to have a high incidence of autoimmune disease (Rosen, 1987). For example, selective IgA deficiency is associated with systemic autoimmune diseases, such as systemic lupus erythematosus (SLE) (Cleland & Bell, 1978; Rosen, 1987). Moreover, drugs with a documented ability to cause systemic autoimmune disorders, i.e., diphenylhydantoin (Seager et al., 1975) and D-penicillamine, have been shown to reduce secretory and/or serum IgA levels. However, the relationship between IgA deficiency and susceptibility to autoimmune disease is not known. It is most likely influenced by other factors as well, since the prevalence of selective IgA deficiency in a normal population is much higher (1 in 700) than the prevalence of systemic autoimmune disease.

Both cyclosporin A and diphenylhydantoin have immunosuppressive activities and affect the thymus. Although neonatal exposure experiments with diphenylhydantoin have been performed (Chapman & Roberts, 1984; Kohler et al., 1987), autoimmune side effects have not been reported. This may be related to the different intrathymic targets of both compounds. Cyclosporin A is thought to disturb thymocyte differentiation by affecting interdigitating and epithelial cells (Schuurman et al., 1992), while diphenylhydantoin affects the more immature cortical thymocytes probably by a glucocorticoid-mediated effect. As pointed out by Schuurman et al. (1992), such differences in intrathymic targets may have different consequences for immune function ranging from immunodeficiency to autoimmune disorders. It illustrates the complex relationship between immunodeficiency, lymphoproliferation and autoimmune effects and the difficulty of immunotoxicological hazard identification (chapter 6) and risk assessment (chapter 7).

2.4.6.2 *Polyclonal activators*

Polyclonal B- and/or T-cell activation has been considered a contributing or initiating mechanism of autoimmunity, particularly in systemic diseases. Although exogenous polyclonal B-cell activators (i.e., lipopolysaccharide) may exacerbate or precipitate SLE, they appear to be insufficient in themselves (Hang et al., 1985; Theofilopoulos, 1995a).

Polyclonal T-cell activation in autoimmune disease is exemplified in graft-versus-host (GVH)-induced autoimmunity, where alloreactive

donor T-cells initiate recipient B-cell differentiation into antibody-secreting cells, particularly those recognizing polymeric "self" antigens (Gleichmann et al., 1989; Goldman et al., 1991; Bloksma et al., 1995). It has been suggested that in this model, as well as in some models of chemically induced systemic autoimmunity, there is a predominant engagement of Th2-cells that promote the humoral response (IL-4 hyperproduction) (Goldman et al., 1991). Polyclonal stimulation of a large set of T-cells by bacterial/viral superantigens is another possible scenario. T-cells that react with MHC Class II-bound superantigens on B-cells may mutually stimulate superantigen-displaying B-cells, thereby leading to production of polyclonal immunoglobulins and, in some instances, autoantibodies (Friedman et al., 1991).

The development of autoimmune reactions as outlined above is only the first step in the production of autoimmune disease. Multiple mechanisms can lead to the same overall clinical manifestations both in organ-specific and in systemic autoimmune syndromes, and therefore, expectations for a single etiological explanation appears unrealistic. For organ-specific autoimmune diseases, the most straightforward explanation to emerge is the concept that these diseases are caused by otherwise conventional immunological responses against self-antigens for which T-cell tolerance is normally not established (i.e., anatomic sequestration, inadequate presentation due to the cryptic nature of the self-determinant, and/or lack of co-stimulatory factors). With regard to systemic autoimmune diseases such as SLE, the situation is less clear, but neither exogenous polyclonal B- or T-cell activators nor immunoregulatory disturbances appear to provide satisfactory explanations. Physical, chemical and infectious assaults may precipitate heterogenous syndromes such as SLE, characterized by an almost all-encompassing autoimmune response against a vast array of mostly dissimilar self-antigens, possibly mediated by the engagement of a large set of non-tolerant T-cells that recognize diverse self-peptides displayed on MHC molecules.

2.5 Type I hypersensitivity diseases and allied disorders

Allergy and atopy have become synonymous for the same set of hypersensitivity disorders, several of which commonly occur in the same individual. They comprise predisposition to develop IgE-mediated immediate (Type I) hypersensitivity responses to common

environmental antigens, in part genetically mediated and manifested as eczema, rhinitis, conjunctivitis and asthma.

Allergic diseases which are considered to result from Type I (immediate) hypersensitivity reactions are shown in Table 12.

Table 12. Examples of Type I hypersensitivity and reaction sites

Disease	Reaction site
Urticaria	Skin
Atopic eczema	Skin
Angioedema	Skin or mucous membranes
Asthma	Respiratory tract
Rhinitis	Respiratory tract
Conjunctivitis	Conjunctiva
Anaphylaxis	Variable, including skin, gastrointestinal tract, respiratory system, cardiovascular system or generalized
Insect venom allergy	
Food allergy	
Drug allergy	

The ability of protein antigens encountered in the environment or workplace to cause IgE antibody-mediated rhinitis and asthma is now well established. Thus, for example, a variety of pollens is known to cause seasonal hay fever in susceptible individuals. It is now apparent that certain chemicals are able to induce similar symptoms in a proportion of exposed individuals (Butcher & Salvaggio, 1986; Karol, 1992). Among chemicals of small relative molecular mass known to cause respiratory allergy in humans are: acid anhydrides such as phthalic anhydride, tetrachlorophthalic anhydride, hexahydrophthalic anhydride and trimellitic anhydride (Bernstein et al., 1982a, 1984; Moller et al., 1985); certain isocyanates including toluene diisocyanate, diphenylmethane-4,4'diisocyanate and hexamethylene diisocyanate (Tanser et al., 1973; Zamit-Tabona et al., 1983; Keskinen et al., 1988); some reactive dyes (Alanko et al., 1978); and platinum salts (Biagini et al., 1985). A number of chemicals induce hypersen-

sitivity disorders that have features similar to Type I hypersensitivity reactions but do not easily fall within the classification of Gell & Coombs (1963).

The characteristics of respiratory allergic hypersensitivity to chemicals are of specific pulmonary reactions usually induced only in a minority of the exposed population and which are provoked by atmospheric concentrations of the allergen that were previously tolerable and that fail to cause symptoms in non-sensitized individuals. Thus, it has been found that with toluene diisocyanate an asthmatic response can be caused by atmospheric concentrations of the chemical far below those that are necessary to induce irritant effects (Newman Taylor, 1988).

Allergic respiratory hypersensitivity induced by chemicals may be of immediate- and/or late-onset. An obligatory universal role for IgE antibody in the pathogenesis of chemical respiratory allergen is uncertain, not least because many symptomatic individuals lack detectable IgE for the relevant allergen. In some cases it may be that insufficiently sensitive or inappropriate methods have been employed for detection of IgE antibody. Nonetheless, it is possible that T-lymphocytes and cell-mediated immune responses may also effect respiratory hypersensitivity reactions to chemicals. There is generally a latent period between the onset of exposure and the appearance of respiratory symptoms. In the case of certain diisocyanates, asthma has been found to develop within a few months. In other instances, however, there may be a latent period of several years. While this is almost certainly the case for protein respiratory allergens, there is no *a priori* reason to suppose that provocation of the immune responses necessary for respiratory sensitization to chemical allergens will result only from exposure via the respiratory tract. Indeed, there is evidence that occupational respiratory sensitization may be caused by dermal exposure to chemical allergens following industrial spillage or splashing (Karol, 1986).

Allergic respiratory hypersensitivity, by definition, results from the induction of a specific immunological response. While there is no doubt that the acute onset of respiratory symptoms associated with hypersensitivity to protein aeroallergens is due to homocytotropic (primarily IgE) antibody, the nature of the immune responses responsible for chemical respiratory allergy is still controversial. Although IgE specific for all recognized chemical respiratory allergens

has been found, and despite a clear association for some chemical allergens between the presence of specific IgE antibody and the development of respiratory symptoms, a clear link between allergic responses and serum IgE antibody has, in some instances (notably with some diisocyanates), failed to emerge. It is nevertheless the case that the induction of acute-onset hypersensitivity reactions in the respiratory tract is usually considered as being dependent upon IgE antibody and the elicitation of classical immediate-type hypersensitivity responses.

In the light of present uncertainties, perhaps the most realistic conclusion that can be drawn is that in many, but perhaps not all, cases the development of chemically induced respiratory allergy is dependent upon IgE antibody and the elicitation of immediate-type hypersensitivity reactions in the respiratory tract. It is possible, however, that in some instances respiratory hypersensitivity to chemical allergens results from the action of T-lymphocytes operating independently of IgE antibody. Irrespective of a putative IgE-independent cell-mediated immune mechanism for the induction of chemical respiratory hypersensitivity, it now appears likely that T-lymphocytes play an important role in late phase reactions and in the pathogenesis of chronic bronchial inflammation.

2.5.1 Asthma

2.5.1.1 Definition

Asthma is a respiratory disease that eludes easy definition. It is characterized by variable airflow limitation due to bronchial responsiveness and often by inflammatory changes in the airways. Asthma has been classified as intrinsic or extrinsic; extrinsic asthma is provoked by sensitivity to a foreign substance, including idiosyncratic drug rections, while intrinsic asthma is characterized by reactivity to non-allergic factors, such as infection and physical and/or psychological stimuli (Barbee, 1987). However, this classification is considered artificial because the clinical signs of both types of asthma are similar.

The US National Institutes of Health (NIH, 1991) published a consensus definition that included the following characteristics: airway obstruction that is reversible (but not completely so in some patients) either spontaneously or with treatment; airway inflammation, and

increased airway responsiveness to a variety of stimuli. In practice, and especially in epidemiological surveys, it has been diagnosed from the replies to questionnaires that have focused on such symptoms as episodic wheezing and shortness of breath (see section 5.2.1.2b). Asthma is distinguished from chronic obstructive pulmonary disease (COPD), i.e., chronic bronchitis and emphysema, by the prominent reversibility of the airways obstruction

The term reactive airways dysfunction syndrome (RADS) was coined to refer to persistent asthma after high-level irritant exposure (Brooks et al., 1985), but the term irritant-induced asthma is just as suitable. To prevent unnecessary confusion, the use of terms other than asthma should be avoided.

The prevalence of asthma has been increasing in a number of countries in recent years (Buist & Vollmer, 1990; Strachan, 1995; ISAAC, 1998). Although some of the increase may be the result of a change in diagnostic classification and increased reporting, a true increase in disease prevalence is likely. The causes of this increase are currently unknown, but environmental pollution is one potential contributory factor.

Allergy is associated with asthma. Up to 80% of patients with asthma have positive immediate reactions to skin-prick testing with a battery of common aeroallergens (Nelson, 1985), although this percentage probably over-represents the importance of allergy in asthma. Whereas allergy clearly plays a primary role in childhood asthma, many adults with asthma do not appear to be sensitized to specific aeroallergens. This observation provided the basis for the traditional characterization of the disease into two major types: i) extrinsic asthma (with sensitization to specific aeroallergens) and ii) intrinsic asthma (without specific sensitization).

There is a genetic component to the risk of developing asthma. Children with one asthmatic parent have an increased risk of developing the disease themselves, and when both parents are asthmatic, the risk is even higher. A parental history of atopy also increases the risk. Up to 40% of the population is atopic: however, many sensitized people do not develop asthma or asymptomatic airway hyperresponsiveness (Witt et al., 1986). Thus allergy alone does not explain the development of persistent asthma, although

continuous or recurrent exposure to allergen may serve to sustain asthma in a genetically susceptible subpopulation.

Infections aggravate asthma and elicit exacerbations of the disease (Johnston et al., 1995). When it comes to the role of viral infections in the induction of the disease, evidence is conflicting (Martinez, 1995). There is evidence that some infections, in particular respiratory syncytial virus (RSV), may predispose for the development of asthma (Sigurs et al., 1995). On the other hand, there is increasing evidence that childhood infections may protect against the development of allergy and allergic diseases, including asthma (Holt, 1996; Shaheen et al., 1996; Shirakawa et al., 1997; Matricardi et al., 1997). Some anecdotal evidence and small studies suggested that childhood vaccination may increase the prevalence of asthma and allergy (Kemp et al., 1997).

2.5.1.2 *Airways inflammation and asthma*

Over the past decade airway inflammation has emerged as an important feature of clinical asthma. It has long been known from autopsy studies of patients that die from status asthmaticus that airway inflammation is present in such patients. The use of fibre-optic bronchoscopy to obtain bronchoalveolar lavage and bronchial-mucosal biopsy specimens has allowed the study of patients with less severe asthma. Airway inflammation is clearly present in these patients as well. Asthmatic airways are characterized by: (a) infiltration with inflammatory cells, especially eosinophils; (b) oedema; and (c) loss of epithelial integrity. Airflow obstruction in asthma is believed to be the result of changes associated with airway inflammation, mucus production and bronchoconstriction. Airway inflammation is believed to play an important role in the genesis of airway hyperresponsiveness in asthma (Holgate et al., 1987).

Much of the research on mechanisms that mediate airway inflammation in asthma has focused on allergen-induced responses. Inhalation of allergen in a specifically sensitized patient with asthma will trigger rapid-onset but self-limited bronchoconstriction, called the early response. In 30 to 50% of extrinsic asthmatic subjects undergoing an allergen inhalation challenge, a delayed reaction will occur 4 to 8 h later, called the late response (O'Byrne et al., 1987). The late response is characterized by persistent airflow obstruction, airway inflammation and airway hyperresponsiveness (Cartier et al.,

1982). Mast cell degranulation and release of mediators such as histamine are believed to be responsible for the immediate response (Liu et al., 1990). The role of the mast cell in the genesis of the late response is more controversial, but the release of chemoattractant substances such as leukotrienes and cytokines (i.e., interleukins: IL-3, IL-4 and IL-5) may be involved in the influx of neutrophils and eosinophils into the airway epithelium, which is a key feature of this response. The infiltration of the airway wall with eosinophils is also a key feature of the late response (Metzger et al., 1987; Djukanovic et al., 1990). The number of Th2-cells in the airway epithelium appears to be higher in patients with allergy-related asthma and may be responsible for the maintenance of chronic airway inflammation (Ollerenshaw & Woolcock, 1992). The Th2-cells are involved in the release of cytokines that may activate both mast cells (IL-3 and IL-4) and eosinophils (IL-5). The eosinophil can release proteins (e.g., major basic protein, eosinophilic cationic protein, eosinophilic peroxidase or eosinophil-derived neurotoxin), lipid mediators, oxygen radicals and enzymes that can cause epithelial injury.

2.5.2 Occupational asthma

Occupational asthma induced by protein allergens is invariably associated with atopy and with the presence of specific IgE antibody. In contrast, occupational asthma induced by chemical allergens is not restricted to atopic individuals and is not always associated with the presence of demonstrable IgE antibody. For both forms of asthma the inflammatory response in the respiratory tract is similar and characterized by T-lymphocyte and eosinophil infiltration.

The immunopathology of occupational asthma has the characteristic features of airway smooth muscle contraction, oedema, and fluid accumulation, resulting presumably from the local release by mast cells of inflammatory mediators such as histamine and leukotrienes. Alternatively, it has been hypothesized that, in some instances of chemically induced respiratory allergic hypersensitivity, the initial inflammatory response results from a chronic cell-mediated immune mechanism operating independently, or in the absence, of IgE antibody (Corrigan & Kay, 1992). Chronic inflammation is recognized as playing an important role in asthma and is associated with infiltration of the bronchial mucosa with inflammatory cells, mucus production, the destruction and sloughing of airway epithelial cells, and subepithelial fibrosis secondary to collagen deposition (Roche et

al., 1989; Beasley et al., 1989). Of particular importance in the development of bronchial mucosal inflammation and injury is the eosinophil, acting in concert with infiltrating T-lymphocytes (Beasley et al., 1989; Gleich, 1990). While the exact role of eosinophils in the development of bronchial hyperreactivity has yet to be established, there is no doubt that the eosinophilia associated with allergen-induced respiratory reactions is influenced markedly by cytokines and, in particular, by IL-5 (Chand et al., 1992; Gulbenkian et al., 1992; Iwami et al., 1993). A role for T- lymphocytes in asthma begs questions regarding the nature of allergen handling in the respiratory tract and the characteristics of local antigen-presenting cells. In the context of primary sensitization following inhalation exposure to the inducing allergen, it is likely that the network of dendritic cells found within the airway epithelium is of vital importance (Holt et al., 1990; Schon-Hegrad et al., 1991).

2.5.2.1 *Occupational asthma and allergy*

Hypersensitivity-induced occupational asthma (see also section 4.3.3) fulfils the criteria for an acquired specific hypersensitivity response:

a) It occurs in only a proportion — usually a minority — of those exposed to the allergen.

b) It develops only after an initial symptom-free period of exposure ranging from days even up to several years.

c) In those who develop asthma, airway responses (both reduction in calibre and induction of hyperresponsiveness to non-specific stimuli) are provoked by inhalation of the specific agent in concentrations that were previously tolerable and that do not provoke similar responses in others equally exposed.

These characteristics have stimulated a search for evidence of a specific immunological response to the causes of occupational asthma, both proteins and chemicals of low relative molecular mass. Attention has been directed towards the identification of specific IgE and IgG antibodies. In general, IgE and IgG_4 have been found in exposed populations to be associated with disease and total specific IgG with exposure. For example, specific IgE and IgG_4 were associated with

asthma and IgG with exposure to acid anhydride workers (Forster et al., 1988).

Studies have suggested a central role for the T-lymphocyte and in particular the Th2-lymphocyte in the development of the eosinophilic bronchitis characteristic of asthma. Evidence for the involvement of T-lymphocytes in occupational asthma was found in nine patients with isocyanate-induced asthma who had activated T-lymphocytes and eosinophils in bronchial biopsy specimens (Bentley et al., 1991). Nonetheless, the IgE antibody-mast cell interaction is probably an important associated response dependent upon Th2-lymphocyte stimulation, and specific IgE remains a valuable marker of the immunological response associated with asthma caused by several agents inhaled at work.

Specific IgE has been identified in the sera of patients with asthma caused by some low relative molecular mass chemicals, particularly acid anhydrides (Newman Taylor et al., 1987) and reactive dyes (Luczynska & Topping, 1986), but not others, notably isocyanates. In a study to examine the determinants of allergenicity of low relative molecular mass chemicals, the properties of two beta lactam antibiotics were compared: clavulanic acid, which is not allergenic; and a carbapenam MM2283, which can cause asthma and stimulate IgE antibody production in man. The characteristics identified as relevant to allergenicity were (a) reactivity with body proteins; (b) hapten of single chemical structure and (c) stability of the conjugate formed (Davies et al., 1977).

Specific IgE antibody has been identified in only some 15% of cases of isocyanate-induced asthma. This may reflect the difficulties of working with reactive chemicals in *in vitro* systems or failure to prepare the relevant *in vivo* chemical-protein conjugate for the *in vitro* test.

Duration and intensity of exposure are the major factors contributing to the development of occupational asthma in populations exposed to its causes. Additional factors such as atopy and tobacco smoking may also contribute. The importance of these factors varies for different causes of the disease. However, for no cause do they adequately explain the development of the disease in the minority who develop it. In part this may reflect the limited knowledge of exposures

experienced but probably also suggests other important, including genetic (such as HLA haplotype), determinants.

2.5.3 Atmospheric pollutants and asthma

There is evidence that air pollutants are involved in exacerbating asthma (Vos et al., 1996). Evidence from laboratory studies suggests that certain air pollutants have the potential to stimulate bronchoconstriction or airways inflammation (see also chapter 5.) Exposure to SO_2 is associated with chest tightness and bronchoconstriction, with the concentration required to induce a response being dependent upon the degree of hyperresponsiveness. It may be that the effects of SO_2 will be augmented in the presence of other pollutants. It has been reported that the sensitivity of mild asthmatics to SO_2 is increased by prior exposure to ozone (O_3). Ozone is a prototype oxidant pollutant that reacts rapidly with tissue components. It is formed by photochemical reactions involving oxides of nitrogen and organic molecules and occurs with other photochemical oxidants and fine particles in the complex mixture called "smog".

Bates & Sizto (1987) studied hospital admissions in Southern Ontario, Canada, an area with a population of seven million people, and observed an association between rates of admissions for asthmatic subjects during the summer season and ambient air levels of both O_3 and suspended sulfates. However, the study design could not separate the 0_3 effects from concomitant effects of acidic aerosol and SO_2. Thurston et al. (1992a,b) found strong associations between elevated summer "haze" pollution (H^+, sulfate, O_3) and increased asthma (and total respiratory) admissions to hospitals in Buffalo and New York City, USA, especially in 1988 when air pollution was severe. However, the specific role of O_3 as opposed to H^+ was less clear.

Controlled (environmental chamber) human exposure studies have clearly demonstrated that some healthy young adults and children respond to O_3 exposure (at levels occurring in ambient air) with irritative cough and substernal chest pain on inspiration and decrements in FVC and FEV_1 (Koren et al., 1989; Folinsbee, 1992). When exercising outdoors in summer such individuals show decrements in FEV_1 that are consistent with the observed ambient air O_3 levels. Controlled exposure to similar levels of ozone has also been shown to cause an inflammatory response of the respiratory tract in all

species that have been studied including humans (Lippmann, 1989). The use of bronchoalveolar lavage (BAL) as a research tool has afforded the opportunity to sample lung and lower airways after exposure to O_3 and to ascertain the extent and course of inflammation and its constitutive elements. The BAL studies (Devlin et al., 1991) have clearly demonstrated that O_3, even at very low concentration, causes increases in numbers of neutrophils, and a variety of other constituents of BAL fluid, some with potential inflammatory properties such as prostaglandin E_2, fibronectin, elastase and IL-6. Inflammation was also detected in the upper airways of O_3-exposed subjects as shown by an increase in neutrophils and other inflammatory indicators in the nasal lavage (NAL) fluid (Koren et al., 1990). Interestingly, both NAL fluid and BAL fluid from non-asthmatic subjects exposed to O_3 have been shown to contain the mast cell marker tryptase. This and another study (Bascom et al., 1990) suggested that O_3-induced inflammation may share certain features of the response observed in subjects with allergic rhinitis challenged with allergen.

A study demonstrated that asthmatic subjects exposed to low levels of O_3 (0.16 ppm) for 7.6 h while performing moderate exercise showed more respiratory symptoms and greater decrements in FEV_1 than did similarly exposed non-asthmatics (Ball et al., 1993).

The concept of influencing the asthmatic response by combining exposure to O_3 with specific allergen challenge has created interest in the potential "indirect" effects of O_3 exposure. In one study, individuals with allergic rhinitis were initially exposed to clean air or 0.5 ppm O_3 for 4 h (Bascom et al., 1990). The high level of exposure to O_3 did not enhance the subsequent acute response to antigen in the nose under these experimental conditions. A study by Molfino et al. (1991) examined the effect of pre-exposure to O_3 (0.12 ppm for one hour at rest) on the subsequent airway response to inhaled ragweed or grass pollen antigen in seven subjects with allergic asthma. They reported O_3-induced increases in bronchial responsiveness to specific allergen challenge. Preliminary data from studies currently conducted examining the effects of pre-exposure to O_3 (0.4 ppm for 2 h at rest) followed by a specific allergen nasal challenge in asthmatics sensitive to house dust mite suggest that the O_3 pre-exposure caused a significant decrease in the dose of allergen needed to induce symptoms (Peden et al., 1994). Eosinophil influx and increase in eosinophil cationic protein were observed 4 h after nasal allergen challenge

following both O_3 and clean air pre-exposure. These changes were more dramatic following O_3 pre-exposure although the mean allergen dose was smaller.

The health relevance of oxides of nitrogen, and in particulate NO_2, has attracted some interest since the gas is present not only outdoors but also indoors. A number of studies suggest mild effects of NO_2 in asthmatics at concentrations less than 1 ppm but others have not found responses at levels up to 4 ppm.

Particulate air pollutants, especially fine particles derived from combustion sources, are also of interest although there have been few controlled exposure studies outside those involving acid aerosols. Bioaerosols, to which an asthmatic is sensitized, are well known to exacerbate asthma. Epidemiological studies describing the increase in mortality associated with particulate matter (PM) provide provocative evidence for adverse pulmonary health effects associated with particulate pollution (Dockery et al., 1993, 1994; Brunekreef et al., 1995; Pope et al., 1995). The association between PM and acute mortality and morbidity has been demonstrated most strongly with elderly people who have chronic cardiopulmonary disease (Pope et al., 1992; Burnett et al., 1995; Schwartz & Morris, 1995). Experimental studies with diesel exhaust particles show that they increase IL-4 and specific IgE production, and exacerbate the response to allergen in allergic individuals (Diaz-Sanchez et al., 1997). Studies in mice have demonstrated that diesel exhaust particles facilitate the induction of allergy (Takafuji et al., 1987; Løvik et al., 1997). Chemicals adsorbed to the diesel exhaust particles, as well as carbon particles with very little chemicals on them appear to enhance the allergic immune response (Diaz-Sanchez et al., 1997, 1999; Løvik et al., 1997).

Environmental air pollutants including tobacco smoke may affect the prevalence and/or severity of asthma in several different ways. In hyperresponsive airways, pollutants may act as triggers of asthmatic reactions without the presence of the specific allergen. Alternatively, a pollutant could induce or increase airway inflammation and, as a result, cause airway hyperreactivity that persists after exposure has ceased. Some pollutants may have a direct toxic effect on the respiratory epithelium leading to inflammation, airway hyperreactivity and the appearance of asthma-like symptoms in previously non-asthmatic individuals. Lastly, there are certain pollutants that may have the ability to augment or modify immune responses to inhaled antigens

or to enhance the severity of reactions elicited in the respiratory tract following inhalation exposure of the sensitized individual to the inducing allergen.

2.5.4 Rhinitis

Rhinitis frequently, but not invariably, occurs in atopic diseases. Similarities and differences between rhinitis and asthma are considered below.

Allergic responses of the nasal mucosa cause an orchestrated set of responses. The acute allergic reaction occurs within minutes and is manifested as rhinorrhoea, pruritus and sneezing, and congestion, due (respectively) to increased vascular permeability, sensory nerve stimulation, and vasodilation with sinusoidal pooling plus oedema formation. These responses are due to mediators released from the mucosal mast cells, and histamine is a major participant.

Following this acute response is the slower development of the late phase allergic reaction which is manifested by congestion and hyperirritability and is due to cellular infiltration with eosinophils, neutrophils and some basophils. There is interest in whether lymphocytes also participate in this reaction, but the data are not clear as yet.

Of the cells that participate in rhinitis, mast cells, neutrophils, eosinophils and lymphocytes may all be important. Mast cells initiate the response through the release of the mediators of anaphylaxis. Work also indicates that mast cells generate a number of cytokines (generally thought of as lymphocyte products, but clearly generated by activated mast cells as well). These products include IL-3, IL-4, IL-5, IL-6 and TNF. Neutrophils are the first cells to infiltrate areas undergoing allergic reactions. The role of the neutrophil in allergy is not clear. However, neutrophils appear to be necessary for the development of increased airway hyperactivity in animal models of asthma. Neutrophils also release factors that activate mast cells (neutrophil-derived histamine releasing factor), and the influx of neutrophils occurs simultaneously with recrudescent histamine release in the late phase reaction. Eosinophils have received a lot of attention, as they are the hallmark of allergic inflammation. Eosinophils infiltrate areas more slowly than do neutrophils, but persist much longer. The eosinophil can cause epithelial denudation, mucus secretion and

histamine release. Both eosinophil and neutrophil infiltrates are inhibited by corticosteroids.

Interest has focused on the possible contribution by lymphocytes to the late-phase reaction. After mast cell activation, about 10% of the superficial lymphocytes express the IL-2 receptor, indicating their activation. There are suggestions that some cytokines are released during this time period, either from mast cells or lymphocytes.

2.5.5 Atopic eczema

In atopic eczema, the patient is much troubled by itching skin; there is a history of chronic or chronically relapsing dermatitis, worst on the flexures, which are excoriated and lichenified, and there is a family or personal history of atopy. This is the typical picture of atopic eczema, though some of the features may be absent (Hanifin & Rajka, 1980). In any discussion of pathogenesis, family history is important because atopic eczema is part of the atopic syndrome that includes genetically determined phenotypes such as extrinsic bronchial asthma, allergic rhinitis, allergic conjunctivitis and gastrointestinal allergy. Important laboratory indices are blood and tissue eosinophilia and antigen-specific IgE bound to mast cells in skin (intracutaneous challenge) or peripheral blood (radioallergosorbence assays). The Wiscott-Aldrich syndrome and hyper-IgE syndrome, which can closely resemble atopic eczema, are usually distinguishable by the associated life-threatening infections.

The clinical course of atopic eczema is unpredictable. Sometimes it remits in childhood, but occasional patients have recurrences throughout life. Some patients (or their parents) are convinced that exacerbations are related to stress and/or exposure to environmental antigens such as food or animals. Secondary skin infection by *Staphylococcus aureus*, herpes simplex virus, varicella virus and, possibly, fungal infections can lead to severe exacerbations. Finally, autonomic nervous system disturbances and changes in fatty acid metabolism and phosphodiesterase activity have been implicated.

Despite the development of numerous theories, the pathophysiology of eczema is still remarkably little understood. Researchers are currently focusing on Langerhans cells, which are thought to be involved in eczema, because these cells possess abundant receptors for IgE. Once in contact with allergen distributed after ingestion or

following direct skin contact, Langerhans cells present the allergen to T-lymphocytes. They may also be directly stimulated to produce inflammatory cytokines, which are responsible for eczematous lesions. Atopic eczema is often accompanied by very high IgE levels. In babies, an elevated IgE level is taken as a reliable predictive sign for the development of asthma and/or hay fever in later life.

The relation between cell-mediated immunity and IgE in atopic eczema was first established by Bruijnzeel-Koomen et al. (1986) who identified the presence of IgE on Langerhans cells in atopic eczema. It is now evident that this binding of IgE is the result of the presence of the high-affinity receptor for IgE on these Langerhans cells (Bieber & Ring, 1992). Langerhans cells and other antigen-presenting cells in skin also express low-affinity Fc receptors that efficiently bind allergen-precomplexed IgE. The functional consequence of the expression of these Fc receptors for IgE on antigen-presenting cells in skin is that the local response to minute quantities of allergens in the skin is amplified. By facilitated antigen-processing, only minute quantities of allergens are needed to be presented to T-cells, because the IgE-receptor-allergen complex aids processing and subsequent presentation up to a 1000-fold (Van der Heijden et al., 1993). Therefore, the onset of atopic eczema as an expression of atopic allergy may result from an interplay between the degrees of expression of one or more Fc receptor types, the serum concentration of allergen-specific IgE, and the number of skin-infiltrating T-cells specific for that allergen and, of course, exposure to the allergen.

Atopic syndrome is genetically determined. When both parents have atopic disease of the same sort, their child has a risk of around 70% of developing a similar phenotype. If parents have different atopic diseases, the incidence of atopic disease in a child is 30% (Björksten & Kjellmann, 1987). With asthmatics as probands in molecular genetic studies, a gene predisposing to atopy has been found on chromosome I Iql3 (Cookson et al., 1989), possibly coding for the β subunit of high-affinity IgE Type I Fc receptor (Sandford et al., 1993). However, the genetic mapping of atopy is far from simple. For example, the increasing prevalence of atopic eczema in the past three decades (Williams, 1992) is difficult to explain on the basis of genetics alone. Furthermore, a maternal pattern of inheritance has been found (Cookson et al., 1992), which might be due to paternal genomic imprinting or to maternal modification of developing immune responses *in utero* or via breast milk. Linkage of atopy with a gene on

I Iql3 could not be shown when patients with atopic eczema were taken as probands. Thus more than one gene seems to be involved.

Environmental factors, such as exposure to allergens, are thought to be involved in the phenotypic expression of atopic eczema. For example, the presence of a strong atopic background has been associated with enhanced protective responses to helminthic infections (Lynch et al., 1998). However, a precise understanding of the environmental factors that determine whether or not the atopic genotype is expressed as an atopic phenotype is lacking.

2.5.6 Urticaria

Urticaria (hives, nettle rash) may be defined as an eruption of short-lived red oedematous swellings of the skin, associated with itching. The relative incidence of the different types of urticaria and angioedema in the general population is unknown.

Urticaria usually involves degranulation of mast cells and release of histamine. Many different elicitors have to be considered. Allergy due to a reaction between a specific antigen and a mast cell-fixed IgE antibody is only one mechanism. Pseudo-allergic reactions, toxic effects and viral infections play a major role.

Acute urticaria resolves within a period of six weeks. If it persists, it is called chronic urticaria. Wheals may be circular, polycyclic or figured. If subcutaneous extension occurs, angioedema is present. Although, like urticaria, angioedema may occur anywhere, the genitalia, eyelids, lips and mucous membranes are especially common sites. Itching is almost always present in patients with urticaria but is inconsistent in angioedema. The duration of urticarial wheals is usually 3 to 4 h, but angioedema lesions may last much longer.

Skin previously involved by wheals or angioedema looks entirely normal apart from occasional purpura or other signs of trauma due to scratching. The mucous membranes are frequently involved including the tongue, soft palate and pharynx. Although discomfort and breathing difficulty may occur, fatalities are almost exclusively associated with hereditary angioedema. Acute urticaria may be associated with systemic anaphylactic symptoms (wheezing, dyspnoea, syncope, abdominal pain, vomiting). Occasionally acute urticaria may merge into serum sickness, arthritis, fever, proteinuria). Common

causes of allergic acute urticaria include ingestion of penicillin, shellfish, soft fruit and nuts.

Urticaria of immunological origin may arise rapidly (often less than 60 min) at the site of contact of the skin or mucous membranes with a specific substance.

Contact urticaria may also be of non-immunological origin, and there are frequent instances in which the mechanism is uncertain. When an immune mechanism is involved, the final common pathway is probably the same. Contact urticaria of immunological origin involves IgE-mediated hypersensitivity as indicated by a positive radioallergosorbent test (RAST). In non-immunological examples, the offending substance may evoke histamine release directly from cutaneous mast cells. Such substances include ammonium persulfate (Mahzoon et al., 1977), dimethyl sulfoxide (Odom & Maibach, 1976) and cinnamaldehyde (Kirton, 1978); however, several other mechanisms are also involved.

Immunological contact urticaria is more frequent in atopic subjects. These patients often give a history of acute oedema of the lips or buccal mucous membrane after ingestion of food items such as fish, egg or nuts. In common with other types of allergy, healthy control subjects are negative on skin testing. The offending allergen is usually a high relative molecular mass substance and skin testing is rarely positive in completely normal skin. Open and closed patch tests and closed patch tests on lightly abraded skin (scratch-patch tests) should be performed. The diagnosis is confirmed by a positive radioallergosorbent test (RAST).

Non-immunological contact urticaria may be elicited in healthy asymptomatic individuals, with the triggering substance frequently being of low relative molecular mass, and contact reactions may be elicitable in clinically normal skin. The danger of such generalized reactions should be borne in mind before skin testing is performed.

2.5.7 *Gastrointestinal tract diseases: mechanisms of food-induced symptoms*

2.5.7.1 *Non IgE-mediated food-sensitive enteropathy*

Slow onset gastrointestinal symptoms are described in children, especially in relation to ingestion of cow's milk. The clinical features

are chronic diarrhoea and failure to thrive. The pathological lesion found in the small intestine is crypt hyperplastic villous atrophy of variable severity. The lesions are often patchy. There is an increased expression of the markers of T-cell activation on the T-cells of the lamina propria, and it is likely that a cell-mediated reaction in the lamina propria is the basis of the abnormality, although IgE involvement has also been described (Walker-Smith, 1992). Nagata et al. (1995) suggested that activated CD4 cells in the lamina propria of the small intestinal mucosa may contribute to the mucosal damage, probably by releasing cytokines.

2.5.7.2 *IgE-mediated food allergy*

Food allergic patients often describe itching and tingling of the mouth and throat as the first immediate symptoms of an allergic food reactions. In addition papules/blisters on the mucosa and swelling of the lips can be seen. These symptoms occur as a result of direct contact between the allergen and the mucosa of the mouth and throat. The concentration of mast cells is very high in the oropharyngeal mucosa and the symptoms are probably caused by degranulation of mucosal mast cells bearing specific IgE towards the offending allergen (Pastorello et al., 1995).

Symptoms like nausea, vomiting, abdominal pains, loose stools and gas production are described in connection with immediate allergic reactions. In a direct challenge of the gastric mucosa using a gastrofibrescope, Romanski (1987, 1989) found gastric changes within 5–20 min of contact with the introduced food. The macroscopic changes were: pale mucosa, oedema, punctate haemorrhage, hyperperistalsis, hypersecretion, erythema. Microscopic examination revealed oedema, hyperaemia, capillary haemorrhage, eosinophilic infiltration and inflammation.

The underlying mechanisms of IgE-mediated gastrointestinal symptoms are a result of degranulation of intestinal mast cells with release of mediators that act directly on the epithelium, endothelium or muscle indirectly through nerves and mesenchymal cells. The result is altered gastric acid secretion, ion transport, mucus production, gut barrier function, and motility (Crowe & Perdue, 1992).

2.5.7.3 *Role of gastrointestinal tract physiology in food allergy*

Many elements of the gastrointestinal tract physiology influence the ultimate allergenicity of food proteins. These include the pH, digestive enzymes, bile, peristalsis, transit time, bacterial fermentation, and the intestinal barrier function, permeability, and absorption. Several food allergens or allergenic determinants were reported to be relatively resistant to acid denaturation and proteolytic digestion (Elsayed & Apold, 1977; Schwartz et al., 1980; Kurisaki et al., 1981; Metcalfe, 1985; Taylor, 1986; Taylor, 1992; Kortekangas-Savolainen et al., 1993). Unfortunately, insufficient information is available on possible differences in susceptibility to acid denaturation and gastrointestinal digestion between strongly allergenic food proteins and proteins that possess weak or virtually no allergenic potential. Attempts have also been made to correlate the susceptibility to enzymatic breakdown of cow's milk proteins, their intestinal permeability and allergenic properties (Taylor, 1986; Marcon-Genty et al., 1989; Savilahti & Kuitunen, 1992). The important role of digestion with respect to food protein allergenicity was clearly demonstrated in mice showing that pre-feeding of an endopeptidase inhibitor (aprotinin) to mice resulted in an inhibition of normally expected oral tolerance induction by protein feeding (Hanson et al., 1993). An abnormal digestive breakdown of proteins may also be of importance, since intragastric administration more easily results in anaphylactic sensitization as compared to *ad libitum* feedings, generally resulting in tolerance induction, as has been shown in rodents (Knippels et al., 1997). However, as digestion of food proteins is part of the normal sequence of events following consumption of food, it is likely that food allergic patients become sensitized to digested allergens rather than to the native proteins. Enzymatically digested food allergens may show the same, more, or less binding to specific IgE from patients (Haddad et al., 1979; Schwartz et al., 1980).

The intestinal barrier function, permeability, and absorption are also hardly, or not, taken into account in the evaluation of the allergenicity of food proteins. Knowledge of the intestinal uptake of specific protein antigens and their fragments may provide some additional information in the evaluation of the potential allergenicity of protein products. There is evidence of limited macromolecular exclusion by the epithelial barrier (Seifert et al., 1974, 1977; Gardner, 1988; Teichberg, 1990).

2.6 Type II hypersensitivity diseases

Pathogenic Type II reactions may occur towards autoantigens, alloantigens (in blood transfusions), infective agents and drugs or chemicals, as described above. As shown in Table 13, these immune reactions may cause corresponding disorders, i.e., autoimmune diseases, transplantation/transfusion reactions or drug-induced haemolytic reactions. As an illustration of Type II reaction-induced diseases, three autoimmune disorders that are also inducible by drugs, i.e., haemolytic reactions, pemphigus and myasthenia gravis, will be dealt with in more detail.

2.6.1 *Drug-induced Type II reactivity*

Some drugs or their metabolites are chemically reactive agents that readily bind to cells and tissues. Such drugs, present on the cell membrane of blood cells, are obvious targets for pathogenic Type II reactivity.

The most frequent allergic reaction occurs with penicillin and its relatives. Benzylpenicillin is a small molecule with a relative molecular mass of 372.47 and with a highly reactive β-lactam ring, which may bind to amino groups on proteins (carrier), forming covalent conjugates. The thus formed penicilloyl hapten is considered as the major determinant in penicillin allergy. Although penicillin is able to induce all types of hypersensitivity reactions (IgE-, immune complex- or T-cell-mediated), haemolytic anaemia with penicillin-specific IgG antibodies reacting with penicillin-coated erythrocytes is a typical example of Type II reactivity.

Interestingly, the specificity of drug-induced antibodies is often much broader than would be expected from the penicillin example. Ultimately, drugs trigger Type II reactivity without being involved in the final destructive reaction. In addition to hapten-specific antibodies, drugs can induce antibodies to metabolites, to drug-carrier combinations or to the carrier alone, resulting in clear-cut autoimmune reactivity. D-penicillamine is a classical example of a drug inducing autoimmunity, but chemicals such as mercury and gold are also able to induce autoimmunity.

Table 13. Clinical disease due to Type II hypersensitivity reactions

	Antigen	Disease	Symptoms
Autoantigens	glomerular basement membrane	Goodpasture's syndrome	vasculitis, renal failure
	epidermal desmosomes (desmoglein-3)	pemphigus vulgaris	skin blistering (intra-epidermal)
	epidermal hemidesmosomes on basal keratinocytes	bullous pemphigoid	skin blistering (subepidermal)
	acetylcholine receptor	myasthenia gravis	striated muscle weakness
	Rhesus antigen	autoimmune haemolytic anaemia	destruction of red cells, anaemia
	platelet integrin gpIIb:IIIa	autoimmune thrombocytopenia purpura	abnormal bleeding
Alloantigens	donor red cell antigens	delayed haemolytic transfusion reaction	destruction of transfused red cells
Infective agents	Streptococcal cell wall antigens cross-reacting with cardiac muscle	acute rheumatic fever	arthritis, myocarditis
	Klebsiella antigens cross-reacting with HLA-B27	ankylosing spondylitis (?)	arthritis involving the spine
Drugs, chemicals	penicillins, cephalosporins trimellitic anhydride	drug-specific haemolytic anaemia	lysis of hapten-coated red cells

The mechanism by which drugs can induce autoantibodies is shown in detail in Fig. 6. By presenting the hapten in or on their MHC-class II molecules, autoreactive B-cells, which are normally present at very low frequencies without being activated, can trigger hapten-specific T-cells to help them (the B-cells) differentiate into antibody-producing plasma cells. Although the induction of the disease is drug-dependent, the Type II effector reaction towards autologous targets may be drug-independent. Hence, in this case the induced autoimmune disease would continue after the exposure to the drug had ceased.

2.6.2 *Transfusion reactions*

Transfusion reactions are examples of the cellular destruction that results from antibody combining with heteroantigens. There are at least 21 blood group systems, with more than 600 antigens within these systems. Some antigens are stronger than others and are more likely to stimulate antibody production. Certain antibodies are produced naturally with no prior exposure to red blood cells, while other antibodies are only produced after contact with cells carrying that antigen.

The ABO blood groups are of primary importance in considering transfusions. Anti-A and anti-B antibodies are so-called naturally occurring antibodies. Individuals do not form such antibodies to their own red blood cells. Thus, an individual who has Type A blood would have anti-B in the serum, and a person with Type B blood has anti-A antibodies. An individual with Type O blood has both anti-A and anti-B in the serum, as O cells have neither of these two antigens.

If a patient is given blood for which antibodies are already present, a transfusion reaction occurs. This can range from acute massive intravascular haemolysis to a small decrease in red blood cell survival. Acute haemolytic transfusion reactions may occur within minutes or hours after transfusion of incompatible blood.

Delayed haemolytic reactions occur 4 to 10 days following a transfusion and are due to a secondary response to the antigen. Antibody-coated red blood cells are removed extravascularly, in the spleen or in the liver, and the patient may experience a mild fever and anaemia.

Haemolytic disease of the newborn appears in infants whose mothers have been sensitized by exposure to fetal blood cells carrying antigens, commonly of the Rhesus family, that differ from their own. The mother makes IgG antibodies in response, and these cross the placenta to cause destruction of fetal red cells. A common antigen involved is the Rhesus D antigen

2.6.3 *Autoimmune haemolytic anaemia*

Drugs account for about 12% of the autoimmune haemolytic anaemia. They can cause haemolysis by three different mechanisms: by acting as a hapten, by inducing a classical autoimmune haemolytic anaemia, or by forming immune complexes with antibodies that can be adsorbed by the patient's red cells, the "innocent bystanders" (Fig. 8).

Antigen-presenting cells phagocytose and process haptenized cells, such as erythrocytes. In addition, free drug molecules may bind to MHC-class II or to peptides within the groove. The hapten is thus presented by MHC-class II molecules to the T-cell receptor (TCR) of T helper cells. Hapten-specific T-cells now proliferate and differentiate, so that they can either attack haptenized cells (The cells causing Type IV reactivity, not shown) or can help nearby B-cells to produce antibodies (in particular The cells).

B-cells ingest and process the antigens to which their immunoglobulin receptors bind and present peptides, including haptenized peptides, derived from these antigens in their MHC-class II molecules. Thus B-cells may trigger hapten-specific T-cells by presenting haptenized peptides. Alternatively, external drug molecules can bind to peptides in the MHC-class II groove. Differentiation of B-cells is dependent on adjacent T-cells providing membrane signals (to CD40, not shown) and the growth factors IL-4 and IL-6.

In drug-specific haemolytic anaemia (on the left of Fig. 8), drug-specific B-cells present the drug to drug-specific T-cells. Mutual activation of T- and B-cells now induces the B-cell to become a plasma cell, producing drug-specific antibodies. Eventually these antibodies lead to destruction of haptenized erythrocytes, while normal cells remain intact.

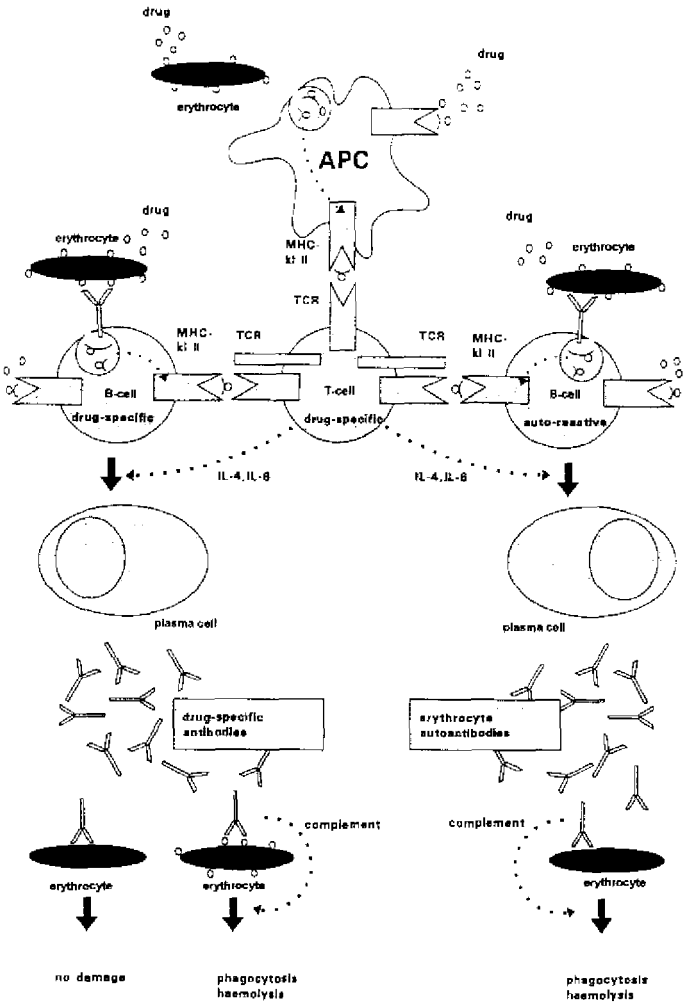

Fig. 8. Drug-induced haemolytic anaemia

In drug-induced autoimmune haemolytic anaemia (on the right of Fig. 8), an autoreactive B-cell ingests and processes erythrocyte membranes, including the haptenized parts. Normally, autoreactive B cells exist but do not become activated by lack of appropriate stimulating T-cells. If drug-specific T-cells are present, however, these B-cells, presenting haptenized peptides in at least some of their class II molecules, may become activated and differentiate into autoantibody-producing plasma cells. These antibodies may induce haemolysis of all erythrocytes in a drug-independent manner.

The hapten type of autoimmune haemolytic anaemia is caused by the presence of drug-specific antibodies. These antibodies may be partly considered as autoimmune since the combination of drug and autologous carrier forms the actual target of the antibodies. When drugs, like penicillin, bind covalently to red blood cells, these drug-specific antibodies bind to the cells and induce their elimination by phagocytosis in the spleen. The induction of high titres of penicillin IgG antibodies typically occurs upon intramuscular administration, rather than upon intravenous penicillin therapy. On the other hand, relatively high intravenous doses of penicillin are required to make the erythrocytes susceptible to immune-mediated haemolysis. Thus, most patients with penicillin-induced haemolytic anaemia have received large doses of drug over a protracted period. After discontinuation of therapy the haemolysis quickly resolves and the antiglobulin test becomes negative within weeks.

Some drugs appear to be able to induce true autoimmune haemolytic anaemia, with Rhesus antigens as the most common targets of the red cell antibodies. It is conceivable that the same auto-specificities are found in drug-induced and idiopathic autoimmune haemolytic anaemia, because the "normally" present (but silent) autoreactive B-cells become activated upon haptenization of the autoantigens, as shown in Fig. 8. As in drug-induced pemphigus and myasthenia, the drug itself does not seem to be involved in the destructive autoimmune reaction. While several drugs (Table 14) have been reported to provoke red cell autoantibodies, α-methyldopa is the best studied example. Only after prolonged therapy anti-red cell autoantibodies (IgG anti-Rh) are formed. Upon withdrawal of the drug the antibody titres usually decline and haemolysis ceases. α-Methyldopa not only induces red cell antibodies, but also antinuclear factors, rheumatoid factors and gastric mucosa antibodies.

Table 14. Summary of drugs causing the different types of immune or autoimmune haemolytic anaemia (from: Foerster, 1993)

Type of drug-induced (A)IHA	Drugs
Drug- or hapten-specific IHA	penicillin, cephalosporins, tetracycline
Autoimmune IHA	α-methyldopa, levodopa, mefanamic acid, procainamide
Immune complex mediated IHA ("innocent bystander" type)	stibophen, *p*-aminosalicylic acid, chlorambucil, quinidine, quinine, phenacetin, sulfonamides, isoniazid, rifampicin, etc.

If soluble drug-specific antibodies are present, they may form immune complexes with administered drugs and fix complement. The complexes are then adsorbed by erythrocytes and thrombocytes resulting in lysis or clearance of these "innocent bystanders". Strictly speaking, this haemolysis is caused by Type III reactivity. The mechanism of adsorption, however, is not completely understood. It is clear that it does not simply involve Fc receptors, since the $F(ab)_2$ domain of the antibodies, in particular, adheres to the target cells. Low-affinity attachment of drugs to the cells seem to make them more susceptible for complex binding, and specific red cell antigens also seem to be involved. Perhaps the bystanders are less innocent than initially thought, and Type II reactivity combines here effectively with Type III reactivity. Many different drugs, usually of low relative molecular mass, are able to induce this type of haemolysis (Table 14) (Foerster, 1993).

2.6.4 *Autoimmune thrombocytopenic purpura*

Autoimmune or idiopathic thrombocytopenic purpura (ITP) is another example of a Type II reaction involving destruction of self-antigens. This disease is characterized by shortened platelet survival and the presence of antibody bound to platelets. It can be classified as acute, intermittent or chronic, depending on the severity and frequency of the symptoms. Acute ITP occurs mainly in children following an upper respiratory viral illness (Karpatkin, 1988). The disease lasts an average of 1 to 2 months. Intermittent ITP may occur in a child or an adult. It is characterized by episodes where the platelet count drops, followed by periods where the count is normal. Chronic ITP is seen in adults, and it may last for years, or indefinitely (Karpatkin, 1988).

ITP can be drug-induced by the following: quinidine, quinine, sulfonamides, *p*-aminosalicylic acid, phenytoin and sedormid (no longer used). The drug acts as a hapten and adheres to the surface of the platelets. This type of ITP is reversed when the drug is withdrawn.

2.6.5 Pemphigus and pemphigoid

Type II reactions in the skin may cause different types of blistering diseases depending on the antigen (location) to which the autoantibodies are directed.

Antibodies towards desmosomal antigens induce intra-epidermal blistering (acantholysis), leading to pemphigus, which is a potentially fatal disease. The presence of these intra-epidermal antibodies can be shown by direct immunofluorescence of perilesional skin and provides the main diagnostic parameter. Most patients also have circulating antibodies with titres reflecting disease activity. Since removal of these antibodies by plasmapheresis reduces disease activity and transfer of positive sera to mouse and monkeys can induce pemphigus-like lesions, it is believed that the anti-desmosomal antibodies are the causative agent of the clinical lesions in pemphigus.

Investigations have unravelled the different desmosomal molecules serving as autoantigens in pemphigus. The most important antigens in pemphigus are the desmogleins; these are transmembrane glycoproteins, which are members of the cadherin gene superfamily. Desmogleins bear the same calcium-binding motifs as other cadherins do, and calcium appears to be essential for the formation of the conformational epitopes that are recognized by pemphigus sera (Amagi et al., 1995). Interestingly, the two clinical variants of the disease, pemphigus vulgaris and pemphigus foliaceus, develop antibodies to different desmogleins, i.e., desmoglein-3 and desmoglein-1, respectively. The differential expression of these two desmogleins in the upper and lower epidermis could explain the different levels of acantholysis seen in the two pemphigus variants (Shimizu et al., 1995).

Drugs may play a precipitating role in pemphigus. Penicillamine-D, thiopronine, ampicillin, rifampicin, phenylbutazone, captopril, pyrazolon, enalapril and piroxicam can all induce pemphigus. It would appear that the presence of certain chemically reactive groups in the drugs, in addition to the pemphigus susceptibility genes in the patient

(HLA-DRB1*0402 or DQB1*0503; Matzner et al., 1995; Wucherpfennig et al., 1995), predispose for drug-induced pemphigus. Sulfhydryl groups (-SH), present in D-penicillamine, and active amide groups (-CO-N-), typically present in enalapril, are held responsible for the acantholytic effects in human skin cultures. In the group of penicillin and cephalosporins, this active amide group is probably more important for the induction of pemphigus than the sulfhydryl group (Wolf & Brenner, 1994).

The mechanism by which the drugs induce pemphigus is still not completely understood. It is clear, however, that in addition to the direct acantholytic effects, which can be observed in human skin cultures *in vitro*, an autoimmune reaction is being induced. The resulting autoantibodies appear to have the same antigenic specificity, i.e., to desmoglein-1 and desmoglein-3, as in idiopathic pemphigus patients (Korman et al., 1991). Together these findings would be in line with involvement of the same mechanism of drug-induced autoimmunity as described for autoimmune haemolytic anaemia.

Antibodies towards antigens present in the lamina lucida of the basement membrane cause a less severe type of blistering disease, called bullous pemphigoid. Direct immunofluorescence of perilesional skin reveals the presence of autoantibodies along the dermo-epidermal junction. Concordantly, sub-epidermal blisters are being formed.

The antigens in bullous pemphigoid have been identified as transmembrane proteins of 180 000 and 230 000 relative molecular mass, present in the hemidesmosomes of the basal keratinocytes (Korman, 1995). These hemidesmosomes are believed to play a role in the epidermal-dermal adhesion. Also in bullous pemphigoid autoantibodies with the same specificity can be detected in the circulation, but their titres do not correlate with disease activity.

The precipitating role of drugs for bullous pemphigoid is not well established, although the disease may occasionally follow drug ingestion (e.g., after furosemide).

2.6.6 *Myasthenia gravis*

Myasthenia gravis is an autoimmune disease that is mediated by IgG antibodies directed to the acetylcholine receptors in the postsynaptic membrane of the muscle (Vincent et al., 1995). The

number of receptors can be considerably reduced by complement-mediated lysis and accelerated internalization. Additionally, the residual receptors may be blocked by autoantibodies directed to the acetylcholine binding site, thus leading to further impairment of the transmission from nerve to muscle. As a consequence, the disease is characterized by weakness and fatigue of the striated muscles. In some patients only few muscles are affected; a well-known localized form of the disease is ocular myasthenia (Weinberg et al., 1994).

In young patients with myasthenia gravis (40-50% of patients, usually female) the thymus is an important site of autoantibody production and T-cell activation. Within the hyperplastic thymus, formation of lymphoid follicles can be observed, with germinal centres surrounded by T-cells. The acetylcholine receptor antigens are here presented to the immune system by muscle-like myoid cells, which bear MHC-class II molecules. As therapeutic treatment, in addition to immunosuppression, thymectomy is beneficial in these patients, since a substantial source of both antigen and antibody-producing plasma cells is thus removed. On the other hand, in late onset (usually male) patients (15–20%), the thymus is rather atrophic and autoantibody production by thymic cells is relatively low. In another minority of patients (15–20%) thymoma may develop. In this last group of patients, autoantibodies to striated muscles are typically found in addition to the acetylcholine receptor autoantibodies.

Like pemphigus, myasthenia gravis can be induced by a number of drugs. D-penicillamine, used for treatment of rheumatoid arthritis, has been most frequently reported as a trigger for myasthenia gravis. A few other drugs are suspected of inducing myasthenia gravis; among them are thiopronin and chloroquin.

Drug-induced myasthenia is characterized by frequent involvement of facial and oropharyngeal muscles (Bonnet et al.,1995). The disease seldom generalizes or results in thymoma. Autoantibodies to acetylcholine receptors are measurable in the circulation in the majority of patients (approximately 80%), whereas almost half of them have blocking antibodies. Similar frequencies of these antibodies are found in idiopathic myasthenia gravis (Morel et al., 1991). The autoantibodies disappear upon discontinuation of the drug, and full recovery may be obtained within a few months.

2.7 Type III hypersensitivity diseases

2.7.1 *Immune complex disease*

Immune complexes are formed every time antibody meets antigen. Generally they are removed effectively by the reticulo-endothelial system but occasionally their formation can lead to a hypersensitivity reaction. Diseases resulting from immune-complex formation can be placed broadly into three groups.

a) The combined effects of a low-grade persistent infection (such as occurs with α-haemolytic *Streptococcus viridans* or staphylococcal infective endocarditis, or with a parasite such as *Plasmodium vivax*, or in viral hepatitis), together with a weak antibody response, leads to chronic immune-complex formation with the eventual deposition of complexes in the tissues.

b) Immune complex disease is a frequent complication of auto-immune disease where the continued production of autoantibody to a self-antigen leads to prolonged immune-complex formation. The mononuclear phagocyte, erythrocyte and complement systems (which are responsible for the removal of complexes) become overloaded and the complexes are deposited in the tissues, such as occurs in systemic lupus erythematosus (SLE).

c) Immune complexes may be formed at multiple sites, such as in the lungs following repeated inhalation of antigenic materials from moulds, plants or animals. This is exemplified in Farmer's lung and Pigeon fancier's lung, where there are circulating antibodies to the actinomycete fungi found in mouldy hay or to pigeon antigens. Both diseases are forms of extrinsic allergic alveolitis, and they only occur after repeated exposure to the antigen. The antibodies induced by these antigens are primarily IgG, rather than IgE, as in immediate (Type I) hypersensitivity reactions. When antigen again enters the body by inhalation, local immune complexes are formed in the alveoli leading to inflammation. Precipitating antibodies to the inhaled actinomycete antigens are found in the sera of 90% of patients with Farmer's lung, but since they are also found in some people with no disease, and are absent from some sufferers, it seems that other factors are also involved, including Type IV hypersensitivity reactions.

The sites of immune-complex deposition are partly determined by the localization of the antigen in the tissues and partly by how circulating complexes become deposited.

Immune complexes trigger a variety of inflammatory processes. They can interact with the complement system leading to the generation of C3a and C5a (anaphylatoxins), which cause the release of vasoactive amines from mast cells and basophils, thus increasing vascular permeability. These anaphylatoxins are also chemotactic for polymorphs. Cytokines released from macrophages, particularly TNFα and IL-1, are also important in localized immune-complex diseases, such as alveolitis, through a mechanism involving neutrophil recruitment. Platelets can also interact with immune complexes, through their Fc receptors, leading to aggregation and microthrombus formation and hence a further increase in vascular permeability due to the release of vasoactive amines. Platelets are a rich source of growth factors, and release of these may contribute to the cellular proliferation found in immune-complex diseases such as glomerulonephritis and rheumatoid arthritis.

The attracted polymorphs attempt to ingest the complexes, but in the case of tissue-trapped complexes this is difficult and the phagocytes are therefore likely to release their lysosomal enzymes to the exterior, causing tissue damage. If simply released into the blood or tissue fluids, these lysosomal enzymes are unlikely to cause much inflammation, because they are rapidly neutralized by serum enzyme inhibitors. But if the phagocyte applies itself closely to the tissue-trapped complexes through Fc binding, then serum inhibitors are excluded and the enzymes may damage the underlying tissue. A classic example of this type of inflammatory response is the Arthus reaction (see section 2.1.3.1).

2.7.2 *Serum sickness*

Serum sickness is a Type III reaction that is seen in humans, although not as frequently as it used to be. Serum sickness results from passive immunization with animal anti-serum used to treat such infections as tetanus and gangrene, usually horse or bovine anti-serum. Approximately 50% of the individuals who receive a single injection develop the disease (Barrett, 1988). Generalized symptoms appear about 1 to 2 weeks after injection of the animal serum and include

headache, nausea, vomiting, joint pain and lymphadenopathy. Recovery takes between 7 and 30 days (Terr, 1994b).

In this disease, the sensitizing and the shock-producing dose of antigen are one and the same, as antibodies develop while antigen still present. High levels of antibody form immune complexes that deposit in the tissues. Usually this is a benign and self-limiting disease, but previous exposure to animal serum can cause cardiovascular collapse upon re-exposure (Terr, 1994b). Antibiotic use has diminished the need for this type of therapy.

2.7.3 *Allergic bronchopulmonary aspergillosis*

Allergic bronchopulmonary aspergillosis (ABPA) is a syndrome characterized by respiratory and constitutional symptoms caused by hypersensitivity reactions to fungal antigens of *Aspergillus fumigatus*. Allergic bronchopulmonary aspergillosis is characterized by episodic wheezing, pulmonary infiltrates, eosinophilia in sputum and blood, markedly elevated serum IgE levels, positive immediate and late skin tests to *A. fumigatus*, serum precipitating antibody to *Aspergillus*, and sputum containing brown plugs or flakes. Not all of these changes may be present during active disease and a diagnosis of allergic broncho-pulmonary aspergillosis is usually considered when asthma is complicated by radiographic or clinical evidence of recurrent pneumonic infiltrates, bronchiectasis or pulmonary fibrosis.

A variety of disease-related immunological alterations have been reported in allergic bronchopulmonary aspergillosis. Antigen extracts of *A. fumigatus, A. niger* and *A. clavatus* have been shown to activate the alternative complement pathway in fresh human serum from healthy humans. Total serum IgE is elevated in most, but not all, instances of allergic bronchopulmonary aspergillosis and Aspergillus-specific IgE is substantially increased as measured by radioimmunoassay.

The immune pathogenesis of allergic bronchopulmonary aspergillosis is thought to involve direct activation of complement by Aspergillus antigen, IgE-antibody production with subsequent release of vasoactive amines from mast cells, as well as IgG-antibody production and deposition of antigen-antibody complexes in the broncho-alveolar tree. Local deposition of immune complexes may activate the complement pathway and generate chemotactic factors for

polymorphonuclear leucocytes in peripheral blood and produce a resultant immune complex-initiated Arthus-type reaction in lung tissues. Also "late phase" eosinophil-mediated IgE-dependent reactions have been suggested to be involved in the pathogenesis of the disease.

2.7.4 Extrinsic allergic alveolitis

Extrinsic allergic alveolitis (EAA) is usually defined in pathological terms as a granulomatous inflammatory reaction which predominantly involves the gas-exchanging parts of the lung and which is the outcome of a specific immunological response to an inhaled substance. The vast majority of reported cases have been caused by inhaled organic dusts, but a few cases have been attributed to inhaled isocyanates, particularly diphenyl methane diisocyanate (MDI) but also hexamethylene diisocyanate (HDI) and toluene diisocyanate (TDI). No reported case has been validated by biopsy evidence of the characteristic pathological appearances; cases have been identified on the basis of:

a) Characteristic clinical history;
b) Changes on chest radiograph;
c) Pattern of functional change following controlled isocyanate inhalation;
d) Proportions of cells received at bronchoalveolar lavage.

Typically, patients present with a history of recurrent episodes of breathlessness associated with systemic symptoms of fever, malaise and chills. A few (but only a minority of reported cases) have had abnormal chest radiographs.

In the majority of cases the diagnosis has been made by the response to inhalation testing or the pattern of cells recovered at bronchoalveolar lavage. Inhalation testing provoked the changes of an "alveolar reaction" with proportionate reduction in forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC) and in transfer factor (TLCO) accompanied by a neutrophil leucocytosis and fever. The cells recovered at bronchoalveolar lavage have, as is characteristic of EAA, shown an increase in the proportion of lymphocytes, on occasion by more than 50%.

In some cases IgG antibody to a human serum albumin conjugate of the relevant isocyanate - MDI-HSA, TDI-HSA and HDI-HSA - has been identified in serum. The outcome of EAA caused by isocyanates has been little reported, but most cases, even if showing significant functional impairment at the time of diagnosis, would seem to have no permanent residual disability after avoidance of isocyanate exposure.

2.7.4.1 Farmer's lung

Farmer's lung affects workers who handle mouldy hay or grain. It originates from poor conditions of storage, involving high dust levels and humidity. Microorganisms responsible for Farmer's lung are moulds, above all *Micropolyspora faeni*, and *Thermoactinomyces vulgaris*. Specific precipitins are found in the blood, especially antibodies of the IgG class. This disease is classified as a Type III hypersensitivity. Better storage and work practices reduce the incidence.

Sensitization takes some time to occur. Clinically, patients suffer respiratory distress accompanied by fever appearing 8–10 h after handling mouldy hay, straw or grain and presenting with fever, shivering, chest pains, lassitude, sweating, headaches and coughing, sometimes accompanied by haemoptysis. Fine auscultatory chest crepitations may be present. In typical forms, the chest X-ray shows miliary infiltrates and micronodules. Later, pulmonary fibrosis appears progressively when the disease reaches a chronic stage. There is also impairment in alveolar gas diffusion (so called restrictive syndrome) and, in the most advanced cases, an alveolar-capillary block, which leads to chronic pulmonary heart failure.

2.7.4.2 Bird-fancier's lung

Bird fancier's lung is another disease of the same type, found especially among pigeon breeders, but also in those handling other birds. The disease is due to the development of precipitating antibodies against serum proteins of relevant avian species, e.g., pigeons, parrots, chickens, pheasants and turkeys.

2.8 Type IV hypersensitivity diseases

Although cell-mediated immunity has fully developed in vertebrates for their benefit by facilitating effective eradication of

microorganisms and abnormal cells, T-cell mediated reactions can, under certain conditions, also cause disease (Table 15). Although allergic contact dermatitis probably represents the most common T-cell mediated disease, a few other pathological conditions are briefly reviewed here.

Table 15. Pathology caused by Type IV hypersensitivity

Type IV induced disease	Antigens, chemicals
Allergic contact dermatitis	low relative molecular mass chemicals, drugs
Protein contact dermatitis	proteins
Granulomatous disease	mycobacterial antigens, beryllium
Autoimmune disease, e.g., diabetes mellitus Type I	autoantigens, e.g., pancreatic islet antigens
Hypersensitivity pneumonitis	toluene diisocyanate, beryllium, heavy metals

Protein contact dermatitis is another example of Type IV hypersensitivity.

One of the more serious complications of Type IV hypersensitivity is the formation of granulomata. In general, T-cell immunity to infectious agents confers a long-lasting state of protective immunity. Macrophages, activated by the T-cell cytokines, can attack the pathogen and should be considered as important effector cells here. If the microorganisms are not readily killed and degraded, however, macrophages may become "frustrated". They fuse to form multinucleated giant cells or develop into large macrophages ("epitheloid" cells). Together these cells can form new structures, so-called granulomata, in which the macrophages with the foreign material are being isolated from the environment by a layer of surrounding T-cells producing cytokines and fibroblasts. The expansion and outgrowth of new granulomata, especially at vulnerable sites, may cause considerable tissue damage and loss of function.

In general, granuloma formation occurs when Type IV reactivity is directed towards persistent indigestible antigens. A number of organisms can induce granulomatous disease: *Mycobacterium tuberculosis* and *M. leprae, Treponema pallidum, Schistosoma*, and

Yersinia enterocolitica. Importantly, a number of exogenous, non-infectious agents can also evoke granulomatous reactions.

T-cell-mediated immune reactions may also cause disease when the T-cell response is directed to autologous tissue. The crucial role of T-cells, for instance, in the breakdown of the insulin-producing β-cells of the pancreatic Islets, leading to insulin-dependent diabetes mellitus, is well established.

Other autoimmune diseases can sometimes be precipitated by Type IV reactions evoked by completely unrelated antigens. Psoriasis may be an example of an autoimmune disease that could be triggered by contact allergens. How exactly these chemicals trigger the disease is not completely clear. It is, however, most likely that any damage to the skin, either toxic, physical or immunological, that recruits sufficient lymphocytes from the circulation to include some auto-reactive, keratin-specific T-cells will trigger a local response in susceptible patients, resulting in a psoriatic lesion.

T-cell-mediated immunity plays a crucial role in the pathogenesis of some lung diseases. Environmental organic chemicals like toluene diisocyanate and trimellitic anhydride, but also inorganic compounds as chromium and nickel are known sometimes to cause pulmonary disease. The extent to which Type IV-mediated immune responses are involved in these disorders is discussed in section 2.1.4.2.

Allergic contact dermatitis is considered to be the most frequent pathological manifestation of Type IV reactivity. In allergic contact dermatitis, T-cells are sensitized to proteins, environmental agents and chemicals, entering the body via the skin. Repeated exposure to such chemicals results in persistent eczematous inflammatory reactions at the site of allergen contact. Although allergic contact dermatitis can be regarded as a prototype of delayed-type hypersensitivity, the sensitization process for chemical contact allergens, which already starts in the most superficial layers of the skin, is very special. The mechanism by which chemicals induce and elicit hypersensitivity reactions in the skin will, therefore, be described in more detail.

2.8.1 Chronic beryllium disease

Chronic beryllium disease is a systemic disorder with primary manifestations in the lungs. The pathogenic beryllium compounds

include metallic beryllium, beryllium alloys and beryllium oxide fume (IARC, 1993). Inhalation of low levels of beryllium dusts or salts over months to years is associated with a chronic interstitial pulmonary granulomatous disorder clinically similar to sarcoidosis (Freiman & Hardy, 1970; Jones Williams, 1988; Williams, 1989). The skin manifestations of beryllium disease consist of contact dermatitis and subcutaneous granuloma formation with occasional ulceration.

The concept that the granulomas of chronic beryllium disease are T-cell-mediated immune granulomas is supported by the observations that:

a) beryllium (i.e., the antigen) persists in the lung for long periods (Jones Williams & Wallach, 1989);

b) large numbers of T-cells and non-caseating granulomas are present in the lung (Williams, 1989);

c) in response to beryllium salts, lung and blood T-cells proliferate and release lymphokines *in vitro*, a parameter also used diagnostically to distinguish beryllium disease from sarcoidosis (Williams & Williams, 1982; Rossman et al., 1988; Newman & Kreiss, 1992; Newman et al., 1994);

d) intradermal administration of beryllium salts induces a local granulomatous response in these individuals.

In chronic beryllium disease, the lung T-cell population is predominantly of the CD4+ phenotype (Rossman et al., 1988; Saltini et al., 1989). These CD4+ T-cells, compared to blood T-cells from the same individual or compared to T-cells from normal individuals, exhibit increased proliferation in response to beryllium (Rossman et al., 1988; Saltini et al., 1989). The T-cells are activated, expressing HLA class II molecules and IL-2R and releasing IL-2 (Pinkston et al., 1984; Saltini et al., 1989). Furthermore, the beryllium-induced lung T-cell proliferation is Class II-restricted. Chronic beryllium disease is strongly associated with HLA-DPB1 *0201, and all beryllium-specific ($BeSO_2$) T-cell clones have been shown to be restricted by this allele.

Analysis of T-cell lines and T-cell clones of individuals with this disease has confirmed that the beryllium-induced response is antigen-

specific and that all the responder cells are CD4+ T-cells (Saltini et al., 1989).

Thus, from the information available, it appears that chronic beryllium disease is a classic example of an immune granuloma host response. Why an element like beryllium should do this is not clear, but two not mutually exclusive hypotheses could explain it. Firstly, it is likely that most disease is caused by dusts of beryllium metal or salts, so that the particulate forms a nidus around which macrophages ingest, allowing the beryllium to be slowly released. Secondly, soluble beryllium salts interact with proteins, such that the beryllium becomes an immunogenic hapten in the context of the protein.

In an epidemiological study of groups exposed to the combustion products of coal containing a high concentration of beryllium, Bencko et al. (1980) found elevated levels of IgG and IgA and increased concentrations of autoantibodies (anti-nuclear and anti-mitochondrial antibodies).

2.8.2 Systemic autoimmune diseases

Several organ-specific autoimmune diseases such as pemphigus and pemphigoid (section 2.6.5) and myasthenia gravis (section 2.6.6) have been discussed above. Many of the major rheumatological disorders are autoimmune in nature. Although systemic lupus erythematosus (SLE) can be ranked under Type III immune complex disorders, for other autoimmune diseases this categorization is less clear-cut.

2.8.2.1 *Systemic lupus erythematosus*

Systemic lupus erythematosus (SLE) is a chronic systemic inflammatory disease that follows a course of alternating exacerbations and remissions. Multiple organ system involvement characteristically occurs during periods of disease activity (Fye & Sack, 1991) (see also section 4.6.2). The disease predominantly affects women (female to male ratio of 9:1) of childbearing age; however, the age at onset ranges from 2 to 90 years. It is more prevalent among non-whites than Caucasians. Family studies have demonstrated a genetic susceptibility to the development of SLE. Autoantibody formation in SLE is partially genetically determined: patients with HLA-DR2 are more likely to produce anti-dsDNA antibodies, those with HLA-DR3

produce anti-SS-A and anti-SS-B antibodies, and those with HLA-DR4 and HLA-DR5 produce anti-Sm and anti-RNP antibodies. Reduced serum complement and the presence of autoantibodies to double-stranded (ds) DNA are hallmarks of active SLE, distinguishing this entity from other lupus variants. Antibodies to single-stranded DNA and particularly against histone proteins are characteristic of some drug-induced forms of SLE, such as procainamide-induced lupus (Rubin, 1989; Rubin et al., 1995).

Although in most cases the etiology of SLE is unknown, a wide variety of medicinal and environmental agents have been associated with the elicitation of SLE at low incidence in susceptible individuals (Kammüller et al., 1989a; Adams & Hess, 1991; Uetrecht, 1992).

2.8.2.2 *Rheumatoid arthritis*

Rheumatoid arthritis is a chronic, recurrent, systemic inflammatory disease primarily involving the joints (Fye & Sack, 1991). It affects 1–3% of people in the USA, with a female to male ratio of 3:1. Constitutional symptoms include malaise, fever and weight loss. The disease characteristically begins in the small joints of the hands and feet and progresses in a centripetal and symmetrical fashion. Elderly patients may present with more proximal large-joint involvement and deformities are common. Extra-articular manifestations such as vasculitis, atrophy of skin and muscle, lymphadenopathy, splenomegaly and leucopenia, are characteristic of rheumatoid arthritis and often cause significant morbidity.

The cause of the unusual immune responses and subsequent inflammation in rheumatoid arthritis is unknown. HLA-D4 and HLA-DR4 occur in approximately 70% of patients with rheumatoid arthritis. Some patients who are negative for HLA-D4 and HLA-DR4 carry the HLA-DR1 gene. It is possible that these and perhaps other genetic determinants impart susceptibility to an unidentified environmental factor, such as a virus, that initiates the disease process. The most important serological finding is the elevated rheumatoid factor titre, present in over 75% of patients (Fye & Sack, 1991).

2.8.2.3 *Scleroderma*

Scleroderma or progressive systemic sclerosis is a disease of unknown cause characterized by abnormally increased collagen

deposition in the skin (Fye & Sack, 1991) (see also section 4.6.3). The course is usually slowly progressive and chronically disabling, but it can be rapidly progressive and fatal because of involvement of internal organs. It usually begins in the third or fourth decade of life. Children are occasionally affected. The prevalence of the disease is 4–12.5 cases per million population. Women are affected twice as often as men, and there is no racial predisposition.

Scleroderma is a manifestation of various diseases, many of them autoimmune (Alarcon-Segovia, 1985). Two primary forms of scleroderma exist: localized or systemic. The systemic form, progressive systemic sclerosis (PSS), has in turn two variants: the diffuse and the CREST syndrome (acronym for Calcinosis, Raynaud's phenomenon, Esophageal involvement, Sclerodactyly and Telangectasia). Autoantibodies to DNA topoisomerase I (scl 70) and centromere may be useful serological markers for these respective diseases. The occurrence of progressive systemic sclerosis with features previously considered characteristic of SLE, rheumatoid arthritis, dermatomyositis and Sjögren's syndrome and associated with high titres of antibodies to nuclear ribonucleoprotein has been termed mixed connective tissue disease (MCTD) (Alarcon-Segovia, 1985). In contrast to other autoimmune diseases, cellular infiltration in scleroderma is minimal or absent in all organs except the synovium, where impressive collections of lymphocytes and plasma cells can be seen. Unfortunately, research on the pathogenesis of scleroderma is severely hampered by the absence of an animal model.

It has been suggested that certain chemicals may be associated with some forms of scleroderma, e.g., tri- and perchloroethylene (Sparrow, 1977; Saihan et al., 1978; Flindt-Hansen & Isager, 1987), vinyl chloride (Lange et al., 1974; Ward et al., 1976; Black et al., 1983), silicone (Rose & Potter, 1995) and epoxy resins (Yamakage et al., 1980).

2.8.2.4 *Sjögren's syndrome*

Sjögren's syndrome is a chronic inflammatory disease of unknown cause characterized by diminished lacrimal and salivary gland secretion resulting in keratoconjunctivitis sicca and xerostomia (Fye & Sack, 1991). There is a dryness of the eyes, mouth, nose, trachea, bronchi, vagina and skin. In one-third of the patients, the disease occurs as a primary pathological entity (primary Sjögren's

syndrome). In the remaining patients, it occurs in association with rheumatoid arthritis or other connective tissue disorders such as SLE. Ninety percent of patients with Sjögren's syndrome are female. Although the mean age at onset is 50 years, the disease also occurs in children.

Patients with Sjögren's syndrome have an abnormal immunological response to one or more unidentified antigens characterized by excessive B-cell and plasma cell activity, manifested by polyclonal hypergammaglobulinaemia and the production of rheumatoid factor, antinuclear factors, including antibodies to SS(A) and SS(B), cryoglobulins, and anti-salivary duct antibodies. Both B- and Th-lymphocytes and plasma cells infiltrate involved tissues. No single immunological test is diagnostic for Sjögren's syndrome, although a spectrum of nonspecific immunological abnormalities occurs in these patients. Histological demonstration of lymphocytic infiltration in a biopsy specimen taken from the minor labial salivary gland is the most specific and sensitive diagnostic test for Sjögren's syndrome (Fye & Sack, 1991).

2.8.2.5 *Hashimoto's disease*

Hashimoto's disease, autoimmune thyroiditis, is the classical example from which much of the knowledge of autoimmune disorders has come (Gell et al., 1975; Roitt et al., 1998).

Antibodies are formed to several antigens in follicular cells of the thyroid, including specific domains of thyroglobulin, thyroid peroxidase and certain surface receptors. Delayed-type cellular hypersensitization also occurs. The consequence is often initial stimulation of the thyroid, followed after a variable period by progressive destruction of the follicular cells, infiltration by lymphocytes and plasma cells, often containing germinal centres, and eventual fibrosis. The clinical disease, which is much more common in women than in men, may be marked by initial thyrotoxicosis, which is invariably followed by progressive hypothyroidism and myxoedema.

Thyroid autoantibodies and variable lymphocytic infiltration are common in many other autoimmune diseases, so other tissues and organs may also be affected and antibodies against these are frequently found.

The cause of Hashimoto's disease is rarely known but it may sometimes follow an overt viral infection of the thyroid and it has been associated with high exposure to iodine.

Thyroid autoantibodies of several types are found in many apparently healthy individuals and are common in patients suffering from other autoimmune diseases.

3. FACTORS INFLUENCING ALLERGENICITY

3.1 Introduction

Allergens can be defined as antigens that give rise to allergy (Sherrill et al., 1994). The molecular properties that distinguish an allergen from an antigen are not known, but certain features appear to be associated with allergens. Induction of allergic responses is highly dependent upon a number of exogenous, as well as endogenous, factors.

3.2 Inherent allergenicity

Most allergens are proteins. The structurally known allergens from pollen, mammals, insects and foods are all proteins (or glycoproteins) with a relative molecular mass of 10 000–40 000 (King et al., 1995). Regarding IgE-mediated allergy, it is known that the IgE antibodies are not formed to an entire allergen, but rather to certain epitopes on the molecule. IgE binding sites are referred to as B-cell epitopes. For a protein to be allergenic, it must be multivalent, expressing more than one B-cell epitope. This allows antigen to bind to more than one IgE molecule on the surface of a mast cell or basophil, and induce these cells to generate and release mediators that initiate the allergic reaction.

B-cell epitopes usually involve 12–15 linear amino acids, although these epitopes may be non-contiguous. In the latter situations, tertiary folding of the molecule provides the epitopes (i.e., conformational epitopes). Allergens must also exhibit T-cell epitopes, the 6–8 amino acid fragments presented to T-cells by antigen-presenting cells such as macrophages. This interaction is necessary to initiate the process of antigen-specific IgE synthesis.

Factors such as "foreignness", size and charge influence allergenicity and sensitization. Allergenic proteins do not possess physicochemical properties that distinguish them from non-allergenic proteins. Foreignness refers to the concept of "non-self". In general, the more foreign the substance, the greater is its immunogenicity. The relationship of foreignness to allergenicity is not known. The larger the antigen, the more likely it is to contain epitopes.

Compounds with a relative molecular mass smaller than 1000 typically are not immunogenic; those with relative molecular mass between 1000 and 6000 may or may not be immunogenic, whereas those with a relative molecular mass greater than 6000 are generally immunogenic (Benjamini & Leskowitz, 1991). Allergens with small relative molecular mass are termed "haptens". Such chemicals are believed to couple to macromolecules to become immunogenic. In general, the nature or identity of macromolecular "carriers" is not known.

Certain inorganic chemicals are particularly potent sensitizers on exposure of the skin, e.g., nickel- and platinum-containing compounds, and, in some instances, of the respiratory tract (Rycroft et al., 1995; Vos et al., 1996). Cross-reactivity has been observed between allergic sensitization to nickel and chromium salts, and between platinum, palladium and related elements.

Physicochemical complexity of a compound also favours immunogenicity, whereas homopolymers of amino acids, such as polylysine, are usually poor immunogens. When the complexity is increased, i.e., by attachment of moieties that of themselves are not immunogenic, the entire molecule becomes immunogenic (Landsteiner & Rostenberg, 1939). For example, attachment of dinitrophenol to polylysine renders the structure immunogenic, (Benjamini & Leskowitz, 1991).

Certain physical and chemical characteristics appear to be associated with allergens. Protein allergens tend to possess biological activity. Haptens tend to have chemical reactivity (or are metabolized into reactive compounds); contact allergens are often lipophilic. Such factors might have functional importance by facilitating access of the allergen to the immune system, and by interfering with regulatory mechanisms of the immune response. For example, many protein allergens have been shown to possess enzymatic activity (Stewart, 1994). The house dust mite allergen *Der p I* is a serine protease (Chua et al., 1988). There is evidence that the proteolytic activity enhances penetration of the allergen through the mucosa (Herbert et al., 1995) and stimulates the synthesis and release of the Th2-associated allergy-promoting cytokine IL-4 from mast cells and basophils (Machado et al., 1996). Furthermore, it has been shown that *Der p I* selectively cleaves the lymphocyte surface membrane molecules CD23 (Hewitt et al., 1995; Schulz et al., 1997) and CD25α subunit (Schulz et al.,

1998) and releases them into the fluids surrounding the cells. Whereas the low-affinity IgE receptor CD23 on the cell surface mediates negative feedback on IgE synthesis, released soluble CD23 promotes IgE synthesis. Thus, the enzymatic cleavage of CD23 by *Der p I* will enhance the synthesis of IgE, a key mediator molecule in allergy. Furthermore, cleavage of the IL-2 receptor CD25α subunit will strongly inhibit the proliferative response and production of IFNγ in Th1-cells. Consequently, the immune response to *Der p I*, and possibly other protein antigens simultaneously presented to the immune system, will be biased towards Th2-cells and an allergic response.

Many of the respiratory chemical allergens possess distinctive functionalities that are thought to endow the chemical with allergenicity. Studies have been undertaken of structural features and physicochemical properties associated with respiratory allergens, and structure-activity relationship (SAR) models have been developed (Graham et al., 1997). Such factors include transport parameters, electron density and chemical reactivities. These models, as well as SAR models of allergic contact dermatitis, are discussed in chapter 6.

The ability of the immune system to recognize and distinguish specific spatial regions (epitopes) on molecules has resulted in the development of reagents and methodology to map these epitopes on molecules such as drugs, proteins and microorganisms (Saint-Remy, 1997). The immune system can distinguish between structures that are almost identical, i.e., that differ from one another by a single amino acid substitution, or by a conformational change. Epitope mapping is performed by generating panels of antibodies of known specificity. Examples of the use of such antibodies are: a) in physiology to identify structures that allow molecules to interact with their receptor, b) in pathology to identify particular T- or B-cell epitopes on antigens, c) in design of vaccines to either increase efficacy or stimulate certain types of responses, such as T-cell responses, d) in microbiology to aid in typing microorganisms.

3.2.1 Inherent properties of chemicals inducing autoimmunity

A variety of medicinal drugs with a relative molecular mass of less than 1000 can elicit systemic hypersensitivity reactions and autoimmune disorders in susceptible individuals at low incidence (Adams & Hess, 1991). Chemical agents, drugs in particular, with a documented potential to induce autoimmune disorders such as SLE,

belong to different chemical classes. These include, among others, derivatives of aromatic amines, hydrazines, hydantoins, thioureylenes, oxazolidinediones, succinimides, dibenzazepines, phenothiaines, sulfoamides, pyrazolines, amino acids (Kammüller et al., 1989a; Adams & Hess 1991; Uetrecht, 1992), amines (Nilsson & Kristofferson, 1989), halothane (Gut et al., 1995), mercuric chloride (Pelletier et al., 1994), gold preparations (Sinigaglia, 1994), occupational or environmental chemicals such as tri- and perchloroethylene (Sparrow, 1977; Saihan et al., 1978) and vinyl chloride (Ward et al., 1976; Black et al., 1983) (see also section 4.4). Environmental nitrophenols have been suggested to be able to elicit or perpetuate human autoimmune disorders (Lauer, 1990). Many of these compounds are heterocyclic and contain at least one aromatic group, suggesting that particular chemical entities may favour induction of immune dysregulation.

From a pharmacological point of view, the majority of auto-immune disease-inducing drugs are β-adrenergic-receptor-blocking compounds, drugs acting on the central nervous system (CNS), anti-thyroid agents and anti-infective agents. In view of the tight functional connectivity between immune, nervous and endocrine systems, which is at least partially effected by shared receptors and mediators among the systems, it is possible that CNS drugs modulate immune responses by acting at these receptors or inducing common mediators.

Lupus-inducing compounds have the capacity to be oxidized by the extracellular myeloperoxidase-H_2O_2 system of activated neutrophils, despite their chemical and pharmacological heterogeneity (Uetrecht, 1992; Jiang et al., 1994). Despite this substrate promiscuity of myeloperoxidase, analogues of lupus-inducing drugs with blocked or missing functional groups such as -NH_2, -$NHNH_2$-, -SH, -Cl or OHC_3 are not metabolized by myeloperoxidase (Jiang et al., 1994).

In order to become antigenic to T-cells, haptens must bind carrier proteins, and whether or not T-cells may require covalent modification of MHC molecules for hapten recognition is a matter of debate. Investigation of mechanisms of allergic and autoimmune reactions has pointed to a major role of trinitrophenol- and gold-hapten-modified MHC-associated peptides as T-cell-antigenic structures (Martin & Weltzien, 1994; Sinigaglia, 1994; Weltzien et al., 1996).

3.3 Exogenous factors affecting sensitization

3.3.1 Exposure

3.3.1.1 Magnitude of exposure

The development of sensitization and the responses in individuals depend upon the frequency and intensity of acute symptomatic episodes (Friedmann et al., 1983; Ollier & Davies 1994). Clinical and experimental evidence indicates that exposure concentration is of critical importance for the development and exacerbation of allergy. For dermal and respiratory sensitization, in animal and human studies, the dose–response concept has been shown to operate at both the induction and elicitation phases of sensitivity.

The role of dose in induction of contact sensitization has been demonstrated in animal models, including guinea-pigs and mice (Chan et al., 1983; Stadler & Karol, 1985). Data revealed a relationship between the amount of chemical applied epicutaneously to the animals and both the severity of the ensuing reaction and the percentage of animals responding. In both species, and with all chemicals tested, a no-effect concentration was also observed.

Both the induction and elicitation phases of respiratory sensitization have been shown to be under the influence of the dose (concentration) of allergen. With protein allergens, sensitization to detergent enzymes was found to diminish as the workplace atmospheric levels of the enzyme dust were reduced (Juniper et al., 1977). With chemical allergens, clinical studies have indicated an association of episodic high (accidental) exposure with development of sensitization (Brooks, 1982). In a study of isocyanate workers, a relationship was found between the number of spills and the percentage of workers displaying symptoms of allergic disease (asthma, bronchitis and decreased pulmonary function). With Western red cedar, an association was also noted between workplace exposure and either the incidence of pulmonary sensitization to the wood dust or the prevalence of occupational asthma (Brooks, 1982). A further indication of the importance of exposure concentration on sensitization is the reported decrease in the number of cases of toluene diisocyanate (TDI) sensitization coincident with the lowering of the permissible occupational exposure levels (Karol, 1992).

Animal studies have established more precisely the relationship between the exposure concentration, the elicitation concentration, and development of respiratory sensitivity (Karol, 1994 a,b). Once again, the concentration of inhaled allergen was shown to be a prime factor controlling the development of sensitivity (Karol, 1983). Exposure of guinea-pigs to monitored concentrations of TDI vapour resulted in development of pulmonary sensitization only when the exposure concentration was ≥ 0.25 ppm (≥ 1.8 mg/m^3) (Karol, 1983). Exposure to lesser concentrations, even for extended periods of time, did not result in sensitization. Both a threshold concentration and a no-effect concentration were observed, suggesting the existence of a safe level of exposure for this potent allergenic chemical (Karol, 1986).

A threshold concentration for sensitization to the allergenic proteolytic enzyme, subtilisin, was also noted in animal studies (Thorne et al., 1986). Groups of guinea-pigs were exposed to atmospheres containing increased concentrations of the enzyme for 15 min per day on each of 5 consecutive days. Sensitivity developed in animals exposed to the high concentrations but not in those exposed to the lesser ones. Even long-term exposure of animals to the lower concentrations failed to produce sensitization, although the animals had received a cumulative exposure comparable to that which regularly induced sensitivity when given over 5 days. This enzyme is believed to be a particularly potent allergen and has a threshold limit value of 0.06 mg/m^3. Clinically, workplace sensitization to the enzyme has been dramatically reduced by lowering workplace exposures, and by changing the formulation of the allergen to make it less readily airborne (Juniper et al., 1977; Thorne et al., 1986; Sarlo & Karol, 1994).

3.3.1.2 *Frequency of exposure*

Increased frequency of inhalation exposure to allergen increased the sensitization rate (Karol, 1986). However, studies clearly demonstrated the importance of the exposure concentration exceeding a threshold level for the chemicals. Repeated inhalation exposure of guinea-pigs to sub-threshold concentrations of subtilisin (Thorne et al., 1986) or TDI (Karol, 1983) failed to sensitize the animals, whereas the same total exposure given over a shorter time span consistently resulted in sensitization. Long-term sub-threshold exposure to TDI resulted in neither respiratory sensitization nor production of specific antibodies (Karol, 1983).

Clinically, chronic low-level exposure has been implicated in the development of respiratory allergy to some airborne chemicals, notably TDI (Karol, 1986). However, at that time the ability to measure low concentrations of TDI was limited. Long sampling periods were often required which eliminated the possibility of detecting sporadic high TDI concentrations (Karol, 1986). As a result, in such studies no conclusion can be drawn regarding the development of sensitization as a result of repeated low-level exposure.

The influence of chronic low-level exposure to detergent enzymes on the development of occupational sensitization to these enzymes has been studied (Juniper et al., 1977). Using skin prick tests as an indication of sensitization, conversion to skin test positivity was observed following 20 months of employment for both high- and low-exposure groups. A reduction in the dust levels in the workplace was coincident with a decreased conversion rate (Juniper et al., 1977).

In the platinum industry, respiratory sensitization to soluble platinum salts has occurred under conditions where exposure is below the official workplace limit. Maynard et al. (1997) examined the possibility that high short-term exposures might be responsible but found there was no evidence for this. In a cross-sectional study of respiratory and dermal sensitization to platinum salts in a population of precious metals refinery workers, skin reactivity was found in workers exposed to permissible levels of platinum salts and was associated with respiratory and dermal sensitization, but not with atopic status (Baker et al., 1990). Merget et al. (1994), in a study of platinum refinery workers, found that in workers who developed immediate-type asthma caused by platinum salts both nonspecific and specific bronchial responsiveness did not decrease after removal from exposure.

Repeated exposure of guinea-pigs to contact allergens resulted in reduced local reactions (Boerrigter et al., 1987) with eventual diminution such that the skin reactions were almost non-existent. However, the state of unresponsiveness disappeared upon discontinuation of the repeated allergen exposures.

In humans, repeated exposure may also down-regulate the local inflammatory response in the skin. This phenomenon is termed "hardening". However, the individual remains sensitized. By contrast,

repeated systemic exposure could also "desensitize". This effect is thought to be due to the high total dose administered.

3.3.1.3 Route of exposure

The route of exposure has an influence on the outcome of exposure to an allergen. In general, exposure by the inhalation or dermal route favours sensitization, whereas exposure by the oral route favours tolerance (unresponsiveness). Immunological unresponsiveness can be induced in animals by non-cutaneous exposure. Induction of "tolerance" in humans to nickel as a result of exposure to nickel-releasing orthodontic braces during early age has been suggested (Van Hoogstraten et al., 1991).

Systemic unresponsiveness after ingestion of antigen has now been described for a large variety of T-cell-dependent antigens (Mowat, 1987). Proteins such as ovalbumin and bovine serum albumin (Silverman et al., 1982; Domen et al., 1987), particulate (erythrocyte-bound) antigens (Kagnoff, 1982; MacDonald, 1983; Mattingly, 1984), inactivated viruses and bacteria (Stokes et al., 1979; Rubin et al., 1981), autoimmune-related antigens (Thompson & Staines, 1990), as well as contact allergens, have been reported to induce oral tolerance (Asherson et al., 1977; Newby et al., 1980; Gautam et al., 1985). Generally, T-cell-mediated delayed-type hypersensitivity responses and IgE production are the types of immune responses most readily tolerized. Persistent tolerance can be induced with relatively low antigen doses of proteins (Heppel & Kilshaw, 1982; Jarrett, 1984; Jarrett & Hall, 1984) and contact allergens (Asherson et al., 1977; Polak 1980; van Hoogstraten et al., 1992; Hariya et al., 1994). The apparent ability of the intestinal immune system to prevent allergic hypersensitivity to soluble, non-replicating antigens seems an important pathway to prevent enteropathies (Challacombe & Tomasi, 1987; Mowat, 1984, 1987). Abrogation of oral tolerance to, for instance, ovalbumin was found to lead to hypersensitivity responses in the intestinal mucosa and gut-associated lymphoid tissues, resembling those observed in food-sensitive enteropathies, e.g., coeliac disease (see section 1.5.1.3).

If mucosal cells in the respiratory tract are the site of initial exposure, the result is frequently production of IgA and IgE antibodies and predisposition to Type I allergic reactions. Initial exposure of mucosal cells in the gastrointestinal tract may have the same effect but

often produces tolerance. By contrast, skin exposure favours Type IV sensitization. It appears that the route of first encounter with the chemical allergen determines whether the outcome is sensitization or unresponsiveness.

Once an individual is sensitized via the skin, subsequent oral exposure does not tolerize, but might contribute to further sensitization by boosting the ongoing immune response. It is even possible to induce systemic allergic reaction via the oral route in skin-sensitized individuals. Overall, all of these factors are dependent upon the nature of the allergen.

3.3.2 Atmospheric pollution

The effect of indoor and outdoor air pollution on allergic disease has received considerable attention. Environmental pollutants have been reported to contribute to the prevalence of allergic disease, the precipitation of allergic symptoms, and their intensity (Ollier & Davies, 1994). Both epidemiological and experimental studies have demonstrated that a variety of atmospheric substances (including sulfur dioxide $(SO)_2$, nitrogen dioxide (NO_2), ozone (O_3) and particles) influence the induction and elicitation phases of the allergic response. Effects have included adjuvant activity for allergen-specific IgE production, modulation of mediator release from inflammatory cells, and irritant effects on effector organs of the allergic response (Behrendt et al., 1995) (see sections 5.13 and 5.14).

The question of whether environmental factors may be involved in the observed increase in the prevalence of allergy is a matter of controversy (Ring et al., 1995b; Behrendt et al., 1995; Vos et al., 1996). There is no doubt that pollutants such as suspended particles, automobile exhaust, ozone, sulfur dioxide and nitric oxides can be measured in rather high concentrations in the air of many countries that show an increasing prevalence of atopic diseases. However, some of these pollutants, like sulfur dioxide, have shown a decrease in air concentrations during the last decades. In a controlled prospective trial comparing different living areas with various degrees of air pollution in western and eastern Germany, striking differences were shown with regard to the prevalence of respiratory atopic diseases, with higher values for western compared to eastern Germany (von Mutius, 1992; Schlipköter et al., 1992; Behrendt et al., 1993, 1996; Ring et al., 1995). In contrast to atopic respiratory diseases, there was a trend to higher

prevalence rates of atopic eczema in eastern Germany. In the same study there was evidence of an increased risk of developing atopic eczema after exposure to natural allergens as well as air pollutants from outdoor and indoor sources (Ring et al., 1995; Krämer et al., 1996; Schäfer et al., 1996).

The mechanisms by which air pollutants influence allergic reactions are not clear. Some pollutants may have a direct toxic effect on the respiratory epithelium leading to inflammation, airway hyper-reactivity and the appearance of asthma-like symptoms in previously non-asthmatic individuals. In cell systems, certain pollutants have been shown to modulate degranulation and histamine release from basophils (Ring et al., 1995). Polychlorinated biphenyls enhance eicosanoid production by granulocytes and platelets (Raulf & Konig, 1991). Certain pollutants may have the ability to augment or modify immune responses to inhaled antigens or to enhance the severity of reactions elicited in the respiratory tract following inhalation exposure of the sensitized individual to the inducing allergen.

High concentrations of air pollutants can have irritant effects and aggravate the symptoms of allergic respiratory and skin diseases (Ring et al., 1995; Behrendt et al., 1996). Laboratory studies suggest that certain air pollutants have the potential to stimulate broncho-constriction and airway inflammation. Exposure to SO_2 is associated with chest tightness and bronchoconstriction, the concentration required to induce a response being dependent upon the degree of hyperresponsiveness of the individual. The effects of SO_2 may be augmented in the presence of other pollutants. It has been reported, for instance, that the sensitivity of mild asthmatics to SO_2 is increased by prior exposure to O_3. Ozone has been investigated extensively and has been found to cause bronchial hyperresponsiveness. In controlled clinical exposure studies, researchers have demonstrated that asthmatics are more responsive to O_3 than normal people (Ball et al., 1993; WHO, in press). Exposure of asthmatics to O_3 for 1 h caused an increase in airway responsiveness to inhaled allergen. The proportion of cynomologous monkeys that developed asthma and a positive skin test after inhalation of complex platinum salts was increased in those animals that inhaled O_3 concurrently (Biagini et al., 1986). The health relevance of oxides of nitrogen, and in particular NO_2, has attracted some interest since the gas is present both outdoors and indoors. Some studies have suggested mild effects of NO_2 in asthmatics at concentrations of less than 1 ppm (< 1.88 mg/m^3); others have not

found responses at levels up to 4 ppm (7.52 mg/m^3). Particulate air pollutants, especially fine particles derived from combustion sources, are also of interest although there have been few controlled exposure studies apart from those involving acid aerosols.

Bioaerosols to which an asthmatic is sensitized are well known to exacerbate asthma. Epidemiological studies describing the increase in mortality associated with inhaled particulate matter (PM-10) provide provocative evidence for adverse pulmonary health effects associated with particulate pollution. The association between particulate matter and acute mortality and morbidity has been demonstrated most strongly with elderly people who have chronic cardiopulmonary disease (Thurston, 1996).

Studies have demonstrated an effect on allergic disease from substances adsorbed to airborne particles. Such substances were found to release histamine from human basophils and had a priming effect on anti-IgE-induced release of histamine and LTC4 (Behrendt et al., 1995). These *in vitro* studies indicated that particle-adherent substances interfere with cells involved in inflammatory processes.

There is evidence of an interaction between pollen and air pollutants. Pollen grains in polluted areas have been shown to be loaded with particles including heavy metals, such as lead, cadmium and mercury. *In vitro*, these pollen grains were found to have altered surface features and increased ability to release cytosolic allergenic proteins (Behrendt et al., 1991).

3.3.2.1 *Tobacco smoke*

Passive exposure to tobacco smoke is a risk factor for childhood asthma (Seaton et al., 1994; Becher et al., 1996). Studies to detect a possible association between passive smoke and allergic disease in adults are much more difficult to design. Asthmatic patients frequently report exposure to passive smoke. In children, there is evidence that tobacco smoke increases the risk for development of wheezy bronchitis and asthma.

Tobacco smoking is associated with an increased risk of developing IgE antibodies and asthma. The mechanism of this effect of tobacco smoke is unknown, but may be a result of injury to the respiratory mucosa. Several studies have indicated that subjects who

smoke cigarettes have higher IgE levels (Zummo & Karol, 1996). Specific IgE antibody or an immediate skin test response was found to be 4–5 times more frequent in smokers exposed to tetrachlorophthalic acid (TCPA) and ammonium hexachloroplatinate. Initially smokers had IgE levels similar to those of controls, but, with age, IgE levels in smokers did not decline at the same rate as they did in the non-smokers (Sherrill et al., 1994). This may provide an explanation for the difference in IgE values observed in adult smokers. Moreover, a relationship was noted between the number of cigarettes smoked and the IgE level, suggesting causality. In female smokers, there was a trend toward increased IgE at older ages (i.e., > 50 years).

Passive smoking has been found to be a risk factor for development of sensitization in children (Halken et al., 1995). The association does not necessarily imply an allergic mechanism, rather the association can be a result of direct irritation and inflammation of the respiratory tract. In children with atopic predisposition, a significant correlation was found between exposure to tobacco smoke and wheezing/persistent wheezy bronchitis. A prospective study of 94 asthmatic children found significantly more asthma symptoms in those exposed to maternal tobacco smoke. A retrospective study with 199 children with asthma found acute exacerbations of asthma increased with exposure to tobacco smoke. In children with past or present atopic dermatitis, asthma was found more frequently in cases where the mother smoked cigarettes (Halken et al., 1995).

3.3.2.2 *Geographical factors*

Exposure to airborne allergens, notably pollens, depends on location, climate and time of year (Emberlin, 1994). Certain types of air pollution reduce the amount of pollen produced, but they can also render the proteins on pollen more allergenic (Ruffin et al., 1986).

3.3.3 *Metals*

Nickel is a frequent cause of contact sensitization, having a sensitization rate of 15–50% in experimental studies. Most cases of nickel allergy can be attributed to exposure to nickel alloys in close skin contact, which release high concentrations of nickel when exposed to sweat. Similarly, chromate dermatitis often relates to exposure to hexavalent chromate in wet cement (Andersen et al., 1995). Investigations of monozygotic female twins, where one or both

were nickel sensitive, have shown that only the twin with a history of contact dermatitis by nickel alloy exposures gives a positive diagnostic patch test to nickel (Menné & Holm, 1983). *In vitro* diagnostic testing failed to demonstrate subclinical nickel sensitization in family members of nickel-sensitive individuals (Silvennoinen-Kassinen, 1981).

3.3.4 Detergents

Reports of respiratory allergic reactions in workers involved in large-scale production of enzyme-containing detergents suggest that the detergent component may contribute to the sensitization to the enzyme component. The symptoms of rhinitis and/or asthma suggested a Type I sensitization. Experimental studies in guinea-pigs, using either inhalation or intratracheal dosing, indicated that detergents and proteolytic enzymes enhance sensitization to allergenic proteins (Ritz et al., 1993; Sarlo et al., 1997) when sensitization was assessed by production of allergic antibody and respiratory responses to allergen challenge.

3.4 Endogenous factors affecting sensitization

3.4.1 Genetic influence

3.4.1.1 Contact sensitization

Although significant genetic influences on contact sensitization have been reported, lack of reproducibility and smallness of these effects suggest their minor importance, as compared to exposure, in clinical contact sensitization. A few studies utilizing different inbred mice and guinea-pig strains noted differences in sensitization rates for some contact allergens (Parker et al., 1975; Andersen & Maibach, 1985). In humans, a well-controlled family study indicated that experimental contact sensitization in children was greater when both parents could be sensitized by the same substance compared to children where only one parent could be sensitized (Walker et al., 1967). A population-based twin study focusing on nickel allergy found a significant genetic effect for the risk of developing this contact sensitivity (Menné & Holm, 1983). However, twin studies, using other designs, have failed to show such an association. Also, studies on frequencies of HLA genes in contact hypersensitive individuals have not revealed consistent patterns (Menné & Holm, 1986). Comparisons

between frequencies of sensitization in different ethnic populations, e.g., for nickel in black and Caucasian groups, revealed either similar or different rates, depending on the study designs (Menné & Wilkinson, 1995).

Histamine releasibility from mast cells and basophils is a critical event in many allergic disorders. In twin studies, this event (which is related to the quantity of IgE present on the cells) was shown to be under genetic control (Bonini et al., 1994).

Products of HLA class II genes are involved in allergen presentation by antigen-presenting cells. Since these genes are highly polymorphic, different HLA genes represent risk factors for development of allergic asthma. Increased responsiveness to the ragweed allergen Ra 5 was found to be associated with the HLA gene DR 2/DW 2.

There is evidence for a genetic contribution to sensitization to some allergens of low relative molecular mass.

3.4.1.2 *IgE-related allergy*

One of the characteristic features of atopy is the production of IgE in an exuberant and prolonged fashion to common largely innocuous environmental allergens, such as house dust mites and pollen. Most atopics are allergic to more than one common environmental allergen and this introduces the concept that the causation of atopy occurs at a variety of levels: generalized hyper-IgE responsiveness; IgE response to specific allergens or epitopes; clinical disease expression (Hopkins, 1997).

The genetics of production of total serum IgE have been studied. In such studies consideration has to be given to the following factors, since each has been shown to affect IgE levels: allergic exposure, parasitic infection, age, sex and smoking. A correlation was found between the total serum IgE of parents and children, suggesting the involvement of one or more genes (Sherrill et al., 1994). However, agreement on the model of inheritance is lacking. Linkage of loci for total serum IgE and BHR to chromosome 5q has been reported (Sherrill et al., 1994). Mapping of this area of the chromosome will be important for further progress. Total serum IgE appears to be under strong genetic control (Bonini et al., 1994), even in the presence of

environmental factors such as smoking. A gene for IgE response with maternal inheritance was identified at chromosome 11q (Cookson et al., 1989). High levels of IgE in cord blood appear to be a strong indicator of subsequent development of atopic disease.

The genetic factors that determine the specificity of the IgE-mediated response are thought to be independent of those governing total serum IgE and may be linked to the human leucocyte antigen (HLA) complex (Sibbald, 1997). Products of HLA Class II genes are involved in allergen presentation by antigen-presenting cells. HLA Class II genes are highly polymorphic. Different HLA genes represent risk factors for the development of asthma associated with sensitization to allergens. Increased responsiveness to ragweed antigen (Ra5) was found to be associated with HLADR2/DW2, and response to ryegrass (Lol pI and Lol pII) with HLADR23 and DR5 (Marsh, 1990). Environmental factors, such as the quality, intensity, route and duration of allergen exposure appear to be more relevant than genetic factors in causing allergic reaction to specific allergens (Bonini et al., 1994).

Twin studies have suggested polyfactorial control of allergy variables such as serum levels of total IgE and IgG4, mediator release from inflammatory cells, and target organ response. Clinical data from 32 monozygotic and 71 dizygotic twin pairs yielded a concordance for allergic disease of 50.0% of monozygotic pairs (16/32) and 35.2% of dizygotic pairs (25/71). The difference was not statistically significant (Cockcroft, 1988). Histamine releasibility from mast cells and basophils is a crucial event in allergic disorders. In twin studies, this event (which is related to the quantity of IgE present on the cells) was shown to be under genetic control (Bonini et al., 1994).

Development of respiratory allergies to small relative molecular mass chemicals, i.e., relative molecular mass less than 5000, such as isocyanates and acid anhydrides has not been found to be associated with atopy (Chan-Yeung, 1995), although atopy has been shown to be a risk factor for development of respiratory symptoms to some chemical allergens, such as hexachloroplatinate (Dally et al., 1980).

Regarding low molecular mass, or chemical allergens, an association between sensitization to acid anhydrides and HLA-DR3 haplotype has been reported (Young et al., 1993). An association of HLA class II alleles and isocyanate asthma was detected (Bignon et

al., 1994). Twenty-eight patients with isocyanate-induced asthma (as documented by positive inhalation challenge) were compared with 16 exposed individuals with no history of the disease. HLA DQB1*0503 and allelic combination DQB1*0201/0301 were associated with susceptibility to asthma. Conversely, allele DQB1*0501 and the DQA1*0101-DQB1*0501-DR1 haplotype conferred protection in exposed healthy subjects. No significant difference was detected in the distribution of HLA Class II alleles and/or haplotypes among the immediate, late or dual responders to TDI. These results are consistent with the hypothesis that immune mechanisms are involved in isocyanate asthma and that specific genetic factors may increase or decrease the risk of development of isocyanate asthma in exposed individuals.

3.4.1.3 Other genetic factors

Another factor that may contribute to susceptibility, or resistance, to sensitization relates to genes that control production of IL-4, a pleotropic cytokine that influences the development of both Th- and B-lymphocytes, the induction of Class II MHC antigens and immunoglobulin class switching from IgM to IgE. Genes for IL-3, IL-4, IL-5 and GM-CSF have been identified on chromosome 5 (Van Lee Uwen et al., 1989). The IL-4 gene, as well as genes that regulate its expression, appear to be prime candidates for predisposition to atopy since there are reports that cells isolated from atopic individuals have the ability to overexpress the IL-4 gene relative to those from non-atopic individuals. In addition, the human IL-4 proximal promoter exists in multiple allelic forms, with one of the alleles having a markedly enhanced promoter activity (Song et al., 1996b). This finding suggests a gene target to screen for genetic predisposition for atopy.

3.4.2 Tolerance

Allergenicity of a given compound may be strongly reduced in individuals who previously developed immunological tolerance. This has been frequently seen when the primary contacts with the allergen were at mucosal surfaces, e.g., by its presence in food. Principles and mechanisms of immunological hyporesponsiveness and tolerance have been dealt with in detail above (see section 1.5).

3.4.2.1 *Orally induced flare-up reactions and desensitization*

Strong and long-lasting oral tolerance can only be achieved in naive individuals, i.e., those who have not been previously exposed to the antigen via the skin. In mice, a single feed of ovalbumin was reported to fully suppress subsequent systemic immune responses, with this state of tolerance persisting for up to 2 years. In contrast, tolerance is hard to induce in primed animals but partial and transient unresponsiveness ("desensitization") may develop after prolonged feeding of the antigen. Similar results have been obtained in guinea-pigs with various chemical allergens, including dinitrochlorobenzene (DNCB) (Polak, 1980), nickel (van Hoogstraten, 1994) and amlexanol (Hariya et al., 1994). Unfortunately, essentially similar results have been obtained in clinical trials aiming at the treatment of autoimmune diseases, e.g., rheumatoid arthritis and multiple sclerosis, by oral administration of putative autoantigens (Weiner et al., 1994). Another problem with oral tolerance induction in previously sensitized individuals arises from the tendency of former inflammatory sites to re-inflame ("flare-up reactions"). These reactions are likely to be due to allergen-specific effector T-cells, which can persist for periods of several months at former inflammatory sites (Scheper et al., 1983).

The differences between immunological responses in naive and primed individuals may reflect changes in expression of cellular adhesion/homing molecules and lymphocyte maturation. A qualitative distinction exists between (difficult to stimulate/afferently acting) naive and (easy to stimulate/efferently acting) effector/memory cells. In contrast to naive lymphocytes, which only are activated by allergen (modified self constituents) if presented by professional dendritic, e.g., Langerhans cells, their progeny, known as effector/memory lymphocytes, can also be stimulated by other cell types presenting allergen-modified MHC class II-molecules, e.g., monocytes, endothelial cells and B-cells. Clearly, effector/memory cells display increased numbers of cellular adhesion molecules (CAMs), allowing for more promiscuous cellular interactions. Amongst these, the most prominent CAMs are the CD28 and LFA-1 molecules, with B7.1 and B7.2 and ICAM-1 as their respective ligands on APC. In addition, priming of T-cells leads to the loss of homing receptors, such as L-selectin, which facilitate interactions with high endothelial venules in peripheral lymph nodes. Apparently, after sensitization, T-cells are less capable of recirculating through the lymphoid organs, but gain ability to migrate into the peripheral tissues. Interactions with endothelia within

inflamed skin are facilitated by the enhanced expression of CAMs, such as the cutaneous lymphocyte-associated antigen CLA, and effector/memory T-cells largely distribute over the peripheral tissues, where conditions may be insufficient to convey effective tolerogenic signals.

3.4.2.2 *Non-specific and specific mechanisms of unresponsiveness*

A preliminary factor contributing to non-responsiveness and/or lack of hypersensitivity reactions at mucosal surfaces is the epithelial barrier function, preventing entry of potentially harmful allergens. Obviously, from an immunological point of view, this is a "null-event", and does not have implications to subsequent encounters with the same allergen. TGFβ, a cytokine locally produced by epithelial cells and immunocytes, plays a pivotal role in maintaining epithelial barrier integrity. Importantly, the same cytokine also has broad nonspecific immunosuppressive functions, e.g., by antagonizing phagocytic effector cell functions of pulmonary alveolar macrophages. Similarly, other immunosuppressive cytokines may be locally released from epithelial cells and may act in concert with TGFβ to down-regulate immune effector functions, such as epithelial cell-derived P15E-related factors which show sequence homology with retroviral envelope proteins (Oostendorp et al., 1993).

In contrast, specific immunological tolerance depends on decreased responsiveness of specific B- or T- cells, or release of immunosuppressive mediators from these cells after specific challenge. So far, no methods of permanent desensitization have been devised. Nevertheless, how T-cells specifically recognize distinct allergens, and how these and other inflammatory cells interact to generate inflammation, is beginning to be understood. Exposure to high doses of antigens may induce clonal deletion or anergy of specific B- or T-cells by induction of apoptosis or antigen-receptor down-regulation (Jones et al., 1990; Schönrich et al., 1991; Ohashi et al., 1991; Melamed & Friedman, 1993).

As IL-4 and IL-13 direct IgE isotype switching, one way to intervene in allergen-specific IgE synthesis and to inhibit or prevent IgE-mediated allergic disease is to inhibit IL-4 and IL-13 production by allergen-specific Th2-cells. In addition to TCR engagement by peptide MHC complexes, optimal T-cell activation and proliferation generally requires co-stimulatory signals provided by interaction

between CD28 or CTLA-4 on T-cells and their ligands CD80 or CD86 on professional APC. Ligation of the TCR in the absence of these co-stimulatory signals can result in T-cell non-responsiveness. Human $CD4^+$ Th2 clones specific for the house dust mite allergen *Der p I* can be rendered non-responsive to subsequent *Der p I* challenges by incubating them with *Der p I*-derived peptides, representing the relevant minimal T-cell activation inducing epitopes, in the absence of professional APC (Yssel et al., 1994). The mechanisms underlying this T-cell unresponsiveness have not yet been determined. Although these cells cannot be activated through their TCR, they proliferate well in response to IL-2 or following activation by Ca^{++} ionophore and TPA, suggesting that TCR activation or signalling pathways immediately downstream of the TCR are disturbed.

This type of tolerance is generally short-lasting, since (functionally) deleted lymphocytes are gradually replenished by newly arising clones in the bone marrow and thymus and, in experimental animal models, cannot be transferred to naive recipients, since these still contain a fully functional repertoire, compensating for any missing clones. On the other hand, mucosal contacts of naive individuals with relatively low amounts of antigens, such as can be the case with environmental or occupational exposure to chemical sensitizers, frequently induce a long-lasting state of specific tolerance. Transfer of lymphoid cells, in particular T-cells, from orally tolerized animals to syngeneic naive recipients prevents their capacity to subsequently mount immune responses to the same allergen, revealing the existence of so-called T-regulator or suppressor cells (Polak et al., 1980; van Hoogstraten et al., 1992, 1994; Weiner et al., 1994).

Although "professional" suppressor T-cells may not exist (Bloom et al., 1992; Arnon & Teitelbaum, 1993) available data support the development of specific "regulatory" T-cells that suppress distinct immune functions. Depending on the experimental models, such regulatory T-cells can belong to either or both the $CD4^+$ or $CD8^+$ subsets (Bloom et al., 1992). Evidence is accumulating that regulatory T-cells most often exert their role, after antigen-specific activation, by releasing distinct cytokines antagonizing distinct effector T-cell functions.

3.4.3 Underlying disease

There is ample evidence that underlying diseases are able to influence the susceptibility of individuals to develop allergy. Both the induction and the manifestation of allergy may be affected.

Conditions that promote sensitization include ongoing inflammatory reactions at the site of allergen contact. It has, for instance, been described that late-phase reactions of the respiratory tract and the associated state of hyperresponsiveness, may facilitate sensitization (priming) to other allergens (Connell, 1969). At skin sites, a pre-existing eczema provides a risk factor for acquiring contact sensitization. The most important factor here is probably the local disturbance of the skin barrier, allowing for an increased penetration of allergen. The fact that all components for an immune response (cytokines, T-cells) have already been attracted to the site of allergen contact may, however, additionally contribute to this increased risk for new sensitization.

The most important diseases affecting the hosts' immune responsiveness, and thus allergic responsiveness, include infectious disease, neoplastic disease and immune deficiencies. The relation between infection and the development of allergic disease is quite complex. On one hand, respiratory viral infections are believed to contribute to the exacerbation of asthmatic disease (Busse, 1990). However, from clinical and epidemiological studies it would appear that under certain conditions viral infections can also protect against asthma. These studies include the observation of incidental spontaneous remission of asthma during hepatitis, fever or measles, as well as the finding of a general inverse relationship between infections and asthma or atopy (Matricardi, 1997; Serafini, 1997). In line with such a "protective" role it is believed that natural infections during early childhood would prevent the development of atopic disease later on, presumably by activation of the Th1 lymphocytes through IFNγ (Serafini, 1997). Reduction in family size and increased hygiene could thus contribute to the increased frequency of atopic disease in developed countries. Interestingly, infectious diseases, which are known to be associated with a predominant Th2 immune responsivenesses, like parasite infections, do not seem to favour the development of atopic disease (Bell, 1996). In contrast, people suffering from severe parasite infection may have less severe reactions to other allergens, due to competition of IgE at the Fc epsilon receptor

level on mast cells. Also in HIV-positive patients, where Th2 responses may become dominant, no clear evidence has been obtained for enhanced atopic sensitization, although allergic manifestations are frequently observed in these patients.

Conditions that suppress allergic reactions have been extensively described, since contact sensitization has been applied as a method for immune status determination in different patient groups. It is a well-known fact that in clinical conditions associated with general immune suppression and anergy, such as malnutrition, immunosuppressive treatment, malignancies and severe physical trauma, Type IV reactivity to recall antigens as well as primary sensitization to contact allergens like dinitrochlorobenzene can be dramatically impaired.

Finally, it should be noted that certain immunological conditions, such as those found in some immunodeficiency diseases, e.g., in the Wiskott-Aldrich syndrome, may predispose for the development of atopic eczema. Atopic disease is also commonly seen in IgA deficiency.

3.4.4 Age

Childhood asthma is becoming more common and doubled in the United Kingdom, New Zealand and Australia between 1970 and 1990. Because of their greater activity and their developing lungs, children may be more susceptible to sensitization as well as to adverse effects of irritants (Zummo & Karol, 1996).

The ability to become sensitized to dinitrochlorobenzene has been shown to be largely unchanged with age. Patch testing with *Rhus* oleoresins in subjects with a history of poison ivy sensitization showed diminished responses in the elderly (Lejman et al., 1984). However, exposure differences as a function of age must always be considered (Menné & Wilkinson, 1995).

IgE levels change with age. Peak levels occur in the first or second decades of life. A longitudinal study of more than 2000 subjects conducted over a 20-year period found no gender difference in total IgE (Sherrill et al., 1994). Both sexes had their highest IgE levels as children. Levels fell gradually up to around age 40 and thereafter remained constant.

3.4.5 Diet

To explain the observed increase in incidence of allergy and asthma during the last two decades, it has been suggested that a change in host resistance to allergy may have occurred (Seaton et al., 1994). A change in the diet in several Western countries has been documented. Specifically, a 20–50% fall in consumption of fresh fruits and vegetables has been noted. Since these foods are sources of antioxidants such as vitamin C and β-carotene, decreased consumption, together with that of red meat and fresh fish, would mean less ubiquinone and fewer cofactors (such as zinc and copper) for antioxidant defence (see section 5.10).

3.4.6 Gender

In general, women appear to have greater immune capability than men (Menné & Wilkinson, 1995). Animal and human studies have indicated a greater incidence of autoimmune disease in women compared with men, as well as higher IgG and IgM levels. Women have also been reported to produce greater cell-mediated immune responses.

In a large, controlled study, men were found more susceptible to sensitization by dinitrochlorobenzene than women (Walker et al., 1967). However, women were more readily sensitized to *p*-aminodiphenyl aniline than were men (Walker et al., 1967). In these studies, the issue of previous exposure to the chemical, and therefore greater susceptibility, could not be dismissed. This factor may also explain greater female sensitization in clinical patch tests with nickel and cobalt. Male and female sensitization rates obtained by maximization testing were comparable (Leyden & Kligman, 1977).

In a study of the influence of sex hormones on sensitization, response to dinitrochlorobenzene was enhanced in women receiving oral contraceptive hormones (Rea, 1979)

4. CLINICAL ASPECTS OF THE MOST IMPORTANT ALLERGIC DISEASES

Allergic diseases give rise to symptoms in many different organ systems and involve many different medical disciplines. The most important allergic diseases comprise allergic contact dermatitis, atopic eczema, allergic rhinitis and conjunctivitis, asthma and food allergy, and autoimmune diseases associated with chemicals.

4.1 Clinical aspects of allergic contact dermatitis

4.1.1 Introduction

Like the mucous membranes and the gut, the skin is an advanced part of the immune system. Together with the skin barrier, the immune system defends the body surface against microorganisms. Skin contact with small molecules (haptens) tends to induce cellular-mediated contact sensitization. The consequence of this contact sensitization is allergic contact dermatitis. If the same molecules are given orally before cutaneous contact, they may induce persistent immunological tolerance. Allergic contact dermatitis is a common disease and the prevalence at any given time varies between 2–4% (Fig. 9, 10, 11). Allergic contact dermatitis of the hands has particularly important implications for society as prolonged sick leave is common.

Most contact allergens are small molecules with a relative molecular mass below 6000. Contact sensitization is not inborn but is always a consequence of earlier cutaneous contact. Contact sensitization is considered to be life-long, but might become weaker if exposure is avoided. Contact sensitized individuals are at risk of developing the skin disease allergic contact dermatitis if re-exposed to the specific chemical. The term dermatitis is used synonymously with eczema and describes either an acute skin disease with redness, oedema and vesicles (water blisters) or a more chronic type with hyperkeratosis, fissures and scaling. The most important differential diagnosis of contact dermatitis is psoriasis, dermatophytosis, and scabies. IgE-mediated immunological contact urticaria is covered briefly.

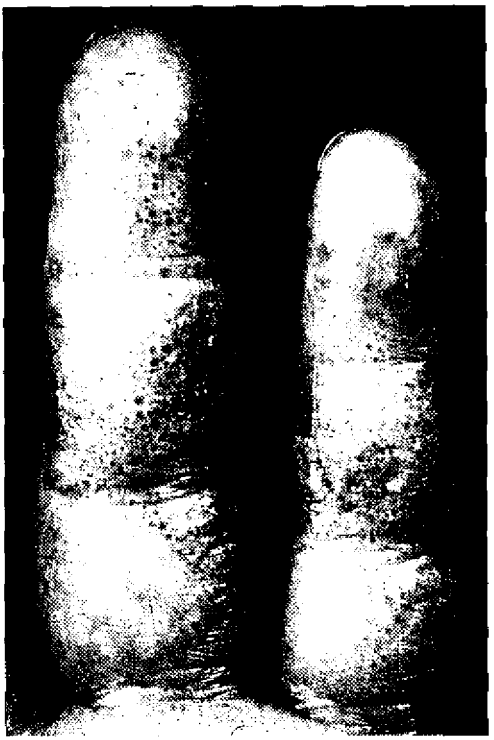

Fig. 9. Acute vesicular hand eczema in a hospital cleaner using rubber gloves. Patch testing showed a positive patch test to thiuram mix in the standard test series (Professor T. Menné).

4.1.2 Regional dermatitis

4.1.2.1 Hand eczema

Epidemiological studies including 20 000 individuals representing the general population showed a one-year prevalence of hand eczema of 10% (Meding, 1990); 20% of cases were classified as caused by contact allergy. The average duration was 12.8 years and 22% had periods of sick leave. Allergic contact dermatitis on the hands is therefore both a common disease and costly for the society, and it can imply significant socioeconomic consequences for the individual.

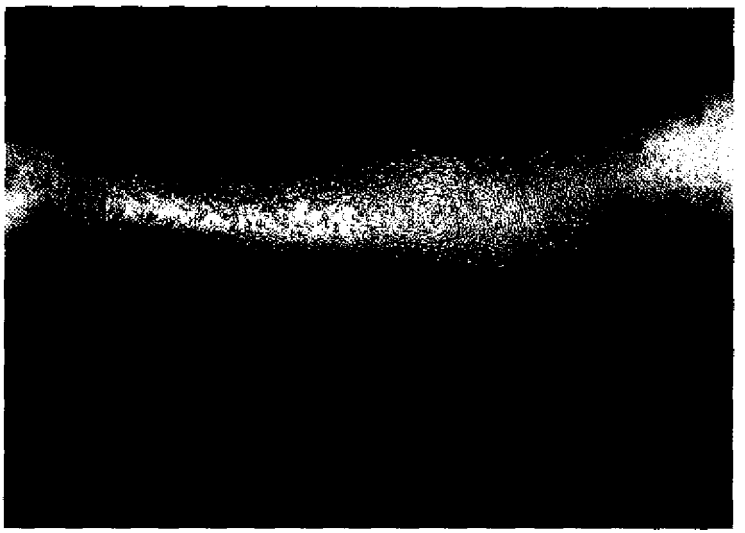

Fig. 10. Severe vesico-bullous acute allergic contact dermatitis in a worker exposed to epoxy resin-containing paint. Later patch testing showed a positive patch test to epoxy resin in the standard test series (Professor T. Menné).

In a survey of 564 cases of permanent disability caused by skin diseases, 222 of the 564 were caused by allergic contact dermatitis of the hands (Menné & Bachmann, 1979).

Frequent causes of allergic hand eczema are nickel, chromate, rubber additives (Fig. 9) preservatives, and fragrances (Menné & Maibach, 1993). It can be acute or chronic, and it can be located on either the dorsal or volar surfaces, or only on the fingers. It can also present as a diffuse dermatitis. Spread to the face and forearms is common.

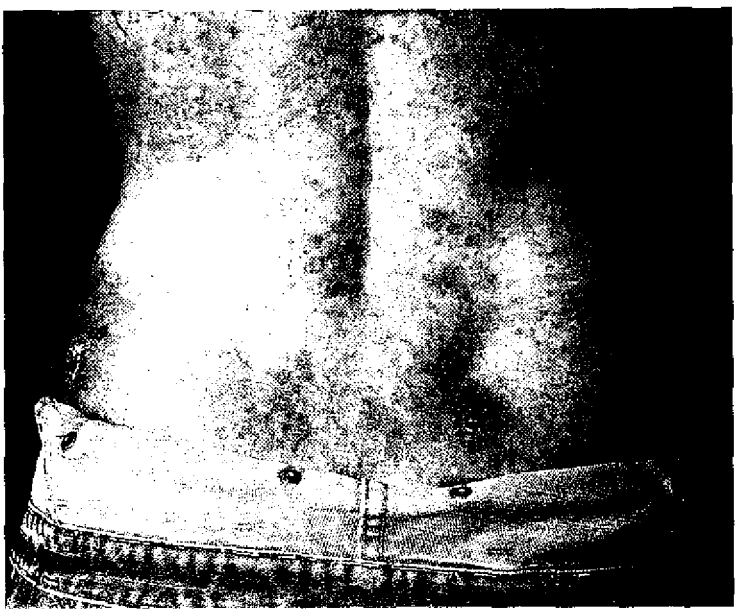

Fig. 11. Chronic generalized dermatitis caused by contact with nickel released from metal buttons on blue jeans. Patch testing showed a positive path test to nickel sulfate in the standard test series (Professor T. Menné).

4.1.2.2 Facial dermatitis

The face is second to the hands in the frequency of allergic contact dermatitis. The exposure can be direct to airborne allergens or indirect by contact with allergens transferred from the hands to the face. Acute allergic contact dermatitis in the face is often dramatic with severe oedema particularly of the eyelid regions. Chronic cases frequently show patchy dermatitis even if the allergen is uniformly spread on the face. Cosmetics, particularly fragrances, are the most common causes of facial dermatitis. Allergic contact dermatitis from medicaments (e.g., eye drops) and airborne occupational dermatitis are

seen. Severe oedema of the eyelids is a common pattern of plant dermatitis. Facial dermatitis causes distress to the individual because of pain, itching and disfiguration.

4.1.2.3 *Other types of dermatitis*

Stasis eczema and leg ulcers are a common disease among the elderly as complications of arterial and venous insufficiency and arteriosclerotic heart disease. Stasis eczema is a consequence of skin malnutrition and can be followed by chronic ulceration. Both entities are treated with topical medicaments such as emollients, steroids, antiseptics and antibiotics. These compounds generally do not have a high sensitizing capacity, but because they are used on damaged skin under occlusion for prolonged periods, contact sensitivity is not uncommon. Patch testing is routinely recommended in the work-up of leg ulcer and leg eczema patients. On average 50% of these patients have a positive patch test of actual or past relevance.

Intertriginous areas such as the axillae, external ear and perianal area are also frequent sites of primary sensitization from topically used medicaments and fragrances because of the natural occlusion.

Shoe dermatitis is located in the skin area in direct contact with the offending material, most frequently chromate-tanned leather, rubber and glues (Podmore, 1995).

Allergic contact dermatitis from textiles gives a characteristic clinical pattern with dermatitis in areas where textiles are in close contact with the skin on the trunk and extremities. The offending sensitizers are textile dyes and formaldehyde-releasing textile resins (Fowler et al., 1992).

4.1.3 *Special types of allergic contact reactions*

4.1.3.1 *Systemic contact dermatitis*

Systemic contact dermatitis can be seen in primary contact sensitized individuals when they are later exposed systemically to the chemical (or drug) either orally, intravenously, by inhalation or by transcutaneous absorption (Menné et al., 1994). The clinical symptoms can either be erythematous flare in areas with earlier contact dermatitis or a combination of symptoms including vesicular hand eczema and inflammatory skin reaction in the flexural and genital area. The

explanation for the flare reaction is probably specific sensitized lymphocytes persisting at the site of earlier allergic contact dermatitis areas. The mechanism behind the other type of reactions is speculative. Histologically this widespread reaction does not have the picture of contact dermatitis but frequently presents the picture of a lymphocytic vasculitis. The pathogenesis may be circulating immune complexes or a general reaction to released cytokines.

Systemic contact dermatitis is mostly seen in patients sensitized to topically used medicaments when they are systemically treated with the medicament or a cross-reacting medicament. Systemic contact dermatitis has been described for a large number of substances.

4.1.3.2 *Allergic photo-contact dermatitis*

Most substances that cause photo-contact allergy are halogenated aromatic hydrocarbons or sunscreen agents (White, 1995). The combination of light, predominantly ultraviolet (UV), and the specific chemical make the complete hapten. Clinical allergic photo-contact dermatitis will therefore present a dermatitis (often severe) in sun-exposed areas. This will typically be on the face, the forearms or the dorsal aspects of the hands. In cases where photo-contact allergy is suspected, patch testing is performed in duplicate and one site is exposed to UVA. If a positive patch test only appears on the UV-exposed site, photoallergy is likely.

4.1.3.3 *Non-eczematous reactions*

Allergic contact sensitivity in the skin can give rise to clinical reaction patterns other than dermatitis (Goh, 1995). These types of reactions are rare and to only a few chemicals. Even if these patients have a clinical reaction type other than dermatitis, they frequently have a positive patch test with the usual eczematous morphology. The most common types of non-eczematous contact reactions are erythema multiforme and lichen planus. Erythema multiforme-like reactions are caused by contact with plant allergens and the lichen planus type by contact with photographic chemicals.

4.1.3.4 *Allergic contact urticaria*

Contact urticaria is an immediate wheal reaction in the skin caused by vasodilatation, with subsequent oedema. Contact urticaria can either be allergic or non-allergic. In the non-allergic types

chemical causes a degranulation of the mast cells without involvement of the immune system. The allergic types are mediated via IgE bound to specific receptors on the mast cells and basophil lymphocytes in the skin. The clinical types are similar with urticaria localized at the contact site. Generalized anaphylactic reactions are rare. Both organic and inorganic substances have now been described as causes of allergic contact urticaria (Amin et al., 1996).

Contact urticaria is a frequent occupational disease among individuals handling animals and animal products. Allergic contact urticaria from proteins in rubber latex is a frequent and troublesome problem among workers, particularly health personnel, due to widespread use of rubber gloves (Taylor & Praditsuwan, 1996; NIOSH, 1997). A sensitization frequency of 2.8 to 10.7% has been reported in health personnel (Turjanmaa, 1996). Individuals occupationally sensitized to rubber latex proteins can develop anaphylactic reactions if exposed to rubber gloves as patients.

4.1.4 Allergic contact dermatitis as an occupational disease

Occupational skin diseases are defined as skin diseases either wholly or partly caused by the patient's occupation (Rycroft, 1995). The epidemiology of occupational skin diseases, which mostly comprise contact dermatitis of the hands, is known from population and cross-sectional studies of specific occupational groups. Information from centralized notification systems exists in some countries, but the quality of data can be questioned. In particular, the problem of under-reporting is difficult to quantify.

Skin diseases comprise between 20 and 40% of all occupational diseases, depending on geographical area. Approximately one-third is caused by allergic contact dermatitis and the rest mainly by irritant dermatitis. The principal occupational contact sensitizing chemicals are listed in Table 16. Not unexpectedly there is an overlap between exposure to chemicals in occupational and domestic environments (see section 4.1 and Table 19). The common high-risk occupations for allergic contact dermatitis, modified from Rycroft (1995), are given in Table 17 (Flyvholm et al., 1996). The prevalence of occupational contact dermatitis in these occupations varies from a few percent up to 15% (Rycroft, 1995).

Table 16. Main allergens related to occupational exposure (from Flyvholm et al., 1996)

Allergens	Sources of exposure
Acrylates	adhesives; bone cement; dental products; UV-curing lacquers, etc.
Amines	hardeners/curing agents for epoxy resin
Chromate	cement; leather; pigments
Cobalt	paints/lacquers
Colophony	adhesives; dental products; paper; tin solder, etc.
Epoxy resin	adhesives; paints; electric insulation
Formaldehyde	disinfectants; preservatives; laboratory chemicals; formaldehyde resins; funeral service
Formaldehyde releasers	metal working fluids; paints; adhesives
Formaldehyde resins	adhesives; paints/lacquers; impregnated textiles and paper; inks
Isocyanates	adhesives; paints; fillings; polyurethane foams
Medicaments	human and animal health care workers
Nickel	coins; nickel plated objects; contaminated oils, etc.
Paraphenylenediamine	hair dyes; rubber additive
Plastics/resins	adhesives; paints; fillings, containers, etc.
Preservatives	water-based products: metal working fluids; paints; adhesives; cleaning agents; cosmetics; polishes; skin protection creams; process water, etc.
Rubber additives	rubber gloves; rubber tubing; washers, etc.

Table 17. High-risk occupations for allergic contact dermatitis

Adhesives/plastics workers	Horticulturalists
Agriculturalists	Leather tanners
Cement casters	Painters
Construction workers	Pharmaceutical/chemical workers
Glass workers	Rubber workers
Graphic workers	Textile workers
Hairdressers	Tilers
Health care workers	Wood workers

It is difficult to give exact data concerning the costs of occupational allergic contact dermatitis, as the compensation regulation differs significantly from one country to another. However,

in the United Kingdom in 1996 it was estimated that 84 000 people had occupational contact dermatitis, and 132 000 working days were lost with a cost to employers of £20 million per year (HSE, 1996).

4.1.5 Diagnostic methods

4.1.5.1 Patch testing

The aim of patch testing is to diagnose contact sensitization to environmental chemicals. The patch test was introduced in 1896 by the Swiss dermatologist Jadahsson (Wahlberg, 1995). The technology is a biological test where contact allergy is proved by re-exposing the skin to the specific chemical under occlusion on a skin area of 0.5 cm^2 on the upper back for 2 days. A positive test is a reproduction of the clinical disease showing redness, infiltration and eventual vesicles. Standardization has taken place, particularly influenced by the Scandinavian and later the International Contact Dermatitis Research Group (ICDRG). The test should only be performed using standardized test materials. All patients are primarily tested with the Standard series including the most frequent sensitizing chemicals such as metals, preservatives, fragrances, rubber additives and topically used medicaments. Testing is frequently supplemented with substances present in the patient's private or occupational environments. Specially trained staff are necessary to obtain high quality outcome of the procedure.

Sensitization can be quantified according to the degree of positive patch test reaction (+ to +++), patch test concentration threshold defined by dilution series, and finally by the "Use test". In the latter test the individual is exposed to the chemical simulating normal use.

The outcome of patch testing defines whether contact allergy is present or not. Quantification of allergy combined with quantitative exposure data is the basis for individual and general risk assessment (Flyvholm et al., 1996).

The frequency of positive patch test reactions in the general population (Nielsen & Menné, 1992) and in eczema patients tested at a dermatological clinic in the same area of greater Copenhagen, Denmark, is shown in Table 18. The allergens causing positive reactions most frequently in eczema patients were nickel, fragrance mix, cobalt chloride, colophony and balsam of Peru. For the general

Table 18. Comparison of frequencies of positive patch test reactions in the general population (Nielsen & Menné, 1992) and in eczema patients at a dermatological clinic in the same area of greater Copenhagen in 1990[a]

Test substance	General population[b] (% positive of tested)			Dermatological clinic[c] (% positive of tested)		
	Men (n=279)	Women (n=288)	Total (n=567)	Men (n=262)	Women (n=410)	Total (n=672)
Potassium dichromate	0.7	0.3	0.5	1.9	2.7	2.4
Neomycin sulfate	0.0	0.0	0.0	3.4	3.7	3.6
Thiuram mixture	0.7	0.3	0.5	4.6	2.7	3.4
p-Phenylenediamine	0.0	0.0	0.0	1.9	2.7	2.4
Cobalt chloride	0.7	1.4	1.1	2.3	2.7	2.5
Benzocaine	–	–	NT	0.4	0.7	0.6
Caine® (local anaesthetic) mix	0.0	0.0	0.0	–	–	NT
Formaldehyde[d]	–	–	NT	1.9	2.2	2.1
Colophony	0.4	1.0	0.7	4.6	5.4	5.1
Quinoline mix	0.4	0.3	0.4	1.9	0.5	1.0
Balsam of Peru	0.7	1.4	1.1	3.4	5.4	4.6
PPD black rubber mix	0.4	0.0	0.2	1.2	0.0	0.5
Wool alcohols	0.4	0.0	0.2	1.2	1.7	1.5
Mercapto mix	0.7	0.0	0.4	1.2	0.2	0.6
Epoxy resin	0.4	0.7	0.5	0.8	0.2	0.5

Table 18 (contd).

Test substance	General population[b] (% positive of tested)			Dermatological clinic[c] (% positive of tested)		
	Men (n=279)	Women (n=288)	Total (n=567)	Men (n=262)	Women (n=410)	Total (n=672)
Paraben mix	0.4	0.3	0.4	0.8	0.2	0.5
p-tert-Butylphenol formaldehyde resin	1.1	1.0	1.1	0.4	1.2	0.9
Fragrance mix	1.1	1.0	1.1	6.1	7.1	6.7
Ethylenediamine dihydrochloride[e]	0.4	0.0	0.2	0.8	0.7	0.7
Quaternium 15	0.4	0.0	0.2	0.0	0.0	0.0
Nickel sulfate	2.2	11.1	6.7	4.2	16.1	11.5
MCI/MI (chloro-methyl- and methyl-isothiazolinone)	0.4	1.0	0.7	0.4	0.7	0.6
Mercaptobenzothiazole	0.4	0.0	0.2	1.2	0.2	0.6
Primin[f]	–	–	NT	0.4	1.5	1.0
Thiomersal[g]	3.6	3.1	3.4	–	–	NT
Carba mix[h]	0.7	0.0	0.4	–	–	NT

[a] Menné, unpublished (personal communication by T. Menné to the IPCS, 1997)
[b] Patch tested with the ready-to-apply TRUE test, Pharmacia (Sweden)
[c] Test substances from Hermal (Germany)
[d] Formaldehyde not included in TRUE test at the time of study
[e] Ethylenediamine dihydrochloride excluded from the European Standard series as of August 1992
[f] Primin not included in the TRUE test at the time of study
[g] Thiomersal not included in the European Standard series
[h] Carba mix excluded from the European Standard series as of January 1989

population, nickel and thiomersal were the most common causes of positive patch test reactions. Contact sensitization is generally more frequent among patients investigated at dermatological centres than it is in the general population.

4.1.5.2 In vitro *testing*

Several attempts have been made to develop *in vitro* methods for testing contact sensitization (von Blomberg et al., 1990; McMillan & Burrows, 1995). Yet, logistical and technical complexities, including allergen toxicities, and the generally low frequencies of circulating allergen-specific T-effector-memory cells, mean that currently available methods are not appropriate for routine clinical use. Nevertheless, *in vitro* tests, in particular the lymphocyte proliferation test (LPT), using patient-derived white blood cell samples, can be of considerable value in answering specific scientific questions, e.g., on the involvement of allergen-specific T-cells or on potential cross-reactivity patterns between allergens (Bruynzeel et al., 1985; Pistoor et al., 1995).

4.1.6 *Assessment of exposure*

To establish the diagnosis of allergic contact dermatitis, the outcome of patch testing needs to be combined with a detailed exposure history (Flyvholm et al., 1996). Both domestic and work-related exposures need to be elucidated. Factory visits are valuable but rarely done (Rycroft, 1995). The most common contact allergens are metals, preservatives, rubber additives, perfumes and medicaments. The main sources of exposure to contact allergens can be divided into groups of substances, products or use categories. Exposure to allergens occurs under many circumstances, such as occupational, domestic work, hobby and leisure time activities, topical medicaments, cosmetics, personal care products, clothing and shoes. Examples of such allergens are listed in Table 19 (Flyvholm et al., 1996). For examples of occupational exposure, see Table 16 (section 4.1.4). Exposure data can be obtained from databases, product labelling or chemical analysis, and by contact with manufacturers and suppliers. The prognosis for the individual patient depends upon the quality of diagnostic patch testing and the ability to prevent contact of the patient with the allergen.

Table 19. Main allergens related to non-occupational exposure

Allergens	Sources of exposure
Domestic work	
Chromium	leather; footwear
Colophony	shoe polish; crayons; plasticine; paper
Flowers/plants	gardening; house plants
Nickel	nickel-plated objects
Plastics/resins	adhesives; paints; containers
Preservatives	cleaning agents; polishes; personal care products
Rubber additives	gloves; other rubber objects
Wood	repairs; handicraft
Hobbies and leisure time activities	
Chromium	leather; footwear
Colophony	adhesive tapes; plasticine; paper; violin bow resin crayons; artists' paints; textiles
Dyes/pigments	gardening; house plants
Flowers/plants	textile resins; preservative in various products
Formaldehyde	nickel-plated objects
Nickel	adhesives; paints; containers
Plastics/resins	paints; personal care products
Preservatives	gloves; sports equipment
Rubber additives	handicrafts
Woods	
Cosmetics and personal care products	
Colophony	mascara.
Dyes	hair dyes; miscellaneous cosmetics
Fragrances	
Glyceryl thioglycolate	permanent waving
Lanolin	
Paraphenylenediamine	hair dyes; creams; lotions; shampoos; liquid soap, etc. (i.e., most cosmetic and personal care products)
Preservatives, e.g., formaldehyde releasers, isothiazolines parabens	
UV filters	sunscreens

Table 19 (contd).

Allergens	Sources of exposure
Topical medicaments	
Antibiotics	
Antihistamines	
Antimicrobials	
Balsams	
Benzocaine	
Colophony	
Ethylenediamine	
Formaldehyde releasers	
Lanolin	
Parabens	
Preservatives	
Tars	

4.1.7 Treatment and prevention of allergic contact dermatitis

The treatment of allergic contact dermatitis requires medical intervention. It usually involves the controlled use of emollients or corticosteroids as well as prevention of further exposure to the offending allergen (Wilkinson, 1995). A distinction is usually made between primary prevention, focusing on the induction of contact sensitization, and secondary prevention, focusing on the eliciting of contact sensitization. In many instances the preventive measures for the two different types overlap.

4.1.7.1 Primary prevention

In the 1960s an epidemic of contact dermatitis from dish-washing products occurred in Scandinavia. The epidemic was resolved by the concerted action of dermatologists and manufacturers. Extensive chemical analysis combined with animal predictive testing, identified highly sensitizing sultones to be present in some products (Magnusson & Gilje, 1973; Ritz et al., 1975). It was determined that these specific chemicals occurred as an impurity in the manufacturing process, when temperature control was not strictly maintained. The evaluation of the problem led to a solution, and there have been no recurrences.

There are examples of exposure to hapten concentrations being legally regulated in an attempt to prevent contact sensitization (Hjorth & Menné, 1990). There is a complex European Union regulation on cosmetic products, forbidding certain substances and regulating others, i.e., preservatives, by a concentration limit (Council of the European Communities, 1976).

Since the 1950s, chromate in cement has been know to be one of the main causes of allergic chromate dermatitis among construction workers. At the start of the 1980s the Scandinavian countries added ferrosulfate at a low concentration to cement to reduce the hexavalent chromate to trivalent chromate. The idea of this initiative was that the trivalent chromate is not absorbed, or only to a minor degree, through human skin, and therefore the risk of primary sensitization from this salt is significantly less than from hexavalent chromate. Epidemiological studies on construction sites performed at the beginning of the 1980s and at the end of the 1980s in Denmark, strongly suggest that this measure has been successful, as the frequency of allergic chromate dermatitis has been reduced in Denmark (Avnstorp, 1992).

Nickel is a common contact allergen on a global scale. This allergy is caused by intimate skin contact with metal alloys, releasing nickel when exposed to human sweat. Under simulated use conditions, some alloys release high amounts and other alloys low amounts of nickel (Lidén et al., 1996). Based on such research, some Scandinavian countries have introduced regulations and quality criteria for nickel alloys intended to be in prolonged skin contact. It is believed that such measures might reduce significantly the frequency of nickel allergy in the population. Regulation of nickel exposure along similar lines has been adopted within the European Union (Council of the European Communities, 1994).

In considering different glove materials to protect against skin irritation and mechanical skin damage, it should be noted that most small sensitizing chemicals rapidly penetrate most rubber and plastic gloves, and appropriate gloves should therefore be used (Estlander & Jolanki, 1988; Mellström et al., 1989; Roed-Petersen, 1989).

There is no method of predicting an individual propensity to contact sensitization to a given chemical. When patch testing with strong sensitizing chemicals is performed, active sensitization from the

test cannot completely be excluded. Pre-employment testing is therefore not a method of preventing contact sensitization.

4.1.7.2 Secondary prevention

The cornerstones of the secondary prevention of allergic contact dermatitis (elicitation of contact dermatitis) are based on sufficient diagnostic procedures and patient information systems. The availability of standardized patch test materials is essential. Furthermore, it is crucial that it is possible for the doctor to inform the patient where exposure to the specific allergen can be expected. Of course, it is even more crucial that the patient is able to understand and use the information over the following years to identify the allergen in the home and occupational environments. It seems obvious that this type of diagnostic follow-up will work, but it has only been evaluated in a limited number of studies. Edman (1988) found that the prognosis for patients sensitive to topical medicaments depended upon whether the patients were able to follow the doctor's advice on the occurrence of sensitizers in different products. Later studies have shown that patients with contact allergy to formaldehyde often continued to be exposed to formaldehyde (Cronin, 1991; Flyvholm & Menné, 1992). When a careful investigation was made, formaldehyde exposure could be demonstrated in nearly all the patients which seemed to be decisive for the prognosis of their hand eczema (Flyvholm, 1997).

4.1.7.3 Ways of preventing contact sensitization

The following ways of preventing contact sensitization have been suggested.

a) replacement of certain chemicals or particular products;

b) regulation of exposure (concentration) to sensitizing chemicals, either general or in specific products, or during particular work processes;

c) optimal diagnostic and information systems; education of either groups or individuals;

d) individual oriented preventive methods; gloves, barrier creams, protective clothing.

The problems of contact sensitization have been identified over many years, and different types of preventive measures have been tried. Some have been successful, but a number of chemicals still give problems to a significant number of people. Different strategies should be considered, whether it concerns common environmental chemicals or chemicals with rare specific exposures. Chemicals frequently used in both the domestic and occupational environment need to be regulated by society, either with suggestion of replacement or regulation of the exposure concentration. For rare chemicals it is often sufficient to focus on specific occupational processes and to educate the exposed individuals in no-touch techniques or introduce individually oriented preventive measures.

4.1.8 Information needed for a preventative programme

The prevention of allergic contact dermatitis should be based on preventing sensitization and, subsequently, on avoiding sufficient exposure to elicit a response in a person already sensitized. This requires information on the following aspects.

a) Occurrence of sensitizing substances

Products used at work or domestically should be labelled to indicate the presence of substances capable of causing sensitization and their concentrations, so that the user may take appropriate precautions.

If there are suitable alternatives there may be no need to use a sensitizing agent.

At present, the potential of new substances to cause sensitization is determined from the results of tests on animals or sometimes on humans (Rycroft, 1995), after databases have been searched for relevant published information. Structure–activity relationships should be assessed and may give valuable indications of sensitizing potential for substances of a similar structure to known contact allergens.

Comprehensive information about the composition of products and the allergenic activity of their ingredients should be collected in each country and be made available to health care professionals and users. This should include the results of surveys of standardized patch

testing of humans so that trends in allergic sensitization can be followed.

b) Avoiding or minimizing exposure

Induction of sensitization and eliciting an allergic disorder both follow dose–response relationships, albeit at very different concentrations.

It is important to minimize initial exposure to sensitizing agents by restricting their availability or, if they cannot be avoided, by minimizing exposure. Exposure can be minimized by ensuring adequate ventilation and using personal protective equipment appropriate to the work situation or in the home, e.g., gloves, masks, etc. (see also chapter 7).

4.2 Atopic eczema (atopic dermatitis)

4.2.1 Definition

Atopic eczema or atopic dermatitis is a chronic pruritic inflammatory skin disease characterized by a typical age-related distribution and skin morphology (Figs. 12, 13). The diagnosis of atopic eczema is based primarily on clinical grounds and the patient's history (Hanifin, 1983; Rajka, 1990). Onset at an early age, pruritus and excoriation, chronic or chronic relapsing course for more than 6 weeks, age-related eczematous morphology and distribution, as well as a positive family history for atopic diseases (allergic bronchial asthma, allergic rhinitis and conjunctivitis or atopic eczema), form the most striking criteria. Together with allergic rhinitis and conjunctivitis and bronchial asthma, atopic eczema forms the classical triad of atopic diseases (Rajka, 1990; Ruzicka et al., 1991). Atopy can be defined as "familial hypersensitivity of skin and mucous membranes against environmental substances associated with increased IgE production and/or nonspecific reactivity" (Ring, 1991). This underlines two components held to be responsible for induction of this disease. Although it is genetically determined, environmental influences may play a role. During the last century many synonyms for atopic eczema/atopic dermatitis have been evolved, e.g., neurodermatitis,

Fig. 12. Typical childhood atopic eczema affecting the face (Dr K. Brockow)

prurigo Besnier, endogenous eczema and diffuse neurodermatitis (Ring, 1991). The diagnosis of this skin disease is based on clinical criteria, family history and/or demonstration of IgE-mediated sensitization.

4.2.2 *Epidemiology of atopic eczema*

Atopic eczema is a common disease among children and adults. In the 1950s the frequency of eczema was estimated to be between 1.1 and 3.1% (Walker & Warin, 1956). In the 1980s and 1990s the frequency of atopic eczema was found to be up to 25% on the basis of questionnaires (Bakke et al., 1990) and up to 9.7% for dermatologically examined cohorts (Varjonen et al., 1992; Schäfer & Ring, 1997). Epidemiological studies on the prevalence of atopic eczema in Germany were conducted with questionnaire, physical and

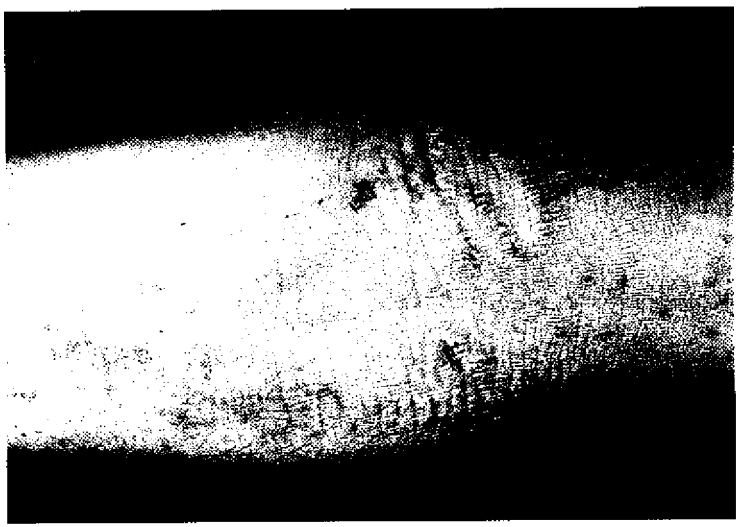

Fig. 13. Adult atopic eczema of the antecubital flexure showing excoriation and lichenification (Dr K. Brockow)

dermatological examination including allergy tests. In 1989–1991 8.3% of 988 Bavarian school children aged 5 to 6 years suffered from atopic eczema (Schäfer et al., 1994), and in a study comparing eastern and western German areas in 1991 atopic eczema was diagnosed in 12.9% of 1086 pre-school children (Ring et al., 1995; Krämer et al., 1996; Schäfer & Ring, 1997). In studies in the United Kingdom, Denmark and Switzerland, the same methodological analyses were applied for a longer time interval to obtain figures on the changes in frequency of atopic eczema. These studies showed a dramatic increase in the prevalence of atopic eczema (Schäfer & Ring, 1995). In the United Kingdom the figures for the prevalence of atopic eczema were 5.1% in 1946, 5.3% in 1964, 7.3% in 1958, and around 12% for 1970–1989. Similarly, in Denmark the prevalence in 1964–1969 was 3.2% compared with 11.2% for 1970–1979. In Switzerland there was an increase from 2.2% in 1968 to 2.8% in 1981.

4.2.3 Clinical manifestations and diagnostic criteria

4.2.3.1 Age-dependent clinical manifestations

In most patients with atopic eczema, the disease begins in infancy between 3 and 12 months of age (Hill & Sulzberger, 1935) as an erythematous, squamous or papulo-vesiculous inflammation, which may worsen to the point of exudation. It is often found on the face, the extremities (especially extensor aspects) and finally the trunk. Oozing and crusted lesions can often be found on the scalp (cradle cap). More and more, itching becomes an essential feature; the infant may be irritable, restless and tries to scratch the affected areas (after 3rd month of life). The course is chronically persistent or relapsing. Later, between 2 and 5 years, the appearance of the lesions changes. They become nummular and infiltrated. The localization changes and affects flexures of popliteal and antecubital fossae, the nape of the neck and the backs of the hands and feet. In severe cases there may be an involvement of the entire skin surface. Dry skin becomes another characteristic feature especially in the adult phase and creates itching followed by scratching. This may lead to severe excoriation with nodule formation and perpetuation of the inflammatory reaction ("Prurigo Besnier"). Chronic inflammation produces thickening (lichenification) of the skin, especially in flexural regions.

4.2.3.2 Diagnosis of atopic eczema

Many diagnostic systems have tried to collect reliable criteria for this disease. The features listed by Hanifin & Rajka (1980) are those referred to most often in the literature. A combination of a number of major and minor criteria allows the establishment of the diagnosis. A more simple selection of criteria for practical purposes has been proposed (Ring, 1991). Williams et al. (1994a) proposed a new arrangement of diagnostic criteria, primarily for epidemiological studies. However, it must be kept in mind that all these diagnostic systems have their drawbacks in this heterogenous disease. Clinical criteria, as well as the patient's history and presence of IgE-mediated sensitizations must be considered together and are the mainstay for establishing the diagnosis. However, minimal forms exist and sometimes do not meet the required criteria. Papular or nodular variants as well as localized forms (e.g., exfoliating cheilitis, infra-auricular rhagades, nipple eczema, finger pad or toe eczema) constitute minimal expressions of this disease (Wüthrich, 1991). Typical eczematous lesions may not only be triggered by IgE-mediated allergic

reactions in patients with a positive family history of atopy, but can also be triggered by food additives. In most patients, establishing the diagnosis is not too difficult. In selected cases, clinical findings, history and IgE-mediated sensitization have to be regarded critically and all important differential diagnoses have to be ruled out thoroughly.

4.2.3.3 *Stigmata of the atopic constitution*

The diagnosis of atopic eczema often depends on further additional features. Stigmata of atopic constitution are prevalent in many patients with atopic eczema, although they are not specific for this disease. Dry skin, hyperlinearity of palms and soles, intraorbital fold, white dermographism, facial pallor, orbital darkening, low hairline and thinning of the lateral portions of the eyebrow are found more often in this group of patients (Przybilla, 1991). They are typical constitutional markers, which may add another clue in establishing the diagnosis (Ring, 1988).

4.2.3.4 *Prognosis*

Variability and chronic relapses are characteristics of the course of atopic eczema. Atopic eczema most frequently begins during infancy (Hanifin, 1983; Rajka, 1990). In about two-thirds of infants with atopic eczema, the disease clears during childhood. In the remaining patients it persists into adult life. Minimal forms and stigmata of the disease often remain throughout life (Vickers, 1991). Sometimes atopic eczema starts only in adulthood. A definite prognosis about the course of an individual patient cannot be made; there is controversy about prognostic factors (Vickers, 1991).

4.2.4 *Etiology*

The manifestation of atopic eczema is subject to a multifactorial genetic predisposition as well as to environmental provocation factors.

4.2.4.1 *Genetic influence*

There is no doubt about the existence of a genetic component favouring the manifestation of atopic eczema (Schnyder, 1960; Küster et al., 1990). Twin studies show a concordance in homozygous twins of 83 and 86%, compared to 28 and 21% in heterozygous twins (Niermann, 1964; Schultz-Larsen, 1991). The chance of developing

atopic eczema depends on the family history of atopy. Whereas about 10–15% of children without a family history of atopy develop atopic eczema, with a positive history of one parent the risk rises to 25–30% and, with a positive history of both parents, to 50–75% (Schultz-Larsen et al., 1986; Björksten & Kjellman, 1987).

4.2.5 Environmental provocation factors

The activity of atopic eczema can be influenced by a large number of environmental provocation factors (Table 20). These can either act specifically in the sense of individual hypersensitivity, primarily IgE-mediated allergy or, more often, as unspecific provocation factors irritating the skin or affecting emotional status. The question of the possible involvement of environmental atmospheric pollution in the increase in the prevalence of atopic eczema remains controversial (see section 3.3.2).

Table 20. Important environmental provocation factors in atopic eczema (adapted from Ring et al., 1996)

Unspecific provocation factors:
Irritants
Microbial skin colonization or infection
e.g., *Staphylococcus aureus*
Pityrosporum ovale
Herpes simplex (Eczema herpeticum)
Psychological stress, emotional factors
Specific provocation factors (individual hypersensitivity):
IgE-mediated allergy
e.g., Food
House dust mite
Animal dander
Pollen
Microbial colonisation?
Contact allergy
Pseudo-allergy (idiosyncrasy) and intolerance
e.g., preservatives in foods
citrus fruits

4.2.6 Pathophysiology

Although knowledge concerning components of the immune system and inflammatory responses in patients with atopic eczema has increased widely in recent decades, the pathophysiology of atopic eczema also remains controversial (Marchionini, 1960; Rajka, 1990, 1996).

4.2.6.1 Dry skin

Dry or rough skin is a major feature of skin alteration in patients with atopic eczema. Although a number of studies have investigated the pathophysiology of dry skin, there is no consensus (Melnik & Plewig, 1991; Lindskov & Hølmer, 1992). An attractive hypothesis is that even clinically non-inflamed "dry skin" shows histologically a mild inflammatory infiltrate, and this is supported by skin biopsies in atopic patients (Uehara, 1991). There seems to be an intimate relation between dry skin, irritability and itch (Rajka, 1990; Ruzicka et al., 1991).

4.2.6.2 Autonomic dysregulation

In addition to immunological abnormalities, signs of dysregulation of the autonomic nervous system have been described (Szentivanyi, 1968; Ring et al., 1988; Ring & Thomas, 1989; Hanifin, 1993). Elevated phosphodiesterase activity in mononuclear leukocytes seems to correlate with increased IgE production and vasoactive mediator secretion (Butler et al., 1983; Cooper et al., 1985).

4.2.6.3 Cellular immunodeficiency

First described by Kaposi in 1895, patients with atopic eczema are more susceptible to infection with viruses (e.g., *Herpes simplex*, Human papilloma) and bacteria (especially *Staphylococcus aureus*) (Kaposi, 1895). Earlier reports about decreased frequencies of allergic contact sensitization in atopic eczema are contradicted by others who claim that the tendency to develop contact allergy is increased (Rajka, 1990). However, Enders et al. (1988) reported that the prevalence of positive patch test reactions for contact allergy in patients with atopic eczema was almost equal to that of patients with allergic contact dermatitis.

4.2.6.4 Increased IgE production

Serum IgE levels are elevated in the majority of patients with atopic eczema (Ogawa et al., 1971). They tend to correlate with the extent and severity of the disease (Johansson & Juhlin, 1970; Wüthrich, 1975). Specific antibodies can be measured against common environmental allergens (Rajka, 1990; Ruzicka et al., 1991). Although often the clinical significance of these antibodies is lacking, in some patients eczematous skin responses can be provoked by aeroallergens (grass pollen, house dust mite or animal dander), a procedure that has been called "atopy patch test" (Reitamo et al., 1986; Adinoff et al., 1988; Ramb-Lindhauer et al., 1990; Ring et al., 1991a,b; Platts-Mills et al. 1991; Vieluf et al., 1993). IgE antibodies to foods are frequently found in patients and may induce urticaria as well as eczematous reactions. Well-controlled clinical trials showed that in a high number of patients with atopic eczema, skin lesions were exacerbated after specific oral provocation with certain foods in double-blind studies (Sampson & Albergo, 1984). Apart from aeroallergens and foods, microbial allergens (*Staphylococcus aureus, Pityrosporum ovale*) might play a role. Chronic colonization of atopic skin could provide a continuing cause of allergen stimulation (Leyden et al., 1974; Ring et al., 1992, 1995; Neuber et al., 1995; Kröger et al., 1995). After allergen stimulation of IgE-bearing mast cells or basophils, the released vasoactive mediators (such as histamine, eicanosoids, etc.) might induce itching, and also eczema via a late-phase reaction (Dorsch & Ring, 1981). Langerhans cells in the epidermis express high affinity receptors for IgE as well as CD23 and IgE binding-protein (Bieber & Ring, 1992). Allergen contact might result in the generation of Th2-helper cells, a subset producing IL-4 and IL-5, thereby maintaining the allergic inflammation. Also other cell types might be involved in the inflammatory process; lymphocytes might act directly through cytokines, and eosinophils through release of pro-inflammatory mediators (Jakob et al., 1991; Kapp, 1995).

4.2.6.5 Psychosomatic aspects

It is well known from clinical experience that psychological and emotional factors can greatly influence the clinical course of this skin disease (Borelli & Schnyder, 1962; Jordan & Whitlock, 1972, 1974; Ring et al., 1986; Cotterill, 1991). There is no convincing evidence that psychological factors *per se* are the primary cause for atopic eczema; however, it is clear that psychological factors may influence

existing eczematous lesions or even trigger new exacerbations of eczema in many patients (Rajka, 1990; Ruzicka et al., 1991). For children, the family situation, e.g., the interaction between parents and the affected child, seems to be of particular importance (Ring et al., 1976; Niebel, 1995).

4.2.7 Diagnostic approach

In atopic eczema diagnosis not only comprises the identification of the disease but should also focus on individual provoking factors able to trigger disease activity (Ring et al., 1991a,b; Morren et al., 1994). Each patient may be susceptible to an individual set of provocation factors. Often, exacerbations can be prevented or the skin condition can be directly improved by avoidance of these factors (Ring et al., 1996). Diagnostic procedures used are intended to reveal provocation factors for the individual patient. Specific provocation of atopic eczema often is the result of an individual hypersensitivity. Although diagnostic tests normally differ from the natural exposure with allergens, they provide useful information in the hands of a trained allergist (Ring, 1988). Allergy diagnosis is based on the four foundations: the patient's history, skin tests, *in vitro* (laboratory) tests and provocation tests.

4.2.7.1 Medical history

The patient's history forms the backbone of allergy diagnosis. Often the patient notices associations between disease activity and specific conditions or actions (e.g., intake of foods, seasonal or daily variations, contact with animals, heavy pollen emission). These observations are very valuable in revealing individual provocation factors. On the other hand, positive allergy tests must always be verified for their clinical significance for the patient's disease by comparing them with the history.

4.2.7.2 Skin tests

Skin test methods are divided into percutaneous (skin-prick, intradermal) tests and epicutaneous (patch) tests (American Medical Association, 1987a). Percutaneous tests search for immediate-type IgE-mediated hypersensitivity and are especially indicated in atopic eczema. The skin-prick test (prick puncture test) has gained the widest acceptance because of its high convenience and safety (Dreborg, 1989). A drop of the test extract is placed on the volar surface of the

forearm and the solution is introduced into the epidermis with a disposable hypodermic needle. After 15 min the reactions are graded in relation to the erythema and wheal that are induced. In intradermal testing 0.02 to 0.05 ml of the test extract is injected intradermally with a syringe. Scratch tests (applying the extract to a superficial scratch) and rub tests (rubbing of the skin with native allergen) are other variants applied only for special indications. Because of the danger of producing anaphylactic reactions these tests should be performed only by trained allergists with experience in emergency treatment. In patients with atopic dermatitis, percutaneous tests are widely used for the detection of hypersensitivity against environmental aeroallergens and foods (Ring, 1988).

Epicutaneous tests primarily focus on the detection of contact allergy by cell-mediated immunity. The extract is put in an aluminum chamber and fixed onto the skin of the patient for 48 h. The test reaction is graded after 48 and 72 h. An eczematous response is regarded as positive. Since it has been shown that in patients with atopic eczema, eczematous skin responses can be elicited by epidermal application with aeroallergens (especially the house dust mite), epicutaneous testing with the atopy-patch test is gaining wide acceptance (Adinoff et al., 1988; Vieluf et al., 1990; Darsow et al., 1995). Although the definite mechanism is still unknown, this test might fill the gap between IgE-mediated hypersensitivity and an eczematous response.

4.2.7.3 *Laboratory tests*

In the serum of patients with atopic eczema, hypersensitivity can be detected by laboratory methods. In atopic eczema the most important hypersensitivity reactions are thought to be IgE-mediated. IgE antibodies can be determined by binding to an allergen in a solid phase and radioactive, enzymatic or fluorometric labelling (Ring, 1988). Specific antibodies against environmental allergens are detected by the RAST (Radio-Allergo-Sorbent Test) and expressed semi-quantitatively in different classes. Positive reactions must be interpreted with regard to their clinical relevance (Pastorello et al., 1989).

4.2.7.4 *Provocation tests*

Oral provocation tests and elimination diets are often necessary for the evaluation of the clinical relevance of a suspected food hypersensitivity (Przybilla & Ring, 1990). Also, allergy-like symptoms to food additives, medications, etc., may be produced by non-IgE-mediated mechanisms ("pseudo-allergy") (Vieluf et al., 1990). In these cases elimination diets and provocation tests are performed. Foods unlikely to produce adverse reactions can be screened by elimination diets or open challenges. Oral provocation by double-blind placebo-controlled food challenges is regarded as the "gold" standard for the diagnosis of food allergies (Sampson, 1983; Bruijnzeel-Koomen et al., 1995). However, there are pitfalls and problems with this procedure (Bindslev-Jensen, 1994a).

4.2.8 *Therapeutic considerations*

The disease can be effectively controlled by a combination of avoidance procedures, basic dermatological therapy and anti-inflammatory therapy for exacerbations (Przybilla et al., 1994; Ring et al., 1996). However, the patient has to accept that there is no simple therapy allowing permanent cure. The integration and active cooperation of the patient in the therapeutic concept ("patient management") is a prerequisite for an effective therapy. In atopic eczema, diagnostic and therapeutic approaches are intimately connected.

4.2.8.1 *Avoidance of provocation factors*

During the first year of life food allergies are frequent. Later, sensitization to aeroallergens becomes more important (Guillet & Guillet, 1992). Food allergies were found in 63% of children with extensive atopic eczema (Sampson, 1982).

Eggs, cow's milk, wheat, seafood and nuts present the most important food allergens. Citrus fruits and preservatives in foods often affect patients via non-allergic mechanisms (Przybilla & Ring, 1990). Individual provocation factors (hypersensitivity) have to be revealed by allergological diagnostic procedures. Therapy consists in the elimination of the relevant allergens from the diet. If extensive interventions are planned, the help of a dietitian is needed. Controversy exists about the value of prophylactic dietary manipulations. Exclusive breast feeding for six months, maternal avoidance of allergens during

lactation, and delay of solid food feeding seem to have a protective influence in postponing or avoiding atopic eczema (Kajosaari & Saarinen, 1983; Arshad et al., 1992; Saarinen & Kajorsaari, 1995).

Sensitization to aeroallergens is frequently found in older children and in adults. As shown by atopy patch tests, in some patients direct contact with house dust mite allergen, animal dander and pollen on intact skin results in eczematous skin lesions (Ring et al., 1991a,b; Darsow et al., 1995). In the case of a suspected allergy to house dust mites, reduction procedures should include encasing of bedding with impermeable synthetic material and removal of carpets and upholstered furniture (Platts-Mills & Chapman, 1987; Platts-Mills et al., 1991; Lau et al., 1995). When allergy to animal dander is shown, contact with the animal must be avoided. In case of exacerbation of atopic eczema due to aeroallergens, rehabilitation in aeroallergen-poor climates (sea level or high altitude mountains) has been recommended (Borelli, 1981). In patients with severe atopic eczema without adequate improvement of skin condition despite therapy, additional contact allergy should be suspected and excluded by epicutaneous (patch) testing.

Furthermore, there are various nonspecific provocation factors influencing the disease activity in patients with atopic eczema. The skin of these patients is highly susceptible to irritants, such as wool, coarse fabrics, soap, detergents, frequent bathing, disinfectants, wet working conditions and others. Patients need to be educated about avoidance of these factors (Ring et al., 1996).

Chronic microbial colonization of the skin (e.g., *Staphylococcus aureus, Pityrosporum ovale*) and superinfection are possible additional provocation factors and should be treated (Cooper, 1994). Psychological factors such as stress are well-known triggering factors for a subgroup of patients. In these patients, psychosomatic intervention has been proven successful and psychosomatic approaches should be supported (Cotterill, 1991; Ehlers et al., 1995).

4.2.8.2 *Basic dermatological therapy*

In patients with atopic eczema there is a defective skin barrier against exogenous substances (Ruzicka et al., 1991; Schöpf et al., 1995). Regular basic therapy with emollients with or without addition

of moisturizers and bath oils is needed for the treatment of the irritable dry skin to prevent the itch/scratch cycle.

4.2.8.3 *Anti-inflammatory therapy*

Recurrent relapses are a characteristic feature of atopic eczema. Anti-inflammatory therapy of exacerbations is aimed to control effectively disease activity and permit a return to basic dermatological therapy as soon as possible. Topical corticosteroids are the drugs of choice for acute exacerbations.

4.2.9 Conclusion

Atopic eczema is one of the most common skin diseases in many countries of the world with an increasing prevalence. Prevalence rates range between 10 and 20% of school children. Owing to the immense suffering caused by the skin disfigurement and the often unbearable itching, as well as the large number of people affected, it presents a major health problem. The role of allergy in this skin disease has been controversial but it has been shown that in the majority of patients, allergic reactions — preferentially by IgE-mediated sensitization — seem to play a clinically relevant role in eliciting and maintaining eczematous skin lesions.

4.3 Allergic rhinitis and conjunctivitis

4.3.1 *Introduction*

Allergic reactions can occur in the respiratory tract and ocular conjunctiva. In the respiratory tract allergic reactions occur in:

a) the upper respiratory tract predominantly involving the nose – *rhinitis*;
b) bronchial airways – *asthma*;
c) gas exchanging parts of the lung – *extrinsic allergic alveolitis*.

Allergic reactions in the nose and airways are characterized by mucosal infiltration with eosinophils and T-lymphocytes, diseases now considered to be the manifestation of a local Th2-lymphocyte-dependant eosinophilic inflammation. In contrast, extrinsic alveolitis is characterized by granulomata and mononuclear cell inflammation within alveoli, centred upon bronchioles; the disease is considered

to be the manifestation of a local Th1-dependant granulomatous inflammation. Both patterns of reaction are predominantly induced by agents suspended in the air, such as dust or fume particulates, aerosol droplets or vapour, inhaled into the respiratory tract. In general larger particles will be deposited and soluble chemicals dissolved in the upper respiratory tract; smaller particles (<5 μm aerodynamic diameter) and insoluble chemicals can penetrate into the gas exchanging parts of the lung.

4.3.2 Definition

Allergic rhinitis and conjunctivitis are common allergic inflammatory conditions induced by hypersensitivity to environmental allergens affecting the nasal (rhinitis) and/or conjunctival mucosa (conjunctivitis) (Mygind, 1986, 1989). Rhinitis, characterized by one or more of the symptoms of nasal congestion, rhinorrhea, sneezing and itching, is defined as the inflammation of the lining of the nose (International Rhinitis Management Working Group, 1994). The symptoms of allergic conjunctivitis consist of redness, lachrymation, itching and burning of the conjunctiva (Ring, 1991). There is an increased likelihood of the development of asthma in these patients.

4.3.3 Clinical manifestations

4.3.3.1 *Seasonal allergic rhinitis and conjunctivitis (hay fever, pollinosis)*

Seasonal allergic rhinitis and conjunctivitis consists of paroxysms of sneezing, nasal itching, nasal congestion and rhinorrhea (Druce, 1993; Mygind, 1986). In severe cases the conjunctiva and mucous membranes of the Eustachian tube, middle ear and paranasal sinuses also may be involved. In these cases additional symptoms usually present with low-grade itching, lacrimation, burning, stinging, photophobia, redness and watery discharge, as well as ear fullness, ear popping and pressure over the cheeks and the forehead. This may be complicated by malaise, weakness and fatigue. Symptoms typically show a periodic distribution manifesting at individual time intervals during the pollen seasons of tree, grass and weed pollen between spring and autumn months. About 20% of patients have asthmatic symptoms as well (Smith, 1983). Food allergy, often manifesting as "oral allergy syndrome" due to cross-reacting allergens, may also be present (see section 4.5.2).

4.3.3.2 *Perennial allergic rhinitis and conjunctivitis*

In perennial allergic rhinitis and conjunctivitis, indoor allergens are the main cause of symptoms, which are similar to those of seasonal allergic rhinitis and conjunctivitis although nasal blockage is more pronounced and itching of the eyes is a common problem. Among the indoor allergens, house dust mites, cockroaches, animal dander and moulds are important. The chronic and persistent symptoms can present as a "permanent cold" and may be accompanied by secondary complaints, such as mouth breathing, snoring and sinusitis (Lucente, 1989). Occupational hypersensitivity to an airborne allergen at the workplace may lead to symptoms only during the week with a disease-free interval at weekends, for example in laboratory animal workers.

4.3.3.3 *Prognosis*

The peak prevalence of allergic rhinitis and conjunctivitis is in adolescents and young adults. The first manifestations of seasonal allergic rhinitis and conjunctivitis develop before 20 years of age in most patients. After 30 years of age, disease severity usually moderates and is only occasionally a problem in the elderly. Repeated exposure to allergens may cause nasal hyperreactivity also to other allergens, thus broadening the spectrum of hypersensitivity (Connell, 1969). A proportion of patients will develop asthma in the course of their disease (Evans, 1993).

4.3.4 *Etiology*

Symptoms of allergic rhinitis and conjunctivitis are provoked by environmental aeroallergens. Typical seasonal allergens are tree pollen in the spring, grass pollen in the early and mid summer and weed pollen in the late summer. In temperate climates of the Northern hemisphere the most important tree pollens derive from birch, alder and hazel; among grass pollens timothy and ryegrass, and among weed pollens mugwort and ragweed are the most important. However, regional differences are also of importance, e.g., cedar pollen in Japan, parietaria pollen in the Mediterranean area and ragweed pollen in the USA being the most important allergens. Sometimes mould spores, e.g., *Cladosporium* and *Alternaria*, cause symptoms during summer and autumn months. In perennial allergic rhinitis and conjunctivitis mainly indoor allergens present in the environment throughout the year are relevant triggers. The house dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, in Southern countries

Bloomia tropicalis, animal dander from horses, cats, dogs and other pets, cockroaches, and moulds such as *Aspergillus* species are the most important allergens.

Epidemiological studies indicate a significant increase in the prevalence of allergic rhinitis and conjunctivitis. There is evidence that outdoor air pollution plays a role in the increasing morbidity from allergic rhinitis and conjunctivitis. The disease seems to be more common in urban than in rural areas (Broder et al., 1974a,b). There is evidence that air pollutants may interact directly with pollen with a possible impact on allergenicity (Behrendt et al., 1992).

Beside exogenous factors, the association of allergic rhinitis and conjunctivitis with other atopic diseases, such as atopic eczema or asthma and a positive family history for atopy clearly demonstrates the genetically determined susceptibility (Coca & Cooke, 1923).

4.3.4.1 *Allergic rhinitis and conjunctivitis caused by contact with chemicals*

Allergic rhinitis and conjunctivitis caused by contact with chemicals is less common than by contact with proteins. The prevalence is unknown. The scope of the problem is probably underestimated because of diagnostic failure (Mygind, 1986). The majority of cases reported in the literature are in association with occupational diseases. Upper respiratory tract hypersensitivity involving the nose often coexists with asthma, conjunctivitis, bronchitis, and occasionally with contact dermatitis, allergic alveolitis or fever. Occupational chemicals may be haptens, allergens, mediator-releasing or pharmacological agents and irritants. Eliciting agents that sometimes are shown to induce an immediate-type IgE-mediated hypersensitivity include anhydrides, metallic salts, dyes, diisocyanates and antibiotics. In many, but not all, workers with trimellitic acid-induced rhinitis and asthma, specific IgE antibodies and positive skin tests can be found, suggesting Type I and Type III allergic mechanisms (Bernstein et al., 1982a). In isocyanate workers with rhinitis, conjunctivitis, asthma, bronchitis, chronic obstructive lung disease, cutaneous reactions or fever, 26% had positive skin-prick tests and in 14% specific IgE antibodies could be detected after conjugation of isocyanates with serum albumin (Baur et al., 1984). In the majority of cases with occupational rhinitis, conjunctivitis and asthma caused by platinum salts, a Type I hypersensitivity was proved by skin tests, *in vitro* histamine release and passive cutaneous anaphylaxis

(Schultze-Werninghaus et al., 1978). In textile workers exposed to reactive dyes, who had respiratory complaints, skin-prick tests and patch tests were positive (Alanko et al., 1978; Estlander, 1988). It is thought that these small molecule chemicals are haptens that combine with proteins to form antigenic determinants.

Symptoms caused by chemicals may also be due to a contact-allergy and delayed-type hypersensitivity. This applies more often for ocular allergy. Rubbing of the eyes after handling detergents or other chemicals may provoke a contact conjunctivitis. Positive patch tests are found to chemicals such as antibiotics, thiomersal, benzalkonium chloride, solutions for contact lenses, and metallic salts. In these cases a Type IV hypersensitivity seems to be the primary allergic mechanism.

However, allergies have to be differentiated from toxic and irritative mechanisms. Strongly toxic chemicals may elicit symptoms by directly damaging the mucosa after single contact. Milder irritants, such as sulfur dioxide, urea formaldehyde, detergents, solvents or dusts may cause hyperreactivity after repeated (cumulative) contact. Exposure to cotton defoliants causes asthma, rhinitis and conjunctivitis, which is thought to be a result of direct histamine release. It is important to note that there is often an overlap between allergic and irritative processes. Hyperreactivity to irritants occurs predominantly after repeated contact in patients with pre-existing atopic diseases, with or without an allergic basis. Chemicals are often not only irritants but also allergens.

4.3.5 Pathophysiology

Allergens transported by the air come into contact with the mucosal surface. Contact with mast cells or basophils leads to IgE-dependent activation and degranulation of mast cells. Preformed mediators stored in the granules (e.g., histamine, tryptase) are released rapidly and elicit immediate symptoms. Other mediators are eluted slowly (e.g., heparin) or are synthesized *de novo* (e.g., prostaglandins, leukotrienes) (Bachert et al., 1995). Afferent nerve stimulation may provoke an axon reflex, and the release of neuropeptides (substance P, tachykinins) may amplify this reaction (Barnes et al., 1991). Mediators that are released slowly induce a late- phase reaction after 6 to 12 h, which results in local accumulation of inflammatory cells including CD4+ T-lymphocytes, eosinophils, basophils and neutrophils

(Dvoracek et al., 1984). These cells and mast cells release cytokines and proteins (e.g., eosinophil basic proteins) that perpetuate the reaction (Bachert et al., 1995). Inflammatory cytokines (e.g., IL-4) may selectively recruit eosinophils by increasing the expression of adhesion molecules on the vascular endothelium (VCAM-1, ICAM-1).

The late-phase reaction results in an increased hyper-responsiveness, which may be specific for an allergen ("priming") or nonspecific to a variety of irritant triggers (Connell, 1969).

4.3.6 *Diagnostic techniques*

Diagnostic techniques are applied for differential diagnosis and verification of a definite diagnosis. The patient's history, physical examination with rhinoscopy and allergy testing represent the basic, readily accessible diagnostic techniques. Rhinomanometry with assessment of nasal resistance and nonspecific provocation tests demonstrating hyperreactivity of the nasal mucosa are also often used for evaluation of clinical relevance (International Rhinitis Management Working Group, 1994).

4.3.6.1 *Medical history*

A careful history of seasonal and/or perennial symptoms provoked by specific exogenous factors is most important for the diagnosis of allergic rhinitis and conjunctivitis. The conditions that precipitate or aggravate symptoms should be asked for in detail. In particular, the presence of allergens in the patient's environment and the possible causal relationship to the symptoms should be evaluated. Exposure factors, such as contact with air pollutants, automobile exhaust emissions or detergents, a history of atopic diseases and the family history provide further important information. The severity of the disease may be estimated by the frequency, distribution and severity of symptoms. Standardized questionnaires are useful in obtaining detailed information.

4.3.6.2 *Clinical examination*

Special devices are usually unnecessary for examination of the eyes, whereas rhinoscopy is obligatory for the examination of the nose. The use of indirect laryngoscopy and full endoscopic ear-nose-throat examination are not mandatory, but may be of value in special patients (International Rhinitis Management Working Group, 1994).

The nasal mucosa is usually reddened, oedematous and produces large quantities of a clear mucous discharge. The periorbital tissues may be oedematous. Cyanosis, conjunctival injection, increased lacrimation and mucous discharge of the eyes are further symptoms. The quality and quantity of the secretions should be noted.

4.3.6.3 *Allergy testing*

Immediate hypersensitivity skin tests (skin-prick test, intracutaneous test) are the primary diagnostic tool, skin-prick tests being the method of choice for the majority of cases (Dreborg, 1989; Ring, 1991). Skin testing with commercially available aeroallergens generally has a high reliability. The number of skin tests that should be performed is confined to a few common environmental allergens tested routinely but should be extended specifically if the individual patient's history indicates a role of other allergens.

The determination of total serum IgE is of limited value for this disease but tests for specific IgE antibodies (e.g., RAST) are useful. Positive results of the skin-prick test and determination of specific IgE antibodies (sensitizations) should always be evaluated in combination with the patient's history. Nasal and conjunctival challenges with commercially available allergens should be used whenever the clinical relevance of a sensitization to an allergen cannot otherwise be estimated. However, there is no universally accepted standard for this technique. As all *in vivo* tests are potentially dangerous, with the risk of anaphylaxis, tests should be carried out only by personnel trained in cardio-pulmonary resuscitation.

4.3.7 *Therapeutic considerations*

The therapeutic repertoire of antiallergic therapy includes environmental control to minimize exposure to the allergen responsible for provoking symptoms, symptomatic medications, and immunotherapy under strict medical supervision (Druce, 1993).

4.4 Clinical aspects of allergic asthma caused by contact with chemicals

4.4.1 Introduction

Asthma is by far the most frequently reported outcome of an allergic respiratory reaction to inhaled chemicals, primarily occurring as the consequence of exposures experienced at work, i.e., occupational asthma. Allergic rhinitis is generally caused by the same agents and may occur in isolation or in association with asthma.

4.4.2 Importance of occupational asthma

The contribution of occupational causes to the prevalence of asthma in the community is not generally known. Estimates in different countries have varied between 2% and 15% but their basis is not secure. In Spain, occupational causes accounted for between 1 in 15 and 1 in 20 of cases of asthma in young Spanish adults aged between 20 and 44 years. Information in the United Kingdom is limited to the numbers awarded compensation and the number of cases reported to voluntary surveillance schemes, both of which are likely to underestimate the true frequency of the disease.

In the United Kingdom a surveillance scheme for work-related diseases (SWORD) with voluntary reporting of new cases of occupational lung disease by respiratory and occupational physicians reported 2101 new cases in 1989 of which 554 (26%) were asthma. The agents most frequently reported to cause occupational asthma were isocyanates, which accounted for 22% of cases, and grain, wood dusts and laboratory animals, which together accounted for a further 17% of cases. The annual incidence rate for occupational asthma in the working population was estimated to be 22 per million. The highest rates in the occupational groups occurred primarily among those encountering chemicals at work, i.e., coach and spray painters, chemical processors, plastics making and processing, metal making and treating, and welders (Table 21).

The incidence reported in this survey is lower than that reported in Finland by Meredith & Nordman (1996); Finland is one of the few countries where occupational lung diseases are registered. The incidence in 1981 of occupational asthma in Finland was estimated to be 71 per million (compared to the rate in the United Kingdom of 22

per million). However, within the United Kingdom there was considerable regional variation in reported rates, and the area of highest incidence, West Midlands Metropolitan Area, had a rate of 63 per million, similar to the reported incidence in Finland. Meredith & Nordman (1996) suggested that the differences in regional rates might at least in part be due to differences in ascertainment and reporting, and that the true incidence of occupational asthma in the United Kingdom was three or more times that reported.

Table 21. Incidence of occupational asthma in high-risk occupational groups reported to the United Kingdom Surveillance of Work-Related and Occupational Respiratory Disease Project (SWORD) in 1989 (Meredith, 1993)

Occupational Group	Cases	Population	Incidence/10^6/year
Coach and spray painters	35	54 737	639
Chemical processors	31	73 189	424
Bakers	29	70 839	409
Plastics making and processing	27	66 005	409
Metal making and treating	14	56 270	249
Laboratory technician and assistant	26	127 478	204
Welders/solderers electronic assemblers	35	220 068	159
Working population			22

4.4.3 Chemical causes of occupational asthma

Many different chemicals encountered at work can stimulate a hypersensitivity response and cause asthma. The more prevalent causes are shown in Table 22.

4.4.3.1 Isocyanates

Diisocyanates are bifunctional molecules used commercially to polymerize polyglycol and polyhydroxyl (polyols) compounds to form polyurethanes. Because each diisocyanate molecule has two reactive isocyanate (NCO) groups, they link adjacent polyols to form a three-dimensional lattice. Isocyanates also react with water to evolve carbon dioxide, a reaction exploited in the manufacture of flexible polyurethane foam. The urethane reaction is exothermic and the heat

Table 22. Examples of occupational chemical respiratory allergens associated with positive bronchial provocation challenges (adapted from Karol et al., 1996)

Isocyanates	**Dyes**
Diphenylamine-4,4'-diisocyanate (MDI)	Brilliant orange GR
Hexamethylene diisocyanate (HDI)	Carminic acid
Isophorone diisocyanate (IPDI)	Reactive orange 3R
Naphthalene-1,5-diisocyanate	Rifafix red BBN
Toluene 2,4-diisocyanate (2,4 TDI)	Rifazol black GR
Toluene 2,6-diisocyanate (2,6 TDI)	
Amines	**Acid anhydrides**
Dimethyl ethanolamine	Phthalic anhydride
Ethanolamine	Tetrachlorophthalic anhydride
Ethylenediamine	Trimellitic anhydride
Triethylenetetramine	
Others	
Abietic acid	Glutaraldehyde
6-Aminopenicillanic acid	Iso-nonanoyl sulfonate oxybenzene
7-Aminocephalosporanic acid	Methyl-2-cyanoacrylate
Ampicillin	α-Methyldopa
Azocarbonamide	Phenylglycine acid chloride
2-(*n*-Benzyl-*N*-*tert*-butylamino)4'-hydroxy	Piperacillin
3'-hydroxymethylacetophenone diacetate	Piperazine
Benzylpenicillin	Plicatic acid
Cephalexin	Spiramycin
Chlorhexidine	Styrene
Complex platinum salts	
Ethyl cyanoacrylate	Tylosin
Natural rubber latex	

generated sufficient to evaporate diisocyanates with high vapour pressures, such as toluene diisocyanate (TDI) and hexamethylene diisocyanate (HDI). Diphenyl methane diisocyanate (MDI) and naphthalene diisocyanate (NDI), whose vapour pressures are lower, evaporate in significant amounts when heat is applied.

It is estimated that approximately 5% of workers regularly exposed to TDI develop asthma, which may be manifested as immediate and/or late onset responses. TDI can act as a direct irritant, can stimulate nerve reflexes, and, in the minority of patients, elicit an IgE antibody response and occasionally an IgG response (Baur & Fruhmann 1981; Baur et al., 1994). In addition, persistent activation of T-cells and continuous expression of pro-inflammatory cytokines seems to maintain a state of chronic inflammation (Maestrelli et al., 1995).

Polyurethanes have widespread applications, and exposure to isocyanates occurs in many different occupations. These include the manufacture of flexible and rigid polyurethane foam, the application of two part polyurethane paints by brush and by spray painting, and in flexible packaging production where isocyanates are used in inks and as laminating adhesives.

Inhaled isocyanates have been reported to cause four different respiratory reactions:

a) Toxic bronchitis and asthma caused by isocyanate inhalation at toxic concentrations. Exposure to TDI at an atmospheric concentration of 0.5 ppm (3.6 mg/m^3) causes irritation of mucosal surfaces – eyes, nose and throat (Henschler et al., 1962). Persistent asthma and reactive airways dysfunction syndrome (RADS) has been reported following a single inhalation of TDI at toxic concentrations (Luo et al., 1990).

b) Bronchial asthma caused by sensitization to isocyanates.

c) Accelerated decline of forced expiratory volume in 1 second (FEV_1). The rate of decline of FEV_1 in an isocyanate manufacturing plant workforce was similar in non-smokers with high cumulative exposures to toluene diisocyanate (TDI) to the rate observed in smokers in both the high- and low-exposure groups. The rate in non-smokers with low cumulative exposure was not different from that expected for control non-smokers. No additive effect of TDI with smoking was observed (Diem et al., 1982).

d) Extrinsic allergic alveolitis, which has been reported particularly in workers exposed to MDI (Zeiss et al., 1980) and also to HDI (Malo et al., 1983).

Of the four reactions, bronchial asthma caused by hypersensitivity to isocyanates has been the most frequently reported and is the most important both in terms of prevalence and morbidity. TDI and MDI have been the most widely used isocyanates and are the major causes of asthma, although, with its increasing use in spray paints, HDI is becoming a more prevalent cause. A study of workers employed at a new TDI manufacturing plant identified 12 workers (4% of the total workforce) who had developed asthma during a 5-year period, with 9 developing it in the first year of employment. The average exposure to TDI monitored by paper tapes was 0.002 ppm (14 $\mu g/m^3$) (Weill et al., 1981). Half of the cases had been exposed to spills; six were maintenance workers, one was a laboratory worker and only five were process workers. A cross-sectional study of a steel coating plant, where TDI had been introduced into the process some years before, identified 21 cases of asthma out of a total of 221, which was probably an underestimate of the true number of cases (Venables et al., 1985a).

Inhalation challenge tests with TDI have shown that asthmatic responses may be provoked in sensitized workers by very low atmospheric concentrations, as low as 0.001 ppm (7 $\mu g/m^3$) (O'Brien et al., 1979). Late asthmatic responses provoked by isocyanates are associated with the development of an increase in nonspecific airway responsiveness (Durham et al., 1987), and cells recovered from bronchoalveolar lavage during a late asthmatic reaction provoked by TDI have an increased proportion of neutrophils, identifying an inflammatory response in the airways provoked by TDI (Fabbri et al., 1987).

4.4.3.2 *Acid anhydrides*

Acid anhydrides are low relative molecular mass chemicals used industrially as curing agents in the production of epoxy and alkyd resins and in the manufacture of the plasticizer dioctyl phthalate. Epoxy and alkyd resins have widespread applications as paints, plastics and adhesives. Six acid anhydrides, i.e., phthalic anhydride (PA) (Maccia et al., 1976), trimellitic anhydride (TMA) (Fawcett et al., 1977; Zeiss et al., 1977), tetrachlorophthalic anhydride (TCPA) (Howe et al., 1983), maleic anhydride (MA) (Durham et al., 1987; Topping et al., 1986), hexahydrophthalic anhydride (Moller et al., 1985) and himic anhydride (Bernstein et al., 1984), have been reported to cause occupational asthma. Inhalation tests with the causal acid anhydride provoked asthmatic responses, and specific IgE or IgG antibodies, or

both, to the specific anhydride conjugated to human serum albumin were identified in the sera of the great majority of cases, although this was less frequent with maleic than with the other anhydrides. Zeiss et al. (1977) suggested that four separate clinical syndromes were caused by TMA, for which they proposed separate immunological mechanisms: i) toxic airway irritation; ii) immediate IgE-mediated rhinitis and asthma; iii) IgG-mediated late asthma with systemic symptoms ("TMA flu"); iv) pulmonary haemorrhage-haemolytic anaemia syndrome as the outcome of antibody binding to circulating red blood cells and to pulmonary vascular cells. The distinction between "immediate" and "late" asthmatic reactions with influenza-like symptoms and their different pattern of immunological response has not been consistently observed by other investigators. It seems more likely that asthma caused by acid anhydrides, including TMA, may be associated with specific IgE or IgG, or both, although specific IgE and IgG_4 seem to be more associated with asthma and IgG with exposure (Forster et al., 1988). A relationship between HLA DR3 and the development of specific IgE to TMA and possibly tetrachlorophthalic anhydride (TCPA), but not phthalic anhydride (PA), has been reported (Young et al., 1995). The pulmonary haemorrhage haemolytic anaemia syndrome is real but rare. It has been reported in individuals exposed to hot trimellitic anhydride fume and may be the outcome of a toxic reaction to inhalation of trimellitic anhydride at very high concentration rather than a hypersensitivity reaction.

4.4.3.3 *Complex platinum salts*

The complex platinum salt ammonium hexachloroplatinate is an essential intermediate in the refining of platinum, a corrosion resistant metal used as a catalyst and in jewellery. Allergy to platinum salts in refinery workers was first reported in 1945 (Hunter et al., 1945). Subsequently, inhalation of ammonium hexachloroplatinate was shown to provoke asthmatic responses and to elicit immediate skin test responses in sensitized individuals (Pepys et al., 1972).

The incidence of occupational allergy in the platinum refining industry was high in United Kingdom in the mid 1970s. In a cohort study of 91 workers who entered employment in a platinum refinery in the two years 1973 and 1974 (Venables et al., 1989), 22 developed respiratory symptoms and an immediate skin test response to ammonium hexachloroplatinate. The risk was greatest in the first year

of employment and smoking was more important than atopy as a predictor of developing a positive skin test reaction.

4.4.4 Diagnosis of occupational asthma

Accurate and early diagnosis of cases of occupational asthma is important. Remission of respiratory symptoms and restoration of normal lung function, including nonspecific airway responsiveness, can follow avoidance of exposure to the specific initiating cause. Furthermore, chronic asthma is more likely to develop in those who remain exposed to the initiating cause after the onset of symptoms. However, avoidance of exposure frequently requires a change of employment. Accurate diagnosis is also essential if those whose asthma is not occupationally caused are to avoid being advised unnecessarily to change or leave their work.

The diagnosis of occupational asthma requires:

a) differentiation of asthma from other causes of respiratory symptoms, in particular chronic airflow limitation and hyperventilation;

b) differentiation of occupational cause from non-occupational asthma;

c) differentiation of asthma initiated by an agent inhaled at work from pre-existing or incidental asthma exacerbated by nonspecific irritants, such as sulfur dioxide and cold air, inhaled at work.

The diagnosis of occupational asthma is usually suggested by the history. It commonly occurs in an individual exposed at work to an agent recognised to cause occupational asthma and only develops after an initial symptom-free period when the patient has been exposed without symptoms to concentrations in air that now provoke asthma. Respiratory symptoms occur during the working week, may increase in severity as the week progresses, and improve during absences from work, at weekends or during holidays. The patient may also be aware of others who have developed similar respiratory symptoms at the place of work.

Nonspecific stimuli provoke asthmatic reactions that usually occur within minutes of exposure to them and resolve within 1–2 h of

avoidance of exposure. Where work-related respiratory symptoms are due to the provocation of asthma by a respiratory irritant encountered at work, the onset of asthma will often have preceded initial exposure to the irritant and the severity of asthma does not significantly improve when away from work. Nonspecific irritants such as organic solvents, which often have a characteristic and unpleasant smell, may also provoke a hyperventilation response, when difficulty with breathing is associated with symptoms that are consequences of a low pCO_2, such as tingling of the fingers, headaches and dizziness.

4.4.4.1 *Investigation of causes of occupational asthma*

In the majority of cases a confident diagnosis of occupational asthma induced by an inhaled chemical can be made from knowledge of exposure at work to a recognised cause of occupational asthma and a characteristic history. Where possible, these should be supported by objective evidence from serial measurements of peak expiratory flow (PEF) or immunological tests or both. Inhalation testing is reserved for occasions when the results of these investigations do not provide an adequate basis for advice about future employment.

4.4.4.2 *Serial peak expiratory flow (PEF) rate measurements*

Asthma can be attributed with confidence to an agent inhaled at work where exposure to it in the work place reproducibly provokes airway narrowing. Repeated measurements of airway calibre, most conveniently made as PEF rates, need to be made during a period long enough to allow observation of the consistency of any changes and their relationship to periods at work. Measurements need to be made repeatedly during each day for a period of several weeks and the patient can take measurements and record the results. Self-recording of PEF measurements is now widely used. Patients are lent a peak flow meter and asked to record the best of 3 measurements of PEF made every 2 h from waking to sleeping over a period of one month in the first instance. To allow sufficient time for lung function to recover from exposure to an agent inhaled at work, it is helpful if the month includes a period away from work which is longer than a weekend, ideally a one or two week holiday. Self-recording requires patient compliance and honesty. The measurements may be conveniently summarized to show the maximum, minimum and mean peak flow measurements for each day and differences between periods at and away from work observed. This method of patient investigation has

proved, in the hands of those experienced in its use, to be reliable and a relatively sensitive and specific index of occupational asthma.

4.4.4.3 *Immunological investigations*

The demonstration of specific IgE antibody is a helpful, but not conclusive, criterion to establish the diagnosis of occupational asthma or rhinitis, because the presence of specific IgE antibody is not unique to clinically allergic individuals. Antigen-specific IgE is rare (5–15% of clinical cases). The presence of specific IgE antibody can be detected by *in vivo* tests such as skin-prick test and *in vitro* tests such as radioallargosorbent test (RAST) or enzyme-linked immunosorbent assay (ELISA). Other *in vitro* tests, such as histamine release from basophils, are less standardised. When allergens are not available, histamine release from basophils can be an alternative to direct measurement of IgE.

The application of immunological tests in the investigation of occupational asthma caused by inhaled chemicals has widened with the preparation of hapten protein conjugates suitable for immunological testing (e.g., acid anhydride and reactive dye-human serum albumin conjugates) and the development of reliable methods for identification of specific IgE antibody in serum. Complex platinum salts such as ammonium hexochloroplatinate can elicit immediate skin-prick test responses without the need for conjugation to human serum albumin.

The value of such tests in the diagnosis of occupational asthma depends upon their sensitivity and specificity in populations exposed to the particular cause. An immediate skin-prick test response and specific IgE antibody identified by RAST to conjugates of the acid anhydride tetrachlorophthalic anhydride (TCPA) with human serum albumin (Howe et al., 1983) were found to be associated with cases of asthma in exposed populations and not simply a reflection of exposure.

4.4.4.4 *Inhalation challenge tests*

Inhalation tests are rarely undertaken because they are potentially hazardous. Inhalation tests with occupational agents should only be undertaken by those experienced in conducting them and who have adequate hospital facilities for continuous monitoring of patients for 24 h after each test.

There are 4 major indications for inhalation challenge testing in the diagnosis of occupational asthma:

a) Where the agent thought to be responsible for causing asthma has not previously been reliably shown to do so.

b) Where an individual with occupational asthma is exposed at work to more than one potential cause, and his future employment depends on knowledge of which one is responsible.

c) Where asthma is of such severity that further uncontrolled exposure in the work environment is not justifiable.

d) Where the diagnosis of occupational asthma remains in doubt after other appropriate investigations, including serial peak expiratory flow rate (PEF) and immunological tests, have been completed.

The aim in an occupational-type inhalation challenge test is to expose the individual under single blind conditions to the putative cause of the asthma in circumstances which resemble as closely as possible the conditions of the exposure at work. Wherever possible, atmospheric concentrations of the inhaled agent should be based on knowledge of the concentrations experienced at work, and the physical conditions of exposure, (e.g., size of dust particles, whether vapour or aerosol, and temperatures to which the materials are heated), should be similar to those encountered at work.

Measurements of airway responses provoked by inhalation challenge tests should ideally include measurements of both changes in airway calibre and in nonspecific airway responsiveness. Changes in airway calibre are most conveniently measured by regular measurements of forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) or PEF rate before and at regular intervals after the test, for at least 24 h. Changes in airway responsiveness can be made by estimating the concentration of inhaled histamine or metacholine that provokes a 20% fall in FEV_1 (PC20) before the test and at 3 and 24 h after the test. The changes in airway calibre and nonspecific responsiveness observed are compared to those following a control challenge test, each test being made on a separate day.

The patterns of change in airway calibre provoked by inhalation testing are distinguished by their time of onset and duration.

Immediate responses occur within minutes and resolve spontaneously within 1–2 h. Such reactions can be provoked by both allergic (e.g., grass pollen) and non-allergic (e.g., inhaled histamine or sulfur dioxide) stimuli. The response depends upon the concentration of the provoking agent and the degree of pre-existing nonspecific airway responsiveness. Lone immediate responses (i.e., an immediate response not followed by a late response) are not usually associated with an increase in nonspecific airway responsiveness. Late responses develop one or more hours after the inhalation test exposure, usually after some 3–4 h, and may persist for 24–36 h. Unlike the immediate response, late responses are often associated with an increase in nonspecific responsiveness which can be identified 3 h post test prior to the onset of the late asthmatic response and less reliably at 24 h after the test (Durham et al., 1987).

A dual response is an immediate response followed by a late response. Recurrent nocturnal responses may be provoked by a single inhalation test exposure with asthmatic responses occurring during several successive nights with partial or complete remission during the intervening days. Such responses are almost certainly a manifestation of a provoked increase in nonspecific airway responsiveness. The question to be answered from the results of an inhalation test is whether or not in the individual case the particular agent inhaled at work has induced asthma. The most reliable means of answering this question is to determine whether or not inhalation of the specific agent at concentrations to which exposure occurs at work reproducibly provokes a non-immediate asthmatic response and increases nonspecific airway responsiveness. In such cases the specific agent can be considered to be the inducing cause in that particular individual. Nonspecific irritants may provoke immediate responses in individuals with hyper-responsive airways, but do not provoke either an increase in nonspecific airway responsiveness or a late asthmatic reaction. Agents that induce specific IgE antibody, however, may provoke lone asthmatic responses; in these cases inferences from the inhalation test result should take the immunological test into account.

4.4.5 Outcome of occupational asthma

Asthma induced by an agent inhaled at work may become chronic, persisting for several years, if not indefinitely, after avoidance of exposure to its initiating cause. This seems particularly, although not exclusively, to occur with asthma caused by low relative molecular

mass chemicals. Asthma caused by isocyanates and acid anhydrides has been reported to have persisted in over half of cases. Six cases of asthma caused by the acid anhydride tetrachlorophthalic anhydride (TCPA) were followed up 4 years after avoidance of exposure: all had chronic respiratory symptoms consistent with persistent airway hyper-responsiveness and a measurable histamine PC20 was present in the five in whom it was assessed. The rate of decline of specific IgE to a TCPA-human serum albumin conjugate during the period of avoidance of exposure was parallel in all 6 subjects and exponential with a half-time of one year, making it very improbable their continuing asthma was caused by further, albeit inadvertent, exposure (Venables et al., 1987).

Chronic asthma in these cases is likely to be a manifestation of persistent airway inflammation which, although initiated by the agent inhaled at work, persists in its absence. Ten patients with TDI-induced asthma, who had continuing respiratory symptoms and airway hyper-responsiveness, were investigated for 4–40 months from their last exposure. Bronchial biopsies obtained from eight patients showed basement membrane thickening with infiltration of the mucosa by eosinophils, lymphocytes and neutrophils. In four patients in whom airway responsiveness had not improved, the proportion of eosinophils in fluid recovered by BAL was increased, whereas this was the case in only one of five patients whose airway responsiveness had improved (Paggiaro et al., 1990).

4.4.6 Management and prevention of occupational asthma

Reduction in the incidence of occupational asthma will follow adequate control of exposure to its causes (Table 22). Substitution of a different paint for one containing TDI halted an epidemic of asthma in a steel coating plant (Venables et al., 1985a). Measures to secure control of exposure to the majority of causes of occupational asthma have, however, been impeded by lack of knowledge of the nature of exposure–response relationships for sensitizing chemicals.

The development of control measures that will significantly reduce the incidence of occupational asthma requires examination of exposure–response relationships and the effects of interventions in longitudinal studies.

Management advice for patients with occupational asthma has been greatly influenced by the results of studies of outcome of occupational asthma which have found evidence of continuing asthma and airway hyper-responsiveness despite many years avoidance of exposure to the initiating cause, and particularly studies, such as those of azodicarbonamide workers, that have identified a relationship between the duration of symptomatic exposure and the risk of chronic asthma. The importance of accurate identification of the specific cause cannot be over-emphasised. Avoidance of exposure often involves relocation or change of employment. Misdiagnosis of occupational asthma can be as hazardous for individual patients as missing the diagnosis because of the implications for employment.

Rigorous prevention of exposure is a key to the prevention of occupational asthma. Patients who develop occupational asthma in whom a specific cause is identified should be advised to avoid further exposure to the cause of their asthma. This seems particularly important where low relative molecular mass chemicals, such as isocyanates, plicatic acid or acid anhydrides, are the cause as these seem particularly, although not exclusively, associated with the development of chronic asthma and airway hyper-responsiveness.

However, environmental changes, unless they involve substitution, are often not able to reduce exposures sufficiently to prevent continuing airway responses in sensitized individuals. Venables & Newman Taylor (1990) examined the relationship between the concentration of TCPA in air and the provocation of asthmatic responses in inhalation challenge tests in 4 sensitized individuals. They observed a log-linear relationship between the magnitude of late asthmatic responses and TCPA concentration, which passed through the origin, suggesting no threshold.

When an individual remains in employment exposed to the cause of the asthma, either directly or indirectly, the effectiveness of relocation or of respiratory protection needs to be monitored. This can be conveniently done by serial self recordings of peak flow to determine whether or not asthma persists and, if so, whether or not it is work related.

4.5 Food allergy

4.5.1 Definitions

Food allergy is an adverse reaction to food occurring in susceptible individuals, which is mediated by a classical immune mechanism specific for the food in question. Therefore, the "true" allergic reaction to a food is caused by an over-sensitive reaction of the body's immune system (in essence, "immunity gone wrong"). *Food intolerances* are all non-immune-mediated adverse reactions to food. The subgroups of food intolerance are *enzymatic* (resulting from an enzymatic defect, e.g., lactase deficiency), *pharmacological* (depending on the direct effect of certain substances found in foods, e.g., caffeine) and *undefined food intolerance* (Bruijnzeel-Koomen et al., 1995) (Fig. 14). Food intolerances will not be described further since, as far as is known, they do not involve immune mechanisms.

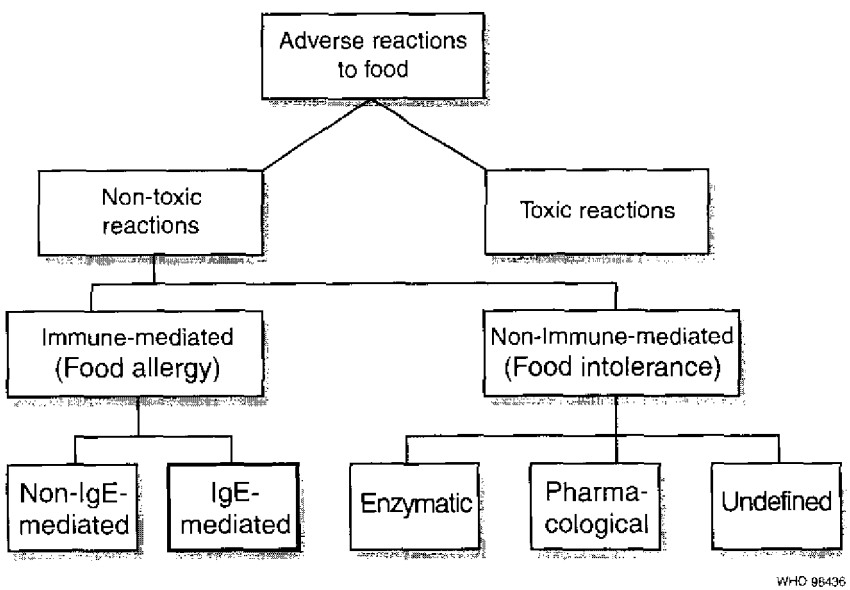

Fig. 14. Allergic reactions to food
(adapted from Bruijnzeel-Koomen et al., 1995)

The foods of animal origin that most commonly cause allergic reactions are milk, eggs, cod fish and shrimps. Those of plant origin that most often cause allergic reactions are peanuts, soybeans, celery, apple, hazelnuts and wheat.

In food allergy the best established mechanism is the presence of IgE antibodies against the offending food (Type I allergy). Other immune mechanisms may be involved in other clinical patterns of food allergy. For example, Type III and Type IV mechanisms may play a role in the genesis of food protein-induced enterocolitis of newborns and infants.

A subgroup of patients with contact allergy (T-cell mediated) to nickel or balsam of Peru can develop cutaneous symptoms (haematogenous contact eczema) to double-blind, placebo-controlled food challenge (DBPCFC) (Veien et al., 1987; Menné & Maibach, 1991). Foods with very high amounts of nickel or containing natural flavours, cross-reacting with balsam of Peru, may provoke skin symptoms.

In the following text only adverse food reactions with a proven involvement of the immune system are covered.

4.5.2 IgE-mediated food allergy

Clinical manifestations of IgE-mediated food allergy can remain localized at the site of the primary direct contact, i.e., the mouth and throat *(oral allergy syndrome)* or the gastrointestinal tract *(isolated gastrointestinal food allergy)*. However, after ingestion of the specific food, food-allergic patients often exhibit symptoms in various organs: the skin, the respiratory tract, the gastrointestinal tract and the cardiovascular system *(systemic anaphylaxis)* are the most frequently affected. Usually, the patients show involvement of two or more organ systems. Among 402 patients with a systemic IgE-mediated allergy to one or more specific foods — the oral allergy syndrome was not included — diagnosed over a 10-year period at the Allergy Unit in the Zurich University Hospital, the affected organ was most often the skin (46%), followed by the respiratory tract (25%), the gastrointestinal tract (20%) and the cardiovascular system (10%)(Wüthrich, 1993). Twenty percent of the food allergic patients had skin symptoms exclusively, 11% had isolated gastrointestinal manifestations and 8% isolated respiratory symptoms. In only 7% of all cases was food

allergy responsible for a chronic condition such as chronic urticaria, perennial asthma or gastroenterocolitis.

4.5.2.1 *Oral allergy syndrome*

Mainly patients with pollinosis may describe, spontaneously or by exact questioning, itching of the lips, mouth, palate and throat immediately after intake of some fresh foods, namely fruits and vegetables. Hoarseness and/or swelling of the lips, tongue, uvula and larynx can occur infrequently. Oral allergy syndrome must be carefully differentiated from the beginning of a generalized anaphylactic reaction in which itching of the mouth and throat may be the first symptoms. These symptoms were described in the USA in ragweed-sensitive patients (6.2% in one series) after ingestion of melon and banana (Anderson et al., 1970; Ross et al., 1991).

Cross-reactivity of pollen-sensitized subjects to various food allergens can occur (Fig. 15). Most patients allergic to birch pollen react to apples, hazelnuts and numerous other vegetables and fruits from the Rosaceae family such as cherries, peaches, pears and almonds (Eriksson et al., 1982; Dreborg & Foucard, 1983; Pauli et al., 1992) ; mugwort pollen sensitive patients may react to celery root (celeriac) and spices, the so called *mugwort-celery-spices-syndrome* (Wüthrich & Hofer, 1984; Wüthrich et al., 1990). Grass pollen allergic patients may react to cereals or tomatoes (de Martino et al., 1988). It has been shown by prick, RAST studies and RAST inhibition experiments that a celery thermolabile allergen seems to be involved in celery-birch-pollen allergic patients whereas a thermostable allergen is involved in celery-mugwort-allergic patients (Wüthrich et al., 1990). Patients with chestnut and banana oral allergy syndrome may be sensitized to natural rubber latex (M'Raihi et al., 1991). Many patients are able to identify the offending fruit or vegetable. Cooking often destroys the reactivity (Wüthrich et al., 1990). Shared epitopes and cross-reactive IgE antibodies are the most probable explanation for the observed clinical symptoms in cases of association between pollen allergy and food hypersensitivity (Calkhoven et al., 1987).

4.5.2.2 *Allergic reactions after ingestion of food*

The main symptoms of gastrointestinal food allergy are vomiting, nausea, diarrhoea and abdominal pains (colics or cramps) (Anderson, 1981; Atkins et al., 1985a,b). Skin reactions include local or

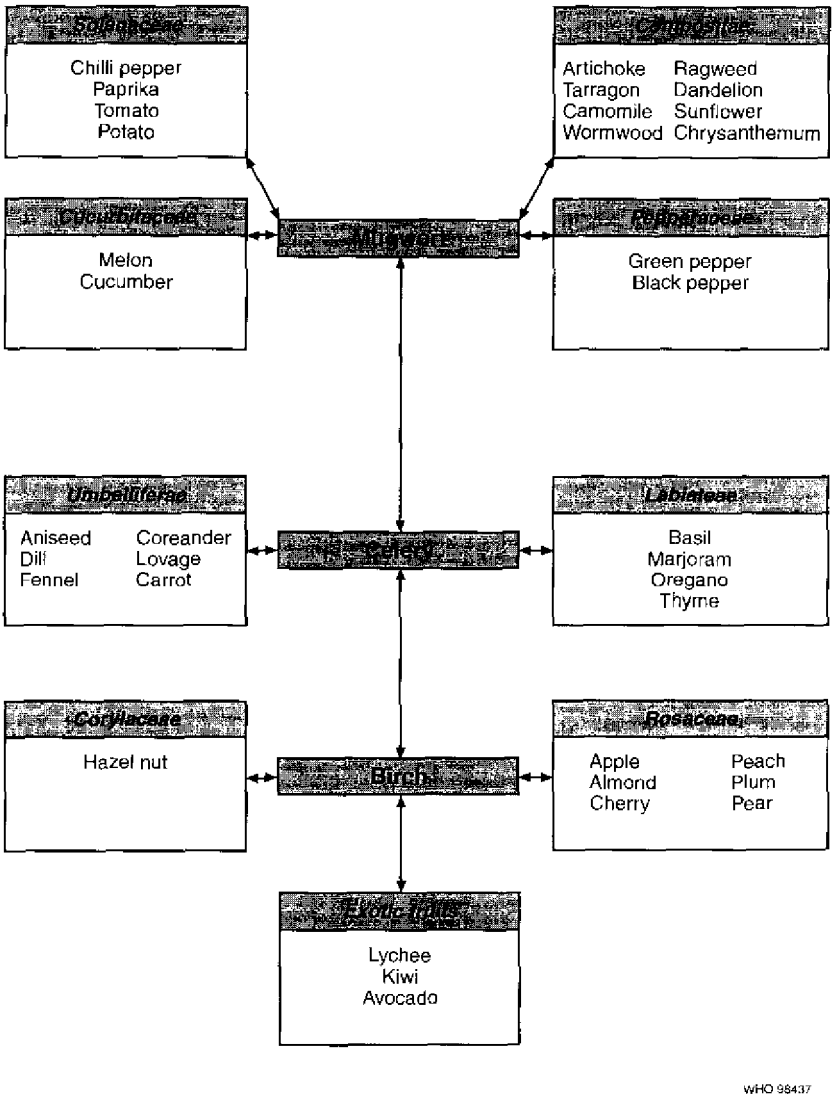

Fig. 15. Cross-reactivity of pollen-associated food allergens

generalized pruritus, flush, urticaria, angioedema, morbilliform exanthema and flare-up of atopic dermatitis. The symptoms of the upper and the lower respiratory tract are rhinitis (sneezing, pruritus of the nose, nasal stuffiness, nasal obstruction), larynx oedema, cough, wheezing and bronchial asthma. Itching, redness and watering eyes (conjunctivitis) is often associated with the above symptoms, but can also appear as an isolated manifestation of food allergy.

Systemic anaphylaxis (cardiovascular collapse) always involves other organ symptoms, e.g., of the gastrointestinal tract, the skin or the respiratory tract. A particular subtype is food-dependent exercise-induced anaphylaxis (Maulitz et al., 1979).

4.5.2.3 *Allergic reactions following skin contact with food*

Urticarial lesions can be provoked by contact with certain foods, such as fish, shrimps, flour and pork meat (Maibach, 1976). Chronic contact with food may induce protein contact dermatitis in food handlers (Hjorth & Roed-Petersen, 1976).

4.5.3 *Non-IgE-mediated immune reactions*

Immune complexes with IgG antibodies and milk antigen inducing complement-mediated damage have been suggested to induce Heiner's syndrome and haemorrhagic gastroenteritis in childhood (Heiner et al., 1962; Gryboski, 1967). Other non-IgE-mediated immune reactions to food include gluten-sensitive enteropathy (coeliac disease), food-induced colitis, and cutaneous allergic vasculitis (purpura).

4.5.3.1 *Gluten-sensitive enteropathy (coeliac disease)*

Gluten sensitive enteropathy or coeliac disease occurs in susceptible individuals. It is characterized by damage to the small intestinal mucosa with symptoms of malabsorption (Kagnoff, 1992). The prevalence of coeliac disease has been estimated to be 0.2–0.5% with considerable geographical variation (Logan, 1992).

Affected individuals develop specific immunological reactions to gliadin, a protein that is a major alcohol-soluble fraction of gluten, present in wheat, rye, barley and oat. Humoral cell-mediated immunity and genetic factors seem to be involved in the pathogenesis.

The peak incidence of symptoms is in infancy after the introduction of cereals, and a second peak occurs during the third decade. In children it is often a semi-acute disease. The symptoms of coeliac disease in children are recurrent abdominal pain, loose stools, anorexia, short stature and delayed puberty. Symptoms of malabsorption are unexplained nutritional deficiencies such as iron and folic acid deficiency, anaemia and rickets. Dental enamel hypoplasia and recurrent aphthae are also associated with coeliac disease.

Patients with coeliac disease must avoid gliadins and related proteins for life. As little as 100 mg gliadin has been described as causing intestinal damage.

4.5.4 Diagnosis of adverse food reactions

4.5.4.1 Case history and elimination diet

The diagnosis of food allergy or food intolerance is the result of a careful case history and clinical examination, and of several *in vitro* and *in vivo* diagnostic procedures. A record of food intake and its relation to clinical symptoms usually provides useful information in the case of acute reactions, occurring from a few minutes to a few hours after ingestion. History is, however, less reliable when symptoms are chronic and caused by food that generally is consumed daily, such as milk, egg, wheat and meat or by additives. An elimination diet based on rice, potatoes and mineral water over a week is useful in patients with chronic symptoms when a food allergy to other foods, hidden allergens, spices or additives are suspected. If there is no evident improvement of the symptoms after the diet period, the role of food is practically excluded. In case of suspicion of an enzymatic intolerance, appropriate laboratory tests must be performed, as well as careful gastrointestinal examinations including mucosal biopsies in the case of a chronic gastrointestinal disease.

4.5.4.2 Skin tests

Besides the case history, skin testing with various methods (skin-prick tests with commercial glycerol extracts, prick-prick or scratch tests with raw food or intracutaneous tests with aqueous extracts) with a panel of routine or selected food is the normal screening procedure for diagnosing food allergy (Metcalfe & Sampson, 1990; Sampson & Metcalfe, 1992). However, the allergen source is of major importance and the standardization of commercial food extracts is not optimal.

When reviewing the literature on sensitivity and specificity of skin-prick tests, as compared with the outcome of double-blind placebo-controlled food challenge (DBPCFC), widely variable results are found, depending on the allergen source (commercial extracts) and the different food items (Sampson & Albergo, 1984; Atkins et al., 1985a,b; Pastorello et al., 1989). Using fresh or raw food, e.g., raw milk, apple, celery, carrot, increases the sensitivity of skin-prick tests (Ortolani et al., 1989). A typical case history (e.g., oral allergy syndrome) or a severe, life-threatening immediate Type I reaction after ingestion of a defined food (e.g., apple or peanut), supported by a clear positive specific skin test with the suspected food, establishes the diagnosis (Metcalfe & Sampson, 1990). The demonstration of IgE antibodies towards the culprit food underlines the IgE-mediated pathogenesis (Johansson et al., 1984).

4.5.4.3 *Specific serum IgE*

The determination of food-specific serum IgE antibodies with different techniques (e.g., RAST = Radio-Allergo-Sorbent-Test, ELISA = Enzyme-Linked Immuno-Sorbent-Assay, FEIA = Fluoro-Enzyme Immuno-Assay) has become a routine diagnostic tool in many Allergy Centres and among practitioners (Johansson et al., 1984). However, a positive IgE determination as well as a positive skin test do not mean actual food allergy, but only a food sensitization. Cross-reactive IgE antibodies to inhaled allergens (birch or mugwort pollen, cat dander, cow epidermis) (Aalberse et al., 1981; Calkhoven et al., 1987) or to botanically related food (e.g., legumes and peanut, soy and chickpea) can be observed in negative challenge tests (Bernhisel-Broadbent & Sampson 1989; Bernhisel-Broadbent et al., 1989). On the other hand, some food allergic patients who show positive results in skin tests have negative results in RAST. Like skin testing, the diagnostic value of IgE determination is hampered by the lack of standardized food extracts. Also the cut-off limit of IgE determination, i.e., the minimal value in kU/litre or class, that should be considered clinically relevant is still a matter of debate.

4.5.4.4 *IgG determination*

Specific IgG antibodies against food (Wüthrich, 1990) can be found in many different physiological and pathological conditions. Their determination does not prove the existence of a clinically relevant immune reaction (Dannaeus & Inganäs, 1981).

4.5.4.5 *Other* in vitro *tests*

The Histamine Release Test (HRT) (Clinton et al., 1986) and the Cellular Allergen Stimulation Test (CAST) (de Weck et al., 1993), which determine the histamine or the sulfidoleucotrienes release from basophil leucocytes in the peripheral blood, are of considerable scientific interest but are too complicated and time-consuming for daily routine use.

4.5.4.6 *Oral challenge tests*

At present, double-blind, placebo-controlled, food challenge (DBPCFC) is considered as the "gold standard" for diagnosis of adverse reactions to foods (Bernstein et al., 1982b; Bock et al., 1988). Although several procedures for performing DBPCFC have been developed, their application in normal clinical practice is hampered by their heavy demands on resources and time.

The use of DBPCFC is necessary to assess objectively food allergy/food intolerance, because several studies have demonstrated a tremendous discrepancy between subjective perception of food allergy/food intolerance and the results of DBPCFC. However, there are difficulties in conducting studies of this nature in large population samples. For example, challenge tests are dangerous with the risk of severe anaphylactic reactions in the case of positive skin-prick tests and/or positive serum IgE (RAST/CAP) against the offending food. Moreover, there may be several potential hazards in the procedure and in the interpretation of food challenges (Bindslev-Jensen et al., 1994b), namely:

a) The nature of the food being tested (raw, freeze-dried, cooked, food allergen extract).

b) The amount of food necessary to provoke objective signs.

c) DBPCFC may bypass important sites, e.g., mouth when using capsules.

d) Additive or synergistic effect of multiple hypersensitivities (e.g., concomitant sensitization to spices or inhaled allergens such as pollen) or trigger factors (e.g., drugs, aspirin, alcohol, exercise, stress).

e) If the patient only reports subjective symptoms, e.g., itch, headache, it may be necessary to increase the number of challenge tests (three active and three placebo provocations) to avoid false positive reactions and to obtain statistical significance (Young et al., 1994).

In clinical practice it is often convenient to apply an open or a single-blind food challenge: a negative test is of high predictive value. A positive test, with the presence of objective signs (e.g., urticaria, asthma) or laboratory markers, increase of serum histamine, tryptase or ECP (Eosinophil Cationic Protein) levels after challenge, is usually sufficient to verify the diagnosis.

4.5.5 Therapeutic considerations

The only proven therapy for food allergy is to avoid the allergen causing the disease. To be able to avoid, for instance, wheat flour, milk or egg in the daily diet, professional advice is necessary both to avoid hidden allergens in processed food and to ensure that the diet is nutritionally adequate.

4.5.6 Prevalence

4.5.6.1 Introduction

It is possible to make more or less well-documented estimates of the prevalence of different adverse reactions to foods. Frequency and duration of breast feeding, eating habits, and flora (e.g., birch trees) are factors influencing the prevalence. It is not known whether the prevalence of food allergy is increasing. The prevalence of pollen rhinitis is increasing (Wüthrich et al., 1995), and it is possible that the prevalence of pollen-related food allergies may also be rising.

4.5.6.2 Children

In many countries cow's milk is the first food allergen newborns meet. The prevalence of cow's milk allergy is 2–5% in one-year-old babies (Jakobsson & Lindberg, 1979; Bock, 1987; Høst et al., 1988; Hill & Hosking, 1997). This figure declines rapidly during the first three years of life (Høst & Halken, 1990).

The overall prevalence of food allergy is somewhat higher with a maximum around one year. Bock (1987) followed a cohort of North

American children from birth to their third birthday; 7.7% had adverse reactions to food not including fruit and fruit juices and 85% of the adverse reactions were found during the first year of life. By the third birthday less than 1% had adverse food reactions. The cumulative prevalence of food allergy/intolerance diagnosed by elimination and open challenge in a group of unselected Danish infants 18 months of age was 6%. In the same study the prevalence in high-risk infants was 17% (Halken et al., 1992).

In a British study of a birth cohort of children age 4 years, 0.7% had allergy to peanuts or tree nuts and 0.5% had allergy to peanuts alone. These figures were based on skin-prick tests and positive history (Tariq et al., 1996).

The estimated prevalence of hypersensitivity reactions in Australia to common foods in infancy and childhood is: cows's milk 2%, egg 3.2%, and peanut 1.9%. Based on food challenge studies from Australia, Japan, Indonesia, Malaysia and the Philippines, milk and egg are the commonest food allergens. Soy, wheat and peanut hypersensitivity are next commonest, but rice allergy is rare (Hill, 1997).

In a study of allergic children, Crespo et al. (1995) found that allergy to cow's milk, egg and fish predominantly began before the second year of age, whereas allergy to fruit, legumes and vegetables predominantly began after the second year. This is in accordance with the findings in a Danish study (Saval et al., 1993) where in older children an increased prevalence of allergic pollen rhinitis was followed by an increase in oral itch, the symptom characteristic for allergic reactions to fruit and vegetables. In the 14–16 year old the prevalence of oral itch was 2–2.9%.

4.5.6.3 *Adults*

a) IgE-mediated allergy

Allergic reaction to foods that cross-react with pollen is probably the most common food allergic reaction in adults, at least in countries where tree pollen allergy is common. In a study of Swedish medical students, Foucard (1991) found that 78% of the students with allergic rhinitis and positive skin test to birch pollen reported clinical sensitivity to nuts and/or apples or other fruits. In another Swedish

study 70% of the patients with birch pollen allergy and 19% of the patients with allergy to other pollen had food-related symptoms (Eriksson et al., 1982). These estimates were mainly based on self-reported symptoms.

In Switzerland approximately 10% (9.1–11.2%) of the adult population currently suffers from hay fever. The prevalence of sensitization measured by skin-prick test, but not necessarily followed by clinical symptoms, is 12.7% for grass pollen and 7.9% for birch pollen (Wüthrich et al., 1995). Other reports of the prevalence of pollen rhinitis vary from 2–15% depending on, among other things, the age group studied (Sibbald, 1993).

Applying Swedish figures for pollen-related reactions to the Swiss data, taking into account the prick test results, approximately 50% of hay fever patients would have adverse reactions to pollen-related foods, suggesting a prevalence of 3–5% in the adult population. In a study by Kremser (1989) only about 10% of subjects allergic to birch pollen had more severe reactions than oral itch. Combining this with the Swiss figure gives a prevalence of pollen-related reactions other than oral itch of approximately 0.5%.

In adults, the prevalence of allergy to non-pollen-related food allergens such as milk, egg, shrimp, meat is probably about 0.1%. In a study by Wüthrich (1993) of 402 patients with food allergy, approximately 75% of the positive reactions were caused by pollen-related food and 25% of the reactions were caused by non-pollen-related food. Patients where the only food-related symptom was itching of the mouth were not included in the study.

In some patients IgE allergy to natural rubber latex causes allergic reactions to ingested banana, avocado, chestnut, etc. (Blanco et al., 1994). The prevalence of natural rubber latex allergy in European health-care workers screened with skin-prick tests has ranged from 2.8 to 10.7%. About half of patients allergic to natural rubber latex have experienced symptoms after eating banana (Turjanmaa et al., 1996). In a study by Beezhold et al. (1996) 36% of a group of patients allergic to natural rubber latex had clinical reactions to related foods. The majority of these patients had anaphylaxis.

In the Netherlands an attempt has been made to estimate the total prevalence of adverse reactions to foods and additives in adults. The

study used double-blind placebo-controlled food challenge (Niestijl Jansen et al., 1994) and 12.4% of the studied population reported food allergy or intolerance to specific food. Food groups mentioned most frequently were fruit, chocolate and vegetables. The prevalence of food allergy or intolerance in the adult Dutch population was estimated to be 2.4%. If the positive reactions to food additives, including menthol and glucose, is disregarded, the estimated prevalence of food allergy/intolerance is 1.2%.

In a British study (Young et al., 1994) approximately 20% of a survey population reported adverse reactions to foods. Eight foods commonly perceived to cause sensitivity were canned or made into candy bars and used for double-blind placebo-controlled challenge. The foods were: cow's milk, hen's egg, wheat, soya, citrus fruits (orange), fish/shellfish (prawn), nuts (peanut, brazil nut, walnut and hazel nut) and chocolate. These eight foods accounted for 49.3% of reactions reported in the study questionnaire. Adding some of the persons with severe reactions and estimating the number of theoretically positive in the non-challenged groups, the estimated prevalence of food allergy/intolerance to the eight foods was 1.4–1.8%. As the eight foods used for challenge accounted for approximately 50% of reported reactions, the total prevalence estimate must be 3–4%.

b) Contact allergens

In dermally sensitized subjects, ingestion of the contact allergen may cause skin flare reactions or other symptoms, for instance, from the gastrointestinal tract. It has been reported that 10% of Danish women have contact allergy caused by nickel (Menné & Holm, 1983). Up to 10% of these may benefit from a nickel-restricted diet (Veien et al., 1993). The prevalence of systemic reactions via the food from other contact allergens is not known. The chemicals of fragrances and of food flavours, natural or synthetic, are often identical. Veien et al. (1987) found an effect of a diet low in flavours in patients having a flare of dermatitis after oral challenge with balsam of Peru.

4.5.6.4 Conclusions

Milk and egg seems to be the most common food allergens in infants and children worldwide. Peanuts are also reported to be common food allergens in the United Kingdom, USA, Australia

and some Asian countries. The overall prevalence of food allergy/intolerance in young children may be around ó–8%. This figure declines before 3 years of age. In older children and young adults the prevalence of allergy to pollen-related fruits, nuts and vegetables increases.

The results from the epidemiological studies, combined with the knowledge on pollen and latex cross-reactions, systemic reactions to contact allergens, and coeliac disease (prevalence estimate 0.2–0.5%), point to an estimated prevalence of food allergy in the adult population of 3–5%, giving that some of the reactions may be coincident. These figures are based on the existing prevalence data, which are mainly European. It is not yet known whether prevalence data in the rest of the world are comparable.

4.6 Autoimmune diseases associated with drugs, chemicals and environmental factors

4.6.1 Introduction

The autoimmune connective tissue diseases include conditions such as systemic lupus erythematosus (SLE), systemic sclerosis, Sjögren's syndrome, rheumatoid arthritis and the systemic vasculitides such as Wegener's granulomatosis, Churg Strauss syndrome and polyarteritis nodosa. The etiology of these conditions remains largely unknown but there is a consensus that several factors may be important, including genetic, ethnic and hormonal; many of these conditions have a female preponderance (Table 23). Some of these conditions may also have an environmental component to their pathogenesis and this particularly applies to systemic sclerosis. Many drugs have been associated with the development of SLE and cutaneous vasculitis. There has been some interest in the possible development of connective tissue diseases associated with silicone breast implants.

4.6.2 Systemic lupus erythematosus

SLE is predominantly a disease of young women (9:1 female: male ratio) and is commoner amongst certain ethnic groups such as Afro-Caribbeans and Orientals (Beeson 1994). Clinically, the disease manifestations include arthritis, serositis, photosensitivity, oral ulceration, malar rashes, recurrent thromboses, glomerulonephritis and central nervous system involvement, e.g., epilepsy, psychoses.

Table 23. Age and sex associations of some autoimmune diseases (adapted from Beeson, 1994)

	Age	% Females
1. Autoimmune diseases that may appear during childhood		
Systemic lupus erythematosus	2–12	70
Dermatomyositis	1–15	70
Sydenham's chorea	5–15	65
Rheumatoid arthritis	1–10	65
Rheumatic fever	5–15	50
Thrombocytopenic purpura	2–5	50
Polyglandular syndromes	2–15	50
Bullous pemphigoid	1–15	50
Diabetes mellitus	2–15	45
Henoch-Schonlein purpura	2–5	40
Post-streptococcal nephritis	5–15	35
2. Autoimmune diseases that usually appear during early adult life		
Systemic lupus erythematosus	15–40	90
Erythema nodosum	15–30	90
Takayasu's arteritis	10–30	85
Myasthenia gravis	20–30	75
Thrombocytopenic purpura	15–45	75
Addison's disease	20–50	75
Rheumatoid arthritis	20–40	65
Multiple sclerosis	20–35	60
Sarcoidosis	20–40	55
Ulcerative colitis	15–40	50
Erythema multiforme	20–40	45
IgA nephropathy	10–30	35
Polyarteritis nodosa	20–50	30
Ankylosing spondylitis	20–30	25
Goodpasture's syndrome	15–35	15
Ankylosing spondylitis	20–30	25
Goodpasture's syndrome	15–35	15
Thromboangiitis obliterans	20–40	5

Table 23 (contd).

	Age	% Females
3. Autoimmune diseases that usually appear during mature adult life		
Sjogren's syndrome	40–60	95
Primary biliary cirrhosis	30–75	90
Hashimoto's thyroiditis	30–50	85
Thyrotoxicosis	30–50	85
Scleroderma	30–50	80
Chronic active hepatitis	30–50	65
Polymyositis/dermatomyositis	40–60	55
Polychondritis	40–60	50
Pemphigus vulgaris	50–80	50
Wegener's granulomatosis	10–80	50
Henoch-Schonlein purpura	30–65	45
Membranous nephropathy	30–60	35
Amyotrophic lateral sclerosis	40–70	35
Tabes dorsalis	30–50	20
4. Autoimmune diseases that usually appear late in life		
Giant cell arteritis/polymyalgia	50–90	65
Pernicious anemia	40–80	60
Bullous pemphigoid	60–75	50
Rapidly progressive glomerulonephritis	50–70	45
Myasthenia gravis	50–80	40
Fibrosing alveolitis	40–70	35

Serologically, SLE is characterized by autoantibody production to nuclear components such as anti-nuclear antibodies, antibodies to double stranded DNA and antibodies to extractable nuclear antigens.

The precise etiology of SLE remains obscure but some environmental factors are known to exacerbate the disease. One of the most important of these is ultraviolet light. In particular UV-B in the 295 to 305 nm range is known to be toxic to SLE patients (McGrath et al., 1994). Furthermore, patients with antibodies to Ro (SSA) are sensitive to UV light, which provokes a photosensitive skin rash and may be followed by a generalized disease flare (Gilliam & Sontheimer, 1982).

Being in a lower socioeconomic group may also increase the risk for the development of glomerulonephritis in patients with SLE (McAlindon et al., 1993), although the reasons for this remain obscure.

Many patients with SLE are allergic to a variety of substances including drugs and chemicals, and a case-control study has supported this clinical observation (Sequeira et al., 1993). Patients with lupus and particularly those with Sjögren's syndrome appear to be sensitive to cotrimoxazole and other sulfonamide-containing drugs. Oral contraceptive pills, especially those with high oestrogen doses, may provoke flares of SLE, and these agents should generally be avoided (Jungers et al., 1982). The progesterone-only pill is associated with fewer flares.

The majority of cases of SLE are idiopathic but certain drugs are known to cause SLE in genetically predisposed individuals. Of the 70 or so drugs reported to be associated with drug-induced lupus, some of which are shown in Table 24, only procainamide and hydralazine

Table 24. Drugs associated with the development of systemic lupus erythematosus

Drugs commonly inducing systemic lupus erythematosus
Debrisoquine
Hydralazine
Quinidine
Procainamide
Drugs with good evidence for inducing systemic lupus erythematosus
Carbamazepine
Chlorpromazine
Hydrazine
Isoniazid
Minocycline
Methyldopa
Penicillamine
Phenytoin

have been studied in detail. Clinically, drug-induced lupus is similar to idiopathic lupus, but there are one or two striking differences. For example, serositis and pleuro-pulmonary involvement is much commoner in drug-induced lupus, whereas renal disease and central nervous system disease is less common in comparison to idiopathic

lupus (Yung et al., 1995). Serologically, anti-nuclear antibodies and antibodies to both single-stranded and double-stranded DNA are found in both drug-induced and idiopathic lupus, but it is uncommon to find very high levels of anti-DNA antibodies in drug-induced lupus. Antibodies directed against certain components of histone are thought to be characteristic of drug-induced lupus. Although anti-histone antibodies may commonly be found in idiopathic lupus, they react to the H1 and H2B subunits of histone. In drug-induced lupus, the specificity appears to be against the H2A-H2B dimer in procainamide-induced lupus, or against H3 and H4 in hydralazine-induced lupus (Burlingame & Rubin, 1991).

Idiopathic lupus erythematosus is associated with HLA DR2 or DR3, at least in Caucasian patients. One of the best clues towards an explanation for a genetic predisposition to drug-induced lupus comes from the study of the acetylator status of these patients. "Slow acetylators" are homozygous for this gene and have low levels of *N*-acetyltransferase in the liver. Both procainamide and hydralazine are metabolized by this pathway and slow acetylators have a higher risk of developing drug-induced lupus following exposure to these drugs (Strandberg et al., 1976; Sonnhag et al., 1979).

The exact mechanisms by which drugs can induce lupus remain unknown and a number of possibilities are being considered. It may be that the ability of both procainamide and hydralazine to bind polynucleotides *in vitro* may render DNA and/or histones antigenic (Dubroff & Reid, 1980; Tomura & van Lancker, 1988). A more specific mechanism has been suggested whereby drugs interfere with the normal process of methylation of DNA. Following DNA replication, cytosine residues are methylated at the 5-position by the enzyme DNA methyltransferase. Failure of methylation of regulatory sequences is associated with gene expression, whereas methylation is associated with suppression of gene transcription. Thus, DNA methylation is a mechanism regulating gene expression (Yung et al., 1995). Studies have shown that procainamide- and hydralazine-treated human T-cells show evidence of hypomethylation (Scheinbart et al., 1991). In particular, procainamide is capable of reversibly inhibiting T-cell DNA methyltransferase in a dose-dependent manner (Scheinbart et al., 1991). Furthermore, these T-cells become auto-reactive in response to procainamide and hydralazine (Yung et al., 1995). These autoreactive T-cells are capable of inducing B-cells to differentiate into IgG-secreting cells without the addition of antigen or

mitogen, thus providing a mechanism for polyclonal B-cell activation that is seen in both idiopathic and procainamide-induced lupus (Richardson et al., 1990).

Experiments have shown that UV light is also capable of inhibiting T-cell DNA methylation, increasing LFA-1 expression and inducing autoreactivity (Richardson et al., 1994). Thus, drugs such as procainamide and hydralazine and environmental factors such as UV light may induce T-cell autoreactivity by inhibiting T-cell DNA methyltransferase, thereby altering T-cell gene expression and making autoimmunity more likely.

4.6.3 Scleroderma: environmental and drug exposure

Scleroderma (progressive systemic sclerosis) is a multisystem connective tissue disease of unknown etiology. The prevalence ranges from 47.9 to 290 per million among females, with an estimated incidence of between 3.6 and 16 per million per year (Silman et al., 1996). It is commoner in women (the sex ratio ranges from 3:1 to 8:1) and some ethnic minorities such as Afro-Caribbeans and is characterized by widespread, diffuse sclerosis affecting the peripheral vasculature, skin, gastrointestinal tract, heart and muscle. Raynaud's phenomenon is a very common early feature, and pulmonary and renal involvement may be serious and life-threatening. Serologically, antibodies to topoisomerase I (anti-Scl 70) and RNA polymerase III may predict prognosis in terms of more extensive disease.

Scleroderma-like conditions (pseudoscleroderma) and scleroderma have been associated with a variety of chemical and environmental agents (Silman & Hochberg, 1996) (Table 25).

4.6.4 Silicone breast implants

Two studies have reported possible interactions between silicone breast implants and the immune system. Tenenbaum et al. (1997) showed the existence of a relationship between the level of anti-polymer antibodies in the serum and the severity of clinical complications (related to connective tissue) in silicone breast implant recipients. Although these antibodies were not directed against silicone polymers this might be the first objective marker to be used as a diagnostic feature in silicone breast implant patients. Smalley et al. (1995, 1997) reported on a lymphocytic response to silica (silicon

dioxide) similar to that for silicone (polysiloxane polymer), a silicon derivative, and present in silicone breast implant material, in silicone breast implant patients and their children.

Table 25. Some agents associated with scleroderma

Organic chemicals:	
	Toluene
	Benzene
	Xylene
	Aromatic mixes e.g., white spirit
	Vinyl chloride
	Trichloroethylene
	Perchloroethylene
	Naphtha-*n*-hexane
	Epoxy resins
	Metaphenylenediamine
	Urea formaldehyde foam insulation
Drugs:	
	Bleomycin
	Carbidopa
	L-5-hydroxytryptophan
	Pentazocine
	Cocaine
	Diethyl proprion
	Fenfluramine HCl

However, several thorough reviews of the subject, including a case-control study (Sanchez-Guerrero et al., 1994, 1995; Gabriel et al. 1994; Hochberg et al., 1996), concluded that there was little or no association between silicone breast implants and either connective tissue diseases or a unique arthralgia/myalgia/fibromyalgia syndrome. They suggested that the connective tissue diseases that had been reported were most likely to have been idiopathic in origin.

In a large retrospective cohort study, the Women's Health Study sent questionnaires to 395 543 women and found 10 830 women who reported breast implants (Hennekens et al., 1996). This study showed

a small but statistically significant increased risk of the combined end-point of any connective tissue disease in patients with breast implants, with a relative risk of 1.24 (95% confidence intervals 1.08–1.41, $P = 0.0015$). Of the specific connective tissue diseases studied, scleroderma was associated with a relative risk of 1.89 (95% confidence intervals 0.98–3.45) but this was not significant ($P = 0.06$). For patients with scleroderma with implants less than 4 years, the relative risk was significant: 2.68 (95% confidence intervals 1.11–6.51, $P = 0.029$) The precise nature of the implants was not ascertained so it is not clear how many of the implants were silicone gel filled. Another possibility for bias is the publicity surrounding breast implants, which may have led to a degree of over-reporting amongst the cases.

Other studies have supported the epidemiological view that there is no association between connective tissue disease and silicone breast implants (Nyren et al., 1998; Edworthy et al., 1998). Both were very large cohort studies that compared patients who had either undergone breast reduction (Nyren et al., 1998) or non-implant cosmetic surgery (Edworthy et al., 1998). Neither found an excess of any connective tissue disease among patients with silicone implants. A United Kingdom review (DOH, 1998) came to the same conclusion.

Overall, apart from one retrospective study, there is no clear evidence that patients with silicone breast implants have any risk of developing a connective tissue disease but the area remains controversial.

4.6.5 Toxic oil syndrome

This epidemic started in May 1981 when large numbers of patients in the Madrid industrial area suffered acute respiratory illnesses that did not respond to antibiotics. The etiological agent was identified as being rapeseed oil that had been denatured with 2% aniline for industrial use but sold illegally by itinerant vendors for domestic food use. Initially 20 688 cases were recorded with 835 deaths — a cumulative mortality of 4%, but over the first 2 years of the epidemic the cumulative mortality was 2.3%. Oleyl-anilide proved to be an excellent marker for case-related oil specimens although the precise nature of the etiological agent has never been described. It has proved impossible to consistently replicate the syndrome in animal studies.

Various clinical features have been described (Kammüller et al., 1984; Philen & Posada, 1993) including severe myalgia, arthralgia, Raynaud's phenomenon, scleroderma-like skin changes, carpal tunnel syndrome, joint contracture, pulmonary hypertension and peripheral neuropathy. The most consistent laboratory features were eosinophilia and high IgE counts and low levels of antibodies such as antinuclear antibodies. HLA DR3-DR4 was associated with the chronic phase of the disease. Histologically, the most consistent finding was a vascular lesion characterized by intimal proliferation with fibrosis, vascular occlusion and thrombosis.

4.6.6 Eosinophilia-myalgia syndrome

This epidemic was first identified in October 1989 in New Mexico (Hertzman et al., 1990). The report was followed by a case-control study that firmly linked the consumption of possibly contaminated L-tryptophan from one source with the eosinophilia-myalgia syndrome (Eidson et al., 1990). L-tryptophan was widely used as a non-prescription food supplement by health conscious individuals for a wide variety of minor ailments. The source of the epidemic was traced to a single manufacturer (Philen & Posada, 1993).

The vast majority of cases occurred in the USA. The majority of these patients were white middle-class middle-aged females, probably reflecting the pattern of use of health supplements rather than any innate risk factor. The mortality rate was reported as 2.7% (37 deaths among 1370 known cases) (Philen & Posada, 1993).

The clinical features resembled those of toxic oil syndrome, although scleroderma-like changes and Raynaud's phenemenon were not reported (Kaufman et al., 1991; Philen & Posada, 1993). These authors also noted that HLA DR4 was associated with an increased risk of chronic disease. Laboratory investigations consistently showed an eosinophilia early in the disease course, although this diminished spontaneously even when the patients continued to be ill (Philen & Posada, 1993). The main factor in treatment was the avoidance of further ingestion of L-tryptophan. Glucocorticoids were used widely and helped the myalgias and reduced the eosinophil count, but there was often a recurrence of symptoms on stopping the steroid treatment, and progression to chronic disease was not altered.

4.6.7 Vinyl chloride disease (occupational acro-osteolysis)

Vinyl chloride (CH_2=CHCl) is a combustible colourless gas at room temperature that is used in the manufacture of a variety of plastics. Several methods can be used to polymerize the gas to make polyvinyl chloride (PVC). In the mid-1960s a new syndrome affecting workers involved in polymerizing vinyl chloride was recognised (Wilson et al., 1967). These patients developed paraesthesia of the fingers, cold sensitivity, Raynaud's phenomenon, pseudoclubbing of the fingers, skin oedema and thickening of the fingers, hands and forearms, and chest X-ray changes (Veltman et al., 1975). The risk of development of symptoms was related to cumulative exposures over time and work practices but was not related to handling the finished PVC product, and in Wilson's (1967) study it occurred in less than 3% of exposed individuals.

An increased prevalence of HLA DR3 and DR3/B8 haplotypes has been noted in patients with vinyl chloride disease (Black et al., 1983). Vinyl chloride is a cause of non-cirrhotic portal hypertension and angiosarcoma of the liver.

The skin changes of vinyl chloride disease resemble morphoea clinically and histologically, and vascular changes were often present with luminal narrowing of the digital arteries and subtotal occlusion of these vessels (Veltman et al., 1975). The most dramatic radiological change is acro-osteolysis seen in the terminal phalanges of the fingers; a transverse lytic band is seen across the distal phalangeal shaft. Ward et al. (1976) reported immunological abnormalities in vinyl chloride disease including polyclonal increases of IgG, cryoglobulins, evidence of complement activation and low titre anti-nuclear antibodies. Vascular endothelial, medial and sub-intimal deposits of IgG, C3, C4 and fibrin/fibrinogen were seen on histology of small and medium-sized arterioles. Reduced T-cell and modestly increased B-cell numbers were also observed (Ward et al., 1976).

4.6.8 Systemic vasculitis: environmental factors and drugs

Various drugs are associated with hypersensitivity reactions and the most common mechanism is an immune-complex-mediated vasculitis (Dubost et al., 1991). Drugs account for approximately 10–20% of dermal vasculitis and this figure is on the increase. The cutaneous lesions most commonly seen include palpable purpura,

although urticarial lesions may be seen in 10% (Dubost et al., 1991). They usually occur symmetrically on the lower limbs extending to the thighs and buttocks (Mullick et al., 1979). In Mullick's series of 30 patients, 19 had disseminated vasculitis with other organ involvement, including renal disease, synovitis, pleuropulmonary and cardiovascular disease with coronary vessel vasculitis and cardiac failure. More than 80% of patients had constitutional features such as fatigue, malaise and fever.

4.6.9 Conclusion

The vast majority of the autoimmune connective tissue diseases have no known etiological agents. Certain drugs, occupational exposures, and UV radiation have been shown either to exacerbate a known autoimmune disease or, occasionally, to trigger the onset of a syndrome that closely resembles one of the established diseases.

5. EPIDEMIOLOGY OF ASTHMA AND ALLERGIC DISEASE

5.1 Introduction

Asthma and allergies, such as atopic diseases (i.e., bronchial asthma, allergic rhinitis, atopic dermatitis) and allergic contact dermatitis, are common medical problems.

Asthma, allergic rhinitis and atopic dermatitis are conditions that have a variety of clinical similarities and epidemiological connections (Montgomery-Smith, 1983). Asthma has frequently been classified as of allergic or non-allergic origin. Different atopic conditions often occur together in the same person. A family history of atopic disorders has consistently been found to be an important predisposing factor for the development of atopic diseases (Croner & Kjellman, 1990). However, epidemiological studies suggest that exogenous factors also play an important role in their etiology (Newman Taylor, 1995). There have been epidemiological studies on geographical variation and time trends in prevalence rates of these disorders, and knowledge about the effects of a variety of environmental factors, including outdoor exposures, indoor exposures, diet and occupational exposures, is increasing.

5.2 Definition and measurement of allergic disease

5.2.1 Asthma

5.2.1.1 Definition

Asthma is a respiratory disease that is not well defined. It is characterized by variable airflow limitation due to bronchial hyper-responsiveness and often by inflammatory changes in the airways (see section 2.5.1)

5.2.1.2 Assessment

a) Questionnaires

In epidemiological studies many different questionnaires have been used to investigate in populations the prevalence of asthma symptoms such as wheezing and shortness of breath, as well as

diagnosed asthma. These include the MRC (United Kingdom Medical Research Council), the ECSC (European Coal and Steel Community), the ATS-DLD (American Thoracic Society and the Division of Lung Disease), and the IUATLD (International Union against Tuberculosis and Lung Disease) questionnaires (Toren et al., 1993).

When questionnaires are used in epidemiological studies to compare prevalence rates between populations, findings may be influenced by differences in language, interpretations of the concept of wheeze in different communities, or the general awareness of the disease in the community (Strachan et al., 1990). Nevertheless, the IUATLD questionnaire has been shown to provide valid and comparable data even when translated (Burney et al., 1989a). A similar written questionnaire was developed for the International Study of Asthma and Allergies in Childhood (ISAAC) (Pearce et al., 1993; Asher et al., 1995; Shaw et al., 1995). In addition, a video questionnaire depicting (in five scenes) adolescents with different symptoms of asthma has been developed to overcome problems with the translation of questionnaires (Asher et al., 1995). The sensitivity and specificity for predicting bronchial hyperresponsiveness of the video questionnaire was similar to the respective questions in the IUATLD questionnaire (Shaw et al., 1992a,b; Shaw et al., 1995). By providing data relatively free from biases due to language, culture, literacy or interviewing techniques, the video questionnaire may prove particularly useful for comparisons of prevalence and severity of asthma in different populations (Asher et al., 1995).

b) Bronchial hyperresponsiveness

In epidemiological settings, a major limitation of using bronchial hyperresponsiveness (BHR), measured by challenge, as a gold standard for the definition of asthma is that a considerable proportion of subjects with bronchial hyperresponsiveness report no respiratory symptoms (Sterk et al., 1993). Thus, bronchial hyperresponsiveness cannot be used synonymously as a diagnosis of asthma (Sterk et al., 1993). The thresholds used to define bronchial hyperresponsiveness — usually a fall in the forced expiratory volume in one second (FEV_1) of more than 15% or 20%, or a fall in peak expiratory flow rate (PEF) of more than 10% — were chosen arbitrarily. Various methods including exercise tests and inhalation of metacholine, histamine or cold air have been described to measure bronchial hyperresponsiveness (Sterk et al., 1993). It is now well recognized that a change

in osmolarity of the periciliary fluid is a potent stimulus to airway narrowing and may be a common cause for provoking an attack of asthma (Anderson & Smith, 1991). This has led to the use of hypertonic saline aerosols to document bronchial hyperresponsiveness in epidemiological studies (Riedler et al., 1994). However, a negative response to any of these provocation methods does not exclude asthma. In general, measuring bronchial hyperresponsiveness in epidemiological studies has a moderate specificity and a relatively low positive predictive value (Sterk et al., 1993).

5.2.2 Rhinitis

There are no clear-cut criteria for defining hay fever and perennial rhinitis. Rhinitis is frequently underdiagnosed and misdiagnosed (Sibbald & Rink, 1991a,b). Patients are generally classified according to the suspected etiology of their conditions. Rhinitis is labelled "allergic" when a causal allergen can be identified, otherwise it is labelled "non-allergic". Subjects with seasonal symptoms are twice as likely to be labelled as having allergic rhinitis by their doctors. The usual complaints of allergic rhinitis are "summer flu", series of sneezing and a stuffy nose. The classification is based primarily on nasal smears and skin-prick tests compared with the patient"s history (Weeke, 1987). Perennial allergic rhinitis in patients who are allergic to pets is easy to diagnose if symptoms such as sneezing and itchy eyes occur immediately after contact with pets. If the symptoms are caused, for instance, by mites, it is more difficult to obtain a clear-cut medical history. Symptoms of patients with non-allergic rhinitis are often different from those with allergic causes. The main symptoms in non-allergic cases are stuffy nose and loss of sense of smell, while in the allergic type, sneezing and watery secretion are more prominent. A standardized questionnaire for assessing the prevalence of rhinitis in children in epidemiological studies has been developed (Asher et al., 1995).

5.2.3 Atopic dermatitis

5.2.3.1 Definition

Atopic dermatitis is a disease that is difficult to define, because of the frequently subtle clinical manifestations, the lack of an identifying laboratory marker, and the lack of a distinguishing primary lesion. Generally, atopic dermatitis is a chronic cutaneous inflammatory

disease with a strong tendency of patients to overproduce IgE (Hanifin, 1987).

5.2.3.2 *Assessment*

The absence of diagnostic criteria for atopic dermatitis prompted Hanifin & Lobitz, (1977) and Hanifin & Rajka (1980) to suggest major and minor diagnostic criteria for atopic dermatitis based on clinical features. Disadvantages of these criteria were that many of them had no precise definition, that some were very infrequent, and that others were nonspecific. A minimum set of diagnostic criteria for atopic dermatitis was derived by the United Kingdom working party composed of dermatologists, family practitioners, paediatricians and epidemiologists (Williams et al., 1994a). These criteria included a history of flexural involvement, a history of dry skin, the onset under the age of 2 years, a personal history of asthma, a history of pruritic skin condition, and visible flexural dermatitis. The criteria were used for assessing the prevalence of atopic dermatitis in children in an international multiple cross-sectional study (Asher et al., 1995). The European Task Force (ETFAD, 1993) on atopic dermatitis has developed a scoring index for the severity of atopic dermatitis, which is based on a combination of symptoms such as erythema, oedema/papulation, oozing/crusts, excoriation and lichenification. Nevertheless, more objective tests, such as raised IgE and/or skin-prick test positivity, remain important tools for epidemiological research.

5.2.4 *Skin-prick test and serum IgE*

Skin-prick testing is commonly used to assess allergic sensitization in epidemiological studies. The test is easy to perform; serious local or systemic reactions occur only very rarely, and different test devices are available. However, results of different studies are often not comparable due to differences in allergen concentrations, type of needles used for pricking, and criteria for defining a positive reaction (Nelson et al., 1993). The most common criterion is that the diameter of the allergen wheal be >2–3 mm after subtraction of the reaction to the negative control (Pepys, 1994). Even well-standardized skin-prick tests may be subject to measurement error arising from different field workers and variations in the degree of skin reactivity in different racial groups or under different environmental conditions. Measurements of total and specific serum IgE may therefore provide valuable additional information on the atopic susceptibility and of the

atopic status of an individual. Total serum IgE, however, may also be raised in association with other conditions, such as parasitic infections.

5.2.5 Allergic contact dermatitis

Allergic contact dermatitis is the condition in which contact with haptens induces cell-mediated contact sensitization.

Patch testing is used to diagnose allergic contact dermatitis. The test should be performed by a skilled operator using standardized test materials and also with substances present in the patient's domestic or occupational environment which are considered to be possible sensitizers.

5.3 Asthma and atopy: prevalence rates and time trends in prevalence rates

Asthma is the most common single chronic disease in childhood. Most of the prevalence studies on asthma have therefore been conducted in children and adolescents. Many of these studies have also determined the prevalence of rhinitis and atopic dermatitis. In the last 20 years prevalence estimates have been reported from many different geographical regions in all five continents. Several studies were repeated after a number of years applying the same methods at different points in time, thereby providing information on time trends in prevalence. Estimates on asthma prevalence were mostly derived by questionnaires, often in combination with a lung function test and determination of bronchial hyperresponsiveness. In addition to questionnaire data, prevalence rates of atopic sensitization were assessed by skin-prick tests and, to a lesser extent, by determination of total or specific IgE. The overview given below is not intended to be comprehensive, but will give some insight into the geographical variation and recent time trends. The comparability of prevalence estimates between study centres, however, is limited as most studies applied different methods.

5.3.1 Europe

5.3.1.1 Prevalences

Between 1989 and 1992 a parental questionnaire, a skin-prick test and a cold air challenge test were administered to 9- to 11-year-old

children in western and eastern Germany. Atopic sensitization was more frequent in western German children living in Munich (5.9%), compared to children living in Leipzig and Halle in eastern Germany (3.9%). Bronchial hyperresponsiveness was also more prevalent in western Germany (8.3%) than in eastern Germany (5.5%) (von Mutius et al., 1994b). Hay fever and rhinitis were reported less often in Leipzig than in Munich (2.4% and 16.6% compared to 8.6% and 19.7%), whereas bronchitis was more prevalent in Leipzig. In contrast to atopic respiratory disease, the prevalences for atopic eczema were similar in the two study areas (von Mutius et al., 1992).

The occurrence of allergic diseases was studied during 1979 and 1980 on the basis of a questionnaire sent to the parents of 20 000 children 7, 10 and 14 years of age in three parts of Sweden with different climatic conditions (Aberg et al., 1989). The prevalence of asthma was significantly higher in the northern part of the country, and this higher prevalence could not be explained by other factors than by the cold and dry climate. In Viborg, Denmark, the frequency of rhinitis was 10.5%, of atopic eczema 7%, of urticaria 3.2%, and of asthma 4.5% among 5- to 16-year-old school children studied in 1990 (Saval et al., 1993). Asthma and rhinitis were more frequent among boys, while atopic eczema was more frequent among girls. For both sexes, the frequency of rhinitis increased during their years at school, while the frequency of skin symptoms decreased.

In a general practice in London, symptoms, atopic state and medical history were compared among 16- to 65-year-old patients with seasonal and perennial rhinitis (Sibbald & Rink, 1991b). The prevalence of rhinitis was 24%, 3% had seasonal symptoms only and 13% had perennial symptoms only. Distinguishing between atopic and non-atopic subjects by skin-prick testing with five common allergens revealed that subjects with seasonal rhinitis were more likely to be atopic. Moreover, subjects with seasonal rhinitis were also more likely to have eczema and to have a family history of hay fever.

Rates of reported eczema during early childhood have been studied by health visitor interview in three national cohorts of children born in England, Scotland and Wales in 1946 (at age 6 years), 1958 (at age 7 years), 1970 (at age 5 years) (Taylor et al., 1984). The reported rates in the three birth cohorts were 5.1%, 7.3% and 12.2%.

5.3.1.2 Time trends

From 1926 to 1961 the prevalence of asthma in Finnish men registered through the defence forces statistics ranged between 0.02 and 0.08%, whereas from 1961 to 1989 the prevalence rose from 0.29 to 1.79% (Haahtela et al., 1990).

In the United Kingdom several studies were conducted in different epidemiological settings to estimate the prevalence and time trends in the prevalence of allergic disorders. The different settings comprised patients of general practices (Fleming & Crombie, 1987; Sibbald & Rink, 1991a,b), national birth cohorts (Taylor et al., 1984), representative samples of school children in England (Burney et al., 1990), in South Wales (Burr et al., 1989), in the London borough of Croydon (Anderson et al., 1983; Anderson et al., 1994), in Aberdeen (Ninan & Russell, 1992), in the South Thames region (Barbee et al., 1987), and hospital admission statistics (Strachan & Anderson, 1992; Anderson, 1989). The latter showed between 1978 and 1985 an increase in the number of hospital admissions because of asthma in infants up to 4 years old (186%) and in 5- to 16-year-olds (56%) in the South West Thames region (Anderson, 1989). Changes in mode of referral, severity on admission and readmission ratio were also explored, but little evidence was found for a reduction in severity or change in readmission rate since 1978. These findings contrast with findings of two identical surveys that were conducted in 1978 and 1991 to explore prevalence changes and use of medical services (Strachan & Anderson, 1992). These data showed a substantial increase in self-referral together with an increase in readmission. A comparison of two surveys of morbidity carried out in 1970–1971 and 1981–1982 in general practices in England and Wales showed an increase in prevalence rates of asthma in men from 8.8 to 15.9% (Fleming & Crombie, 1987).

Between 1973 and 1989 the lifetime prevalence of wheezing among 12-year-old children in South Wales increased from 17 to 22%, a history of asthma rose from 6 to 12%, and current asthma from 4 to 9% (Burr et al., 1989). Increases occurred also in the frequency of history of eczema (5 to 16%) and of hay fever (9 to 15%), while wheezing not attributable to asthma remained constant. Increasing prevalences of asthma were also observed between 1973 and 1986 in English school children (relative increase in boys of 6.9% and in girls

of 12.8%), which could not simply be explained by changes in diagnostic fashion (Burney et al., 1990).

In Aberdeen, Scotland, the prevalence of wheeze among 8- to 13-year-old school children rose from 10.4% in 1964 to 19.8% in 1989, and shortness of breath rose from 5.4 to 10.0%. Wheeze and shortness of breath were more prevalent in boys than in girls. Asthma rose from 4.1 to 10.2%, hay fever from 3.2 to 11.9% and eczema from 5.3 to 12% (Ninan & Russell, 1992). Between 1978 and 1991 significant relative increases in the 12-month prevalence rates of attacks of wheezing and of asthma were found in the London borough of Croydon (Anderson et al., 1994). Results from a repeated cross-sectional study performed in 1991–1992 and 1995–1996 in 10-year-old children in Leipzig, Germany, showed a rise in hay fever and atopic sensitization. However, no increase in the prevalence of asthma or bronchial hyperresponsiveness was observed over the 4-year period (von Mutius et al., 1998).

5.3.2 Oceania

5.3.2.1 Prevalences

Several cross-sectional surveys were performed in Australian and New Zealand school children to assess the prevalence of respiratory symptoms and bronchial hyperresponsiveness. A comparison of 769 children living in Wagga Wagga (inland Australia), and 718 children living in Belmont (coastal Australia) carried out in 1982, showed that respiratory symptoms, asthma, bronchial hyperresponsiveness, hay fever and atopy were all more common in the dry inland area than in the humid coastal area. In both areas 38% of the children were atopic (Britton et al., 1986).

The prevalence of wheeze in Melbourne school children was 23.1% for 7-year-olds, 21.7% for 12-year-olds, and 18.6% for 15-year-olds. History of wheeze was more common for boys than for girls at age 7 years, but not at age 15 years. A history of asthma among 7-year-olds was reported for 46% of the children in 1990 compared to 19.1% in 1964 (Robertson et al., 1991).

When comparing school children living in New Zealand with school children living in South Wales in 1990, the prevalence of a history of asthma at any time was higher in New Zealand (17%) than

in South Wales (12%) (Barry et al., 1991). Wheeze ever and wheeze brought on by running were also higher in New Zealand than in South Wales. The sex ratio of asthmatic and wheezy children was similar. In a comparison of 12- to 15-year-old school children living in five regions in four countries, the prevalence of wheezing during the last 12 months, as assessed by both a written and a video questionnaire, was similar in West Sussex, England (29% and 30%), Wellington, New Zealand (28% and 36%), Adelaide, Australia (29% and 37%), and Sydney, Australia (30% and 40%), and was lower in Bochum, Germany (20% and 27%) (Pearce et al., 1993). One year prevalence of severe wheezing limiting speech was greater in Wellington (11%), Adelaide (10%) and Sydney (13%) than in West Sussex (7%) and Bochum (6%). In addition, the one year prevalence of frequent attacks of wheezing, frequent nocturnal wheezing, and doctor diagnosed asthma were higher in Australia and New Zealand than in the European centres.

5.3.2.2 *Time trends*

Between 1982 and 1984 the prevalence of bronchial hyperresponsiveness assessed by histamine challenge test among 2363 Australian children was 17.9% (Salome et al., 1987). The prevalence of respiratory symptoms, bronchial hyperresponsiveness, severity of bronchial hyperresponsiveness and bronchial hyperresponsiveness combined with symptoms was compared between children living in Auckland, New Zealand and two locations in Australia: Wagga Wagga in the inland and Belmont on the coast (Asher et al., 1988). The prevalences were similar in Auckland and Wagga Wagga, but lower in Belmont. When the same study was repeated 10 years later with Australian 8–10 year olds, the prevalence of wheeze had increased from 10.4% in 1982 to 27.6% in 1992 in Belmont and from 15.5 to 23.1% in Wagga Wagga. Bronchial hyperresponsiveness increased twofold up to 19.8% in Belmont and 1.4 fold up to 18.1% in Wagga Wagga (Peat et al., 1994). The prevalence of atopy remained unchanged. The reported asthma or wheeze in a Maori population rose from 26.2% in 1975 to 34% in 1989 (Shaw et al., 1990).

5.3.3 *Eastern Mediterranean*

The prevalence of asthma was studied in Israeli adolescents by means of computerized medical draft records of conscripts aged 17–18 years who were born over a 9-year period and examined up to the end

of 1989 (Laor et al., 1993). Asthma was more prevalent in males than in females and the prevalence of asthma increased over time. Risk of asthma was higher for subjects of Western origin and lowest for those of African origin. By area of residence the risk for asthma was highest in coastal and lowest in inland regions.

5.3.4 Africa

In Africa, 694 children from a Cape Town township and 671 from a rural area in Transkei performed an exercise tolerance test. Of the children living in the urban area 3.2% had asthma compared to 0.14% living in a rural district (Van Niekerk et al., 1979). The prevalence of reversible airway obstruction after running for 6 min was studied in 7- to 9-year-old school children living in three areas in Zimbabwe. Prevalence of airway obstruction was 5.8% in northern Harare, an urban area with more people of higher socioeconomic status, 3.1% in southern Harare and 0.1% in Wedza, both more rural areas. Prevalence in white children living in northern Harare was similar to the prevalence in black children in that region, (5.3% and 5.9%, respectively) (Keeley et al., 1991).

5.3.5 Asia

5.3.5.1 Prevalences

In 1992, the prevalences of hay fever, eczema and wheeze were estimated by parental questionnaire in secondary school students, ages 12 to 19 years, in the three south-east Asian cities Hong Kong, Kota Kinabalu and San Bu (Leung & Ho, 1994). The prevalences for hay fever were 15.7%, 11.2% and 2.1%, for eczema 20.1%, 7.6% and 7.2%, and for wheeze 11.6%, 8.2% and 1.9%, in the respective cities. Skin test reactivity to one of five common allergens was common and present in 49.0–63.9% of subjects. In Singapore, allergic rhinitis was studied in a cross-sectional population-based study of 2868 adults, aged 20–74 years (Ng & Tan, 1994b). Allergic rhinitis was reported by 4.5% of subjects.

5.3.5.2 Time trends

Two surveys in 7- to 15-year-old school children in Taipei, Taiwan, were conducted in 1974 and 1985 (Hsieh & Shen, 1988). The prevalence of childhood asthma increased from 1.3% in 1974 to 5.1% in 1985, with boys showing higher rates in both studies.

5.3.6 North America

5.3.6.1 Prevalences

Significant variation in asthma mortality between different geographical regions was reported. Regions with elevated rates included the central plain states and three large urban metropolitan areas: Chicago IL, New York NY and Phoenix AZ (Weiss et al., 1989).

5.3.6.2 Time trends

Asthma morbidity in Indian children and adults in Saskatchewan was registered using hospitalization data from 1970 to 1989. Asthma hospitalization was higher among boys than girls at age 0–4 years, but this was reversed at the ages of 15–34 and 35–64 years. Hospitalization for asthma had significantly increased for the age groups 0–4 and 35–64 years. Increases of asthma morbidity in recent years were also observed (Senthilselvan & Habbick, 1995).

The reported prevalence of ever having asthma increased among 6- to 11-year-old children between the first (1971 to 1974) and second (1976 to 1980) US National Health and Nutrition Examination Surveys (NHANES) from 4.8 to 7.6% (Gergen et al., 1988). Asthma was more common in blacks than in whites, in boys than in girls, and in urban than in rural areas.

Changes in the asthma prevalence between 1981 and 1988 were studied in the US National Health Interview Survey (NHIS) in 0- to 17-year-old children (Weitzman et al., 1992). The prevalence of parent-reported childhood asthma increased from 3.1 to 4.3%. Trends towards a lower rate of hospitalization and better overall health status of the asthmatics from 1981 to 88 were reported. The overall prevalence increased by 40%, which was mainly due to a prevalence increase in white children. However, the prevalence was still higher in blacks. There was no evidence of an increase in severity of asthma.

An example of the uncertainties in collecting and interpreting epidemiological data is provided by consideration of the age-adjusted death rate for asthma in the USA, which increased by 40% between 1982 and 1991. The increase was higher in females (59%) than in males (34%). The death rates were consistently higher in blacks than in whites. Prevalence rates for self-reported asthma increased also by

42%, with a much greater increase in females (82%) than in males (29%). Death rate and self-reported asthma increased but hospitalization rates remained stable. This was possibly due to an improved outpatient treatment and changed billing practices reflecting changes in the classification of cases of asthma under other diagnostic categories. Ethnic differences in self-reported morbidity may potentially be explained by accessibility to health services and socioeconomic factors (CDC, 1995). This is an example of the difficulties in collecting and interpreting epidemiological data.

5.3.7 The International Study of Asthma and Allergies in Childhood

The International Study of Asthma and Allergies in Childhood (ISAAC) was initiated to maximized the value of epidemiological research into asthma and allergic disease by establishing a standardized methodology and facilitating international collaboration (Asher et al., 1995). Its specific aims are:

a) to describe the prevalence and severity of asthma, rhinitis and eczema in children living in different centres, and to make comparisons within and between countries;

b) to obtain baseline measures for assessment of future trends in the prevalence and severity of these diseases;

c) to provide a framework for further etiological research into genetic, lifestyle, environmental and medical care factors affecting these diseases.

The first phase of the collaborative studies was completed by 155 centres in 56 countries and included more than 720 000 participants (Strachan et al., 1997). The core questionnaires used were designed to assess the prevalence and severity of asthma, allergic rhinitis and eczema in children. A 20-fold difference in the 12-month-period prevalence of wheezing in 13- to 14-year-old school children (range 1.6–36.8%) and an 8-fold variation between the 10th and 90th percentiles (3.9–30.6%) were observed (ISAAC Steering Committee, 1998) (Fig. 16). The 12-month-period prevalence of symptoms of allergic rhinitis in 13- to 14-year-olds varied from 1.4 to 39.7% with a four-fold variation seen between the 10th and 90th percentiles (4.9–21.0%) (Strachan et al., 1997; ISAAC Steering Committee, 1998). There was also a high variability in the 12-month-period

prevalences of atopic eczema (defined as flexural dermatitis), which varied from 0.3 to 20.5% (ISAAC Steering Committee, 1998).

5.3.8 Conclusion

Despite the differences in methodology between epidemiological studies conducted in the last 20–30 years, there is evidence that the prevalence of asthma and other atopic disorders is increasing in many countries (e.g., Burr et al., 1989; Burney et al., 1990; Ninan & Russell, 1992) (Fig. 17).

A change in genetic susceptibility of populations to the manifestation of these diseases is unlikely to explain the observed time trend. However, although the studies suggest an increase in prevalence, this may partly reflect changes in diagnostic fashion and in public awareness of these diseases. The scientific evidence for increases in the prevalence of asthma in children and young adults since 1970, for example, is still weak because the measures used are susceptible to bias (Magnus & Jaakkola, 1997). Such methodological problems can only be minimized if a methodology using standardized questions on disease severity, including objective measurements, is applied (see also section 5.15).

The ISAAC study in which such a standardized methodology was applied found a high worldwide variation in the prevalence and severity of asthma, rhinitis and eczema. In addition, countries such as Australia and New Zealand that have high prevalence rates of asthma in the ISAAC study are similar to those countries, including the United Kingom, with the highest prevalence rates for asthma symptoms in adults in the European Community Respiratory Health Survey (ECRHS) (Burney et al., 1996) and countries such as India or Algeria are in the lowest quartile for asthma prevalence in both studies. Together with the observed increases in the prevalences of these diseases in several countries during relatively short time periods of one or two decades, this variability in prevalence rates probably is a reflection of different lifestyle and environmental factors which seem to play an important role in the etiology of allergic diseases.

There are worldwide differences in the distribution of these diseases. Among the exogenous factors that have been suggested are changes in allergen exposure, outdoor and indoor pollutants, cigarette smoke, work place exposure, personal hygiene and changes in diet.

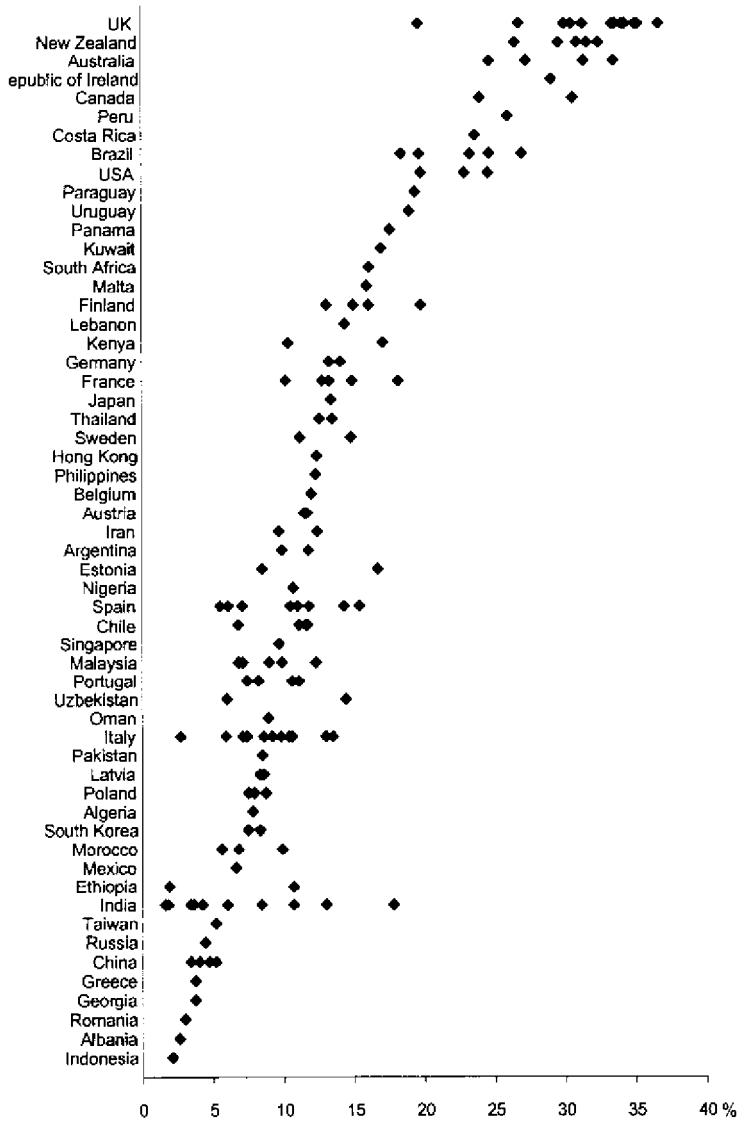

Fig. 16. The 12-month-period prevalence (%) of self-reported asthma symptoms in 13–14-year-olds (written questionnaire) for each ISAAC centre by country (ISAAC Steering Committee, 1992; with kind permission of *The Lancet*)

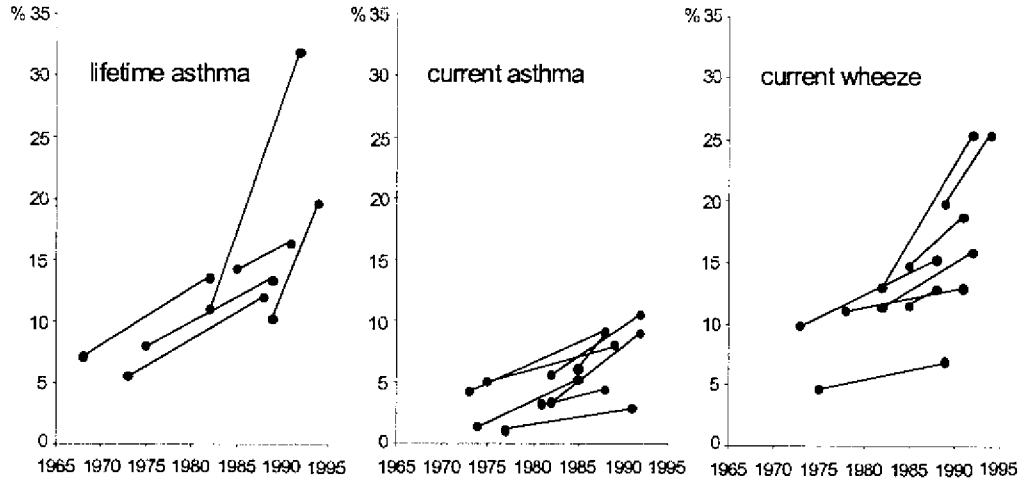

Fig. 17. Changes in the prevalence of asthma and wheezing among children and young adults in a number of repeated cross-sectional studies (adapted from Duhme et al., 1998b)

5.4 Age and gender distribution

The natural history of asthma is not well understood (Martinez et al., 1995). Many infants have episodes of wheezing associated with viral infections soon after birth and during the first years of life. However, the majority of these children have transient conditions (Martinez et al., 1995). The period prevalence of wheezing has a peak before the age of 10 years (Anderson et al., 1992). During infancy boys are more often affected than girls, but this difference seems to disappear during and after adolescence (Barbee, 1987; Anderson et al., 1992). Several studies have shown a higher prevalence of asthma symptoms and bronchial hyperresponsiveness (BHR) in female than in male adolescents (Shaw et al., 1991; Anderson et al., 1992; Pearce et al., 1993; Riedler et al., 1994).

Hay fever has a median age of onset of around 15 years. The prevalence has a peak in people aged 16–24 years. For perennial rhinitis the median age of onset is around 20 years and the prevalence reaches a peak in 20–30 year olds (Broder et al., 1974; Schachter & Higgins, 1976; Viner & Jackman, 1976; Sibbald & Rink, 1991a,b). A number of studies reported a slightly higher prevalence of allergic rhinitis in males than in females (Weeke, 1987). Atopic dermatitis starts typically during the first year of life (Hanifin, 1987). The onset of the disease is before the age of 7 in 60 to 90% of the cases (Schultz Larsen & Hanifin, 1992). The prevalence appears to be higher among females than among males (Schultz Larsen & Hanifin, 1992; Schultz Larsen, 1993).

5.5 Migration

Migration studies have suggested that the environment has a strong effect on the development of atopic disorders. Analyses of interregional migrants in the United Kingdom showed that the regional variation in cohorts, aged 5–7 years, was primarily related to the region of current residence, and not to the region of birth (Strachan et al., 1990). Ethnic group differences in the prevalence of atopic dermatitis were studied in London school children. It appeared that, compared to white children, London-born black Caribbean children were at increased risk of atopic dermatitis (Williams et al., 1995a).

Striking differences in prevalence rates of asthma were found in young urban and rural Xhosa children (Van Niekerk et al., 1979). In

this society, asthma was found mainly in urban communities and there appears to be a striking absence of asthma in the rural environment. Whether this is due to alterations in lifestyle or the possibility that the rural traditional way of life exerts a protective effect in the prevention of asthma is not clear.

The prevalence of asthma in Tokelauan children has been studied in two environments, in Tokelau and in New Zealand (Waite et al., 1980). Prevalence of asthma assessed by an interview of the mothers was much higher among those children who were examined in New Zealand than among those examined in Tokelau. Furthermore, for the children examined in New Zealand, there was no significant difference in the asthma prevalence between those children born in New Zealand and those born in Tokelau.

Asthma in children and adolescents living in the New Guinea Highlands was extremely uncommon in the sixties and early seventies. In 58% of the observed asthma cases the onset of the disease was not before the age of 30 years. Multiple sensitivities as assessed by skin-prick test were common and did not diminish with increasing age at onset. One possible explanation was that the degree of exposure to allergens is high enough to cause sensitization, but not high enough to cause lower respiratory symptoms until prolonged exposure in a perhaps especially sensitive person has taken place (Anderson, 1974). The prevalence of asthma among adults but not children living in the Eastern highlands of Papua New Guinea has risen drastically between 1975 and 1985. Allergy to house dust mite appeared to be a significant feature in the disease pathogenesis, and it is likely that this is associated with modifications to traditional life style due to the introduction of blankets and changes in sleeping habits, which promote a more fertile environment for growth and multiplication of mites (Dowse et al., 1985).

5.6 Viral infection

Findings of different studies suggest that viral infections in early life may play a part in the prevention of allergic sensitization (Martinez, 1994). Interesting observations with regard to a possibly preventive role of viral infections in the development of asthma were made in the population of Tristan da Cunha. The prevalence of asthma among these islanders had earlier been reported to be one of the highest in the world (Mantle & Pepys, 1974). After the evacuation

from Tristan da Cunha because of a volcanic eruption immunoassays in blood samples of the islanders revealed a low prevalence of serum antibodies against common viruses. During the years of evacuation, when the islanders lived in the United Kingdom, a high incidence of respiratory infection was observed. Thus, this population with a high prevalence of asthma and a high prevalence of allergic sensitization had a very low incidence of respiratory infections while living on the remote island, and became heavily infected after being exposed to respiratory viruses that they had probably rarely encountered before. Similar observations were made in the Western Carolina islands where the prevalence of asthmatic symptoms among children was very high, but viral infections presumably very uncommon (Martinez, 1994). A study from the United Kingdom found an inverse association between hay fever and the number of older siblings (Strachan, 1989; Strachan, 1995). Factors directly or indirectly related to the number of siblings may decrease the susceptibility of children to become atopic. Again, infections acquired during early childhood were proposed to be protective against allergic sensitization. It has been speculated that declining family size may in part contribute to the increasing prevalence of atopic diseases reported in Western countries over the past few decades, because of a lower chance of infection by older siblings. In children from eastern and western Germany, allergic sensitization as assessed by skin test reactivity was also inversely associated with the number of siblings (von Mutius et al., 1994b).

5.7 Socioeconomic status

Hay fever has been recognized as a complaint of the more affluent classes since the 19th century. A study of adults in south London found little difference in the prevalence of rhinitis symptoms or skin-prick reactions by social class, but a greater use of the label "hay fever" by doctors for patients of higher socioeconomic status (Sibbald & Rink, 1991a).

Eczema is more prevalent in British school children of higher socioeconomic status than in those of lower status. Exposures associated with social class are probably at least as important as genetic factors in the expression of childhood eczema (Williams et al., 1994b). The authors suggest that most or even all of the reported changes in the prevalence of atopic dermatitis are due to a secular trend in diagnosis. Population studies have used a range of different methods and definitions for atopic dermatitis ranging from

questionnaire-based recall of eczema as a child or parental recall to health visitors' and general practitioners' records. It is likely that the term eczema is heavily biased by social class (allergy being a more acceptable term in higher social classes) and with time (eczema was a less acceptable label in earlier years due to connotations of uncleanliness and arthropod infestation).

Hospital admissions for eczema have fallen over the last 20 years, but such in-patient data are misleading as much of this reduction is probably due to the success of treatment with topical corticosteroid preparations (Williams, 1992).

The prevalence of wheeze varied little by socioeconomic group in an investigation of 5472 children, aged 5–17 years, in the United Kingdom (Strachan et al., 1994). However, marked trends of severity towards increased morbidity in poorer families were observed. Diagnostic labelling and drug treatment of wheezy children did not differ substantially with socioeconomic status. Thus, a degree of socioeconomic equality existed in the process of medical care for childhood asthma in the United Kingdom (Strachan et al., 1994; Strachan, 1996).

No effect of socioeconomic status on the prevalence of asthma was noted in the first and second US National Health and Nutrition Examination Surveys (NHANES) (Gergen et al., 1988). In the Auckland region, 1050 children aged 8–9 years were examined by parental questionnaire and histamine inhalation challenge (Mitchell et al., 1989). There was no relationship between socioeconomic status and asthma diagnosis, bronchial hyperresponsiveness, or any combination of bronchial hyperresponsiveness with symptoms or diagnosis. The relative importance of socioeconomic status and several other factors in the etiology of wheezing illness in the first 5 years and on the persistence of this illness at the age of 16 years was studied in over 15 000 children born in the United Kingdom during one week of April 1970. Persistence of wheeze at age 16 years was related to high social status (Lewis et al., 1995).

During a study of the prevalence of asthma and bronchitis in Sydney school children, some social and environmental factors were documented to ascertain if these affected the prevalence of either of these diseases (Peat et al., 1980). No consistent relationship was found between social class and lung disease with the exception of increased

prevalence of asthma in boys and girls of higher socioeconomic status. Differential access and utilization of medical care by the poor and rich may contribute to differences in asthma prevalence. The relationship of socioeconomic status to various indicators of asthma was studied in Canada in the context of universal access to medical care. As compared with children from the most advantaged homes, children from the least advantaged homes were more likely to present exercise-induced bronchospasm, while there was no excess of reported wheeze or diagnosed asthma. This result was interpreted as indicating that there are unidentified environmental factors that contribute to the excess asthma morbidity in children (Ernst et al., 1995). In general, the association of socioeconomic status with hay fever and eczema seems to be more consistent than with asthma and respiratory symptoms.

5.8 Occupational exposure

Many prevalence studies have been conducted among workers in high-risk occupations. Asthmatic workers may differ with regard to the frequency of attacks, to the occurrence and to the onset of airway obstruction. Diagnostic guidelines for occupational asthma were internationally proposed in the USA and in Europe (Cartier et al., 1989; Burge, 1989; EAACI, 1992). The diagnosis of occupational asthma is based both on the clinical signs of the disease and the demonstration of the occurrence of a recognized allergen in the patient's workplace. Objective tests, such as skin testing, spirometry and serial measurements of the peak expiratory flow, should be performed to ascertain the occupational nature of the disorder (see also section 4.4.4). Other diagnostic tools in epidemiological surveys are standardized questionnaires inquiring information on work-related symptoms. Typical symptoms of occupational asthma are symptoms comprising difficulty in breathing, chest tightness, wheezing, a period of initial exposure of 2 weeks or longer before the first onset of symptoms, or evidence of airflow obstruction, and improvement of symptoms when the subject is not working for days or longer.

Surveillance programs in the United Kingdom and Canada indicate that occupational asthma is the most frequently reported occupational lung disease accounting for 26 to 52% of the reports (Chan-Yeung & Malo, 1995a). The proportion of asthma attributable to occupational exposure is not known. Estimates range from 2 to 15%. The role of occupational exposure is difficult to ascertain, because: a) occupational asthma is still poorly recognized; b) affected

workers are scattered through many small workplaces often employing few workers; c) methods of confirming work relationships are often rather crude; d) there is no good reporting system of occupational asthma in many countries; e) there are very few screening or surveillance programmes for this condition.

Owing to such methodological problems it is often not possible to give unbiased estimates of the true prevalence and incidence of occupational asthma in populations if official government statistics such as disabling benefit awards or worker's compensation boards are used and comparisons within and between countries may be distorted (Meredith & Nordman, 1996).

Occupational asthma falls into two categories: a) pre-existing asthma that is aggravated by irritant or physical stimuli in the workplace and b) asthma that is specifically induced by sensitization to a workplace chemical (Jarvis et al., 1996). About 250 agents that can give rise to occupational asthma are recognized (Chan-Yeung & Malo, 1995a). Isocyanates are responsible for the most common form of the disease, i.e., occupational asthma with latency. Occupational asthma without latency follows exposure to high concentration of irritant gases, fumes or chemicals; chlorine and ammonia are the most common agents (Chan-Yeung & Malo, 1995a,b). Some substances may give rise to asthma by inducing specific IgE antibodies. These allergens are mostly substances of high relative molecular mass (>5000) such as proteins in the urine of laboratory rats. Others are compounds of low relative molecular mass, such as complex halogenated platinum salts or acid anhydrides; these agents act as haptens and combine with a body protein to form a complete antigen (Venables & Chan-Yeung, 1997). It has been proposed that certain chemical allergens may cause sensitization of the respiratory tract via an IgE-independent mechanism. However, it is possible that new or technically refined IgE measurements may reveal a much greater association between IgE antibodies and chemical respiratory sensitization than is presently assumed (Kimber & Wilks, 1995). Intermittent exposure to high levels of an occupational agent is associated with a higher risk of development of work-related asthma than a steady exposure to lower concentrations, typical for isocyanate exposure.

Not all subjects develop occupational asthma under the same exposure conditions. Various host markers (genetically determined)

and factors (acquired) have been incriminated in occupational asthma. In general, atopy appears to be an important risk factor for occupational asthma due to compounds of high relative molecular mass, such as enzyme detergents or exposure to laboratory animals (Chang-Yeung, 1990). In a survey of workers exposed to flour in bakeries or mills, the relation with symptoms was independent of atopic status (Cullinan et al., 1994). Atopy has little predictive value in occupational asthma due to chemicals of low relative molecular mass, and routine screening for atopy in high-risk workplaces may not be justified (Chan-Yeung & Malo, 1995b).

The effect of smoking on occupational asthma is not clear and appears to be dependent on the type of occupational agent. When the agent induces asthma by producing specific IgE antibodies, cigarette smoking may enhance sensitization. However, cigarette smoking was not associated with increased work-related asthmatic symptoms in workers exposed to detergent enzymes, laboratory animals or colophony (Chan-Yeung, 1990; Chan-Yeung & Malo, 1995a,b).

As part of the European Community Respiratory Health Survey (ECRHS), the risk of occupational asthma has been estimated in a random sample of 2646 young Spanish adults aged 20–44 years. Depending on the definition of asthma, 2.6–6.7% of asthma cases were attributable to occupational exposures (Kogevinas et al., 1996). Incidence rates for occupational asthma for 1992 were estimated to be approximately 153 cases per million workers in Finland (Meredith & Nordman, 1996). Incidence rates reported for the United Kingdom in the Surveillance of Work-related and Occupational Respiratory Disease Project (SWORD) between 1989 and 1991 showed a high risk of occupational asthma among paint sprayers, chemical and food processors, laboratory staff, plastics and metal treatment workers, and in welders and electronic assemblers (Meredith, 1993). For 1993 the incidence was estimated to be 37 per million working persons per year (Meredith & Nordman, 1996). The most frequent agents causing asthma among the organic agents were flour, grain, hay, wood dust and laboratory animals; among the chemical agents were isocyanates and glutaraldehyde, and some miscellaneous compounds such as solder, colophony, glues and resins (Meredith & McDonald, 1994).

In the UK SWORD project there is a high level of national coverage by chest physicians participating in the surveillance project and estimates of the working population at risk are available, so that

incidence rates can be calculated by age, gender, region and occupation. However, there is underestimation, because some patients are seen only by general practitioners. Questions remain regarding diagnostic accuracy and etiology.

In the following sections, studies on specific occupational exposures in relation to occupational atopic diseases, with emphasis on occupational asthma, are reviewed. The selection of exposures is based on findings of the SWORD project and the availability of epidemiological data.

5.8.1 Chemicals with low relative molecular mass

A total of 314 cases of occupational asthma were diagnosed at the Institute of Occupational Health in Helsinki, Finland during the period 1987 to 1990 (Savonius et al., 1993). By far the most common causes of occupational asthma were low relative molecular mass chemicals such as the isocyanates (76 cases), followed by formaldehyde (18 ases), epoxy resin and epoxy resin hardeners (17 cases), and cyanoacrylates (6 cases).

5.8.1.1 *Diisocyanates*

The main occupational hazards caused by polyurethane chemicals are asthma and rhinitis, but contact dermatitis and urticaria may also develop (Estlander et al., 1992). Exposures to toluene diisocyanate were studied for effects on respiratory health of workers in two plants manufacturing polyurethane foams (Jones et al., 1992). Intensive personal monitoring was performed to characterized job exposures. Initial questionnaire and spirometry data were obtained in 386 workers. Multiple regression analyses showed significant adverse effects of cumulative toluene diisocyanate exposure on airway responsiveness. According to Diller (1987), the incidence of isocyanate asthma reported from different studies varies between 0 and 25%. Reasons for differences in observed incidence are intensity of isocyanate exposure, criteria for diagnosis, mode of calculation, sensitizing capacity of different isocyanates, individual predisposition and confounding factors. No geographical or ethnic difference was observed.

5.8.1.2 Acrylates

Acrylate monomers are used in a variety of industrial fields. Their industrial use is increasing, since they have many features that make them superior to formerly used chemicals (Savonius et al., 1993). Contact sensitization is a well-known adverse health effect of exposure to acrylates, but they may also cause respiratory symptoms. The main acrylic compounds currently in use are acrylates, cyanoacrylates and methacrylates. While methacrylates are well-known contact sensitizers, cyanoacrylates have caused only few cases of contact allergy. Acrylates may also have other harmful health effects. Hand and finger symptoms and paraesthesiae have been reported among dental personnel preparing acrylates with their hands. The domestic use of acrylates is limited and the sensitizing problem is mainly occupational, but probably occurs in many different industries. Cyanoacrylates are used mostly as a component of a high strength glue used for joining different materials. Methacrylates are used as adhesives, as dental and orthopaedic fillings, as material for protheses and as an embedding material for different purposes, including histological preparations.

5.8.1.3 Anhydrides

Methylhexahydrophthalic anhydride (MHHPA) and methyltetrahydrophthalic anhydride (MTHPA) are dicarboxylic anhydrides used as hardeners for epoxy resins. MHHPA and MTHPA typically require an elevated curing temperature (50–200 °C), which facilitates escape of anhydride vapours. Anhydrides are low relative molecular mass chemicals that have been reported to cause immunologically mediated respiratory diseases (Tarvainen et al., 1995). Contact urticaria and other skin symptoms have also been described. Some anhydrides, e.g., phthalic anhydride, have caused generalized urticaria, in connection with respiratory symptoms, after high exposure.

5.8.1.4 Solder flux

Questionnaires and lung function measurements were administered to 104 electronic workers in the USA who soldered printed circuits boards. Symptoms of eye, throat and nose irritation occurred in nearly half of the group. Lower respiratory tract symptoms, including cough, phlegm production and wheezing, also occurred with increased frequency, compared with reported rates among a general population sample (Greaves et al., 1984).

5.8.2 Metals

5.8.2.1 Cobalt

Several clinical and experimental findings point to cobalt as a main sensitizer and causal agent of hard metal asthma (Nemery et al., 1992; Swennen et al., 1993; Cirla, 1994; Lauwerys & Lison, 1994). Clinical features have been clearly identified by bronchial provocation tests. IgE and IgG antibodies with cobalt specificity have been demonstrated. Clinically, the ability of cobalt to induce delayed hypersensitivity is well known for contact dermatitis (Cirla, 1994). Occupational exposure to cobalt occurs mainly by inhalation in various industries and occupations involved in the production and processing of metal and various cobalt-containing alloys and salts. The potential for exposure to cobalt is particularly important during the production of cobalt powder, the production and processing and use of hard metals, the polishing of diamonds with cobalt-containing disks, the use of pigments and dryers containing cobalt salts and the processing of cobalt alloys. In a cross-sectional survey of 194 workers from 10 diamond polishing workshops and 59 workers from 3 other workshops, a questionnaire was administered and spirometry was performed to assess whether exposure to cobalt was associated with respiratory impairments (Nemery et al., 1992). Spirometry showed significantly lower indices of ventilatory function in the group with the highest exposure to cobalt. These differences were not due to differences in smoking habits.

5.8.2.2 Metal-polishing industry

A comparative study of spirometric measurements in 104 polishers and 90 unexposed controls was carried out in 25 brass and steel ware polishing industries in Moradabad, India (Rastogi et al., 1992). The polishing process generates dust, containing fine particles of emery and metal, which are mainly composed of copper and zinc and constantly inhaled by the polishers. Of the polishers 58.6% had one or more respiratory symptoms as compared to 25.5% of the controls. Occupational asthma was found to be confined to polishers, 4.8% being affected. The polishers exhibited significantly greater reduction in various lung function parameters over the work shift, which was larger in smokers than in non-smokers. The duration of exposure was directly correlated with acute fall in lung function.

5.8.2.3 *Aluminium*

Occupational asthma is a major respiratory health problem within the primary aluminium industry (O'Donnell, 1995). In Australia and New Zealand the incidence of occupational asthma in primary aluminium smelting varies between smelters, estimates ranging from 0 to 2%. Cases showed no association between the frequency of the symptoms or the severity of bronchial hyperresponsiveness and a family history of asthma, atopic skin test, tobacco smoking or age. Current evidence suggests that occupational asthma in the aluminium industry is irritant induced and caused by inhalation exposure to gaseous or particulate fluoride compounds.

5.8.2.4 *Platinum salts*

Nonspecific and specific bronchial hyperresponsiveness in immediate-type asthma caused by platinum salts did not cease after removal from exposure (Merget et al., 1994) (see also section 4.3.4.1).

5.8.3 *Natural rubber latex*

Populations at increased risk of developing natural rubber latex hypersensitivity include health care workers, rubber industry workers and subjects undergoing multiple surgical procedures, especially children with spina bifida and urogenital abnormalities. Prevalence figures for natural rubber latex allergy in studies using skin-prick tests range from 2.9 to 17% among hospital employees and are around 11% among glove-manufacturing workers (Vandenplas, 1995). Natural rubber latex allergy has been demonstrated in 32 to 50% of the children with spina bifida by skin-prick test or serological testing. The prevalence of sensitization to natural rubber latex in the general population ranged from 0 to 9% according to the atopic status of the populations under study (Turjanmaa, 1994; Vandenplas, 1995). The observed rise in incidence of sensitization to natural rubber latex during the last decade is probably related to the increased use of natural rubber latex devices as a protective barrier against infections. Other possible determinant factors include increased recognition of natural rubber latex allergy by exposed workers and clinicians, changes in manufacturing methods, and discontinuation of steam sterilization.

Complications were thought to be limited to contact dermatitis due to irritation and sensitivity to certain rubber additives, and only

few reports of phenomena consistent with natural rubber latex sensitivity were published before 1984. A possible explanation for the abrupt rise in the incidence of natural rubber latex sensitivity is a change in rubber manufacturing or rubber processing. There is a lack of epidemiological studies observing the longitudinal trends in prevalence and natural history of natural rubber latex allergy. The risk for natural rubber latex allergy among health care workers appears to vary with the frequency and intensity of exposure. Cross-reactivity between natural rubber latex and certain foods, particularly banana, avocado, chestnut and other fruits such as kiwi, papaya and passion fruit, have been described (Charous, 1994).

A questionnaire and skin-prick tests with natural rubber latex and common inhalant allergens were administered to hospital personnel (201 nurses, 50 members of the cleaning staff, 38 laboratory technicians), of whom 4.7% showed skin reactivity to latex. Among those with a negative skin test to natural rubber latex no one had a history of occupational asthma. For the latex-sensitive subjects (N=12), a histamine challenge test was performed, which showed bronchial hyperresponsiveness in all subjects. Seven of the twelve developed significant bronchial response to a challenge test with latex gloves. The overall prevalence of occupational asthma due to natural rubber latex was estimated as 2.5% (Vandenplas et al., 1995).

A skin-prick test was performed on 77 surgeons and nurses with an allergen solution made from latex gloves and rubber latex catheters. In addition, subjects answered a questionnaire on the history of hand dermatitis, atopic eczema, rhinitis or asthma, and symptoms when using natural rubber latex gloves. The prevalence of relevant immediate allergy assessed by skin-prick test was 5.2%. The reliability in detecting sensitized persons was limited. The agent used previously in skin-prick testing to demonstrate natural rubber latex sensitization gave unreliable results; the use of a standardized reagent is necessary (Cormio et al., 1993).

Among 512 hospital employees 7.4% of the doctors and 5.6% of the nurses working in operational units were allergic to natural rubber latex. The frequency was lower in non-operating units and among laboratory personnel (Turjanmaa, 1987).

5.8.4 Flour

Occupational respiratory diseases are common among bakers (Keskinen et al., 1978; Meredith, 1993; Reijula & Patterson, 1994; De Zotti et al., 1994; Lauwerys & Lison, 1994). In Finland, for example, the mean annual incidence of occupational respiratory diseases was 31 per 100 000 among the general work force compared to 374 per 100 000 among bakery workers (Reijula & Patterson, 1994). The annual incidence of occupational asthma as reported to the SWORD project in the United Kingdom was 334 per million for bakery workers as compared to 658 per million for paint sprayers or 175 per million for electronic assemblers (Meredith, 1993). A substantial prevalence of wheat flour allergy was found in bakers and pastry cooks (Armentia et al., 1990). Not only wheat allergens, but also α-amylase must be considered as the causative agent (De Zotti et al., 1994). Other allergens such as storage mites are suspected to play a role in the development of occupational asthma (Tee et al., 1992a,b). Findings from the initial cross-sectional phase of a cohort study of employees exposed to flour in bakeries or mills showed that subjects without previous exposure to flour expressed related symptoms especially to flour aeroallergen (Cullinan et al., 1994). Forty-four male workers exposed to flour and 164 unexposed controls were examined by using personal samplers measuring inspirable dust concentrations. The proportion of subjects with one or more symptoms and with bronchial hyperresponsiveness was significantly greater among workers exposed to flour than among controls. The conclusion was drawn that despite exposure to relatively low concentration levels of inspirable flour dust, subjects working in the baking industry are at risk of developing both respiratory symptoms and airway hyperresponsiveness (Bohadana et al., 1994).

5.8.5 Animals

The occurrence of respiratory disease was studied in 257 active veterinarians and 100 control subjects who had no occupational animal contact. Asthma was significantly more prevalent in veterinarians than in controls (Lutsky et al., 1985). Selected indicators of allergy and atopy were studied to determine predictors of laboratory animal allergy in a prospective study of laboratory technicians. Although the prevalence of atopy and allergic symptoms had increased in exposed technicians after the follow-up period of 2 years, this was also found in an unexposed matched control group, and there were no significant

differences between the groups in any measured variable at follow-up (Renstrom et al., 1994).

5.8.6 Other agents

The association between occupational exposure to low levels of airway irritants and bronchial responsiveness to histamine was assessed in 688 male workers of synthetic fibre plants (Kremer et al., 1995). According to job titles, exposure status was grouped into 7 categories: 1) reference group, 2) white collars, 3) SO_2, HCl, SO_4^{2-}, 4) polyester vapour, 5) oil mist and oil vapour, 6) polyamide and polyester vapour, 7) multiple exposures. A higher prevalence of airway responsiveness was associated with history of allergy and respiratory symptoms. A slight trend was seen for subjects with more than 5 years of exposure to polyester vapour and oil mist and oil vapour towards a higher prevalence of bronchial hyperresponsiveness. No overall association could be demonstrated.

5.9 Allergic contact dermatitis

5.9.1 Epidemiology of allergic contact dermatitis

Only a few studies have investigated the frequency of allergic contact dermatitis in the general population (Coenraads & Smit, 1995). Nielsen & Menné (1992) studied the distribution of allergic contact sensitization in an unselected sample of 793 individuals. Of these, 567 participants were patch tested with the standard series (TRUE-test). This series included the most common contact sensitizers, such as metals, fragrances, preservatives and medicaments. It has been found that 50–80% (depending on patient selection) of all cases of allergic contact dermatitis will be diagnosed using this series (Menné et al., 1992). Among the 567 volunteers were 15.2% who positively reacted to one or more of the substances included in the test series. Multiple contact sensitizations were thus observed more commonly than expected (Nielsen & Menné, 1992). Patients evaluated at patch test clinics often have multiple contact allergies. In experimental studies individuals with one contact allergy were found to be more easily sensitized to a second one (Friedmann, 1990). In the clinical situation the causes of multiple sensitivities are difficult to evaluate, because factors such as genetic susceptibility, broken skin barrier and multiple exposures are mixed up. Multiple contact sensitivities are typically seen in individuals with long-lasting chronic dermatitis.

In the following paragraphs, specific exposures closely related to allergic contact dermatitis are discussed.

5.9.1.1 *Nickel*

Nickel-containing metal alloys and nickel-plated surfaces release free nickel ions when in direct contact with human sweat. The typical nickel dermatitis is therefore located beneath metal items, such as buttons, jewellery, suspenders, glasses and similar objects. Among the 567 participants of an unselected sample of 793 subjects were 11.1% women and 2.2% men affected with a nickel allergy (Nielsen & Menné, 1993) (see Table 18; section 4.1.5.1). Individuals primarily sensitized from consumer items might, at a later stage, develop occupational nickel dermatitis when occupationally exposed to this metal. Tools and equipment used in different jobs by workers such as carpenters, electricians, painters and plumbers were found to release nickel (Lidén et al., 1996).

5.9.1.2 *Chromates*

Chromium salts, but not metallic chromium, are sensitizing. The hexavalent salts are the main sensitizers as they penetrate the skin more easily than the trivalent chromate (Gammelgaard et al., 1992). Chromate was found to be a cause of occupational hand eczema among employees in the construction industry, mainly because of the presence of hexavalent chromate in wet cement (Irvine et al., 1994). The irritating capacity of cement (abrasive and high pH), combined with the potent allergen hexavalent chromate, caused occupational hand eczema in up to 10% of the workers. Addition of ferrosulfate to cement reduces hexavalent chromate to the trivalent state which has a minimal bioavailability. Introduction of ferrosulfate addition to cement in the Scandinavian countries has significantly reduced occupational hand eczema (Avnstorp, 1992).

5.9.1.3 *Fragrances*

Allergic contact dermatitis can also be caused by fragrances (Johansen & Menné, 1995; Johansen et al., 1996a, 1997; Scheinman, 1996). In a study by Nielsen & Menné (1992), 1%–2% of the 567 participants were affected by fragrance allergy (Table 18). Geier & Schnuch (1996) found a frequency of fragrance allergy of 8 to 17% among eczema patients.

5.9.1.4 Preservatives

Preservatives are widely used in water-based cosmetics, households, and industrial products. Preservatives have antimicrobial effect against a wide range of microorganisms. The most widely used preservatives in cosmetics are the parabens, formaldehyde and formaldehyde releasing substances (quaternium 15, diazolidinyl urea, imidazolidinyl urea) and the isothiazolones (Andersen & Rycroft, 1991). Formaldehyde and isothiazolones are also frequently used in industrial products. In a British study, the frequency of contact allergy in dermatology patients varied between 1 and 3% for different preservatives (Jacobs et al., 1995). In another study the prevalence of contact allergy to formaldehyde in eczema patients was as high as 8% (Flyvholm et al., 1997).

5.9.1.5 Medicines

The frequency of allergic contact dermatitis to topical medicines varies considerably from country to country, and even within regions, depending upon the availability and product preference by local prescribing doctors. Most cases of allergic contact dermatitis to medicines are caused by substances having doubtful documented effect or which can be replaced by other medicines (Angelini, 1995). Systematic studies have shown that 1–3% of eczema patients are contact-sensitized to topically used steroids (Dooms-Goossens & Morren, 1992; Lauerma, 1992). In addition, traditional remedies, such as balsam of Peru and Propolis, are well-known sensitizers (Li, 1995).

5.9.1.6 Plants and woods

Plants and woods contain a diversity of strong and weak allergens (Ducombs & Schmidt, 1995). Allergic contact dermatitis to plants and woods usually presents itself with acute oedematous bullous lesions that spread to skin areas distant from the primary contact. Airborne and dustborne patterns can be seen with severe facial dermatitis and flexural dermatitis. In North America poison ivy and poison oak are important plants because of their high content of urushiols, which are potent allergens (Kligman, 1958a,b). These allergens are also present in plants and trees in Asia and Australia. Allergic contact dermatitis from these plants represents an occupational health problem for outdoor workers.

The *Compositae* family comprises 13 000 species. It includes decorative flowers such as chrysanthemums and dahlias, but also a number of common weeds and salads. Some cases of *Compositae* dermatitis present an airborne pattern often with a photo-aggravated facial dermatitis. This type of dermatitis is caused by different weeds in Europe, North America and Australia. Another variant of the disease has been recognized, giving a high morbidity and some mortality, in rural areas in India. The disease has been termed parthenium dermatitis after the offending plants (*Parthenium hysteropherous*) (Lonkar et al., 1974). The main allergens in *Compositae* plants are different sesquiterpene lactones. In consecutive patch-tested eczema patients in Northern Europe 1–3% reacted to the sesquiterpene lactone mix (Ducombs et al., 1990). Most of these were hobby gardeners with severe hand eczema, but there were some cases among professional gardeners. The development of the sesquiterpene lactone mix illustrated how new diagnostic technologies can change the understanding of allergic skin diseases. The development of these techniques for routine testing has dramatically changed the prognosis for these patients, as the correlation between plant contact and their hand eczema was unrecognised earlier.

5.9.2 Lack of a relationship between atopy and allergic contact sensitization

Atopic hospital employees, who performed wet work, had a similar prevalence of contact allergy (22%) to that of their non-atopic colleagues (21%) (Lammintausta et al., 1982). In a hospital-based case series, the prevalence of contact allergy against ingredients of topical medications was common in subjects with atopic dermatitis. However, the sensitization rate to multiple other substances was low among these patients (Lammintausta et al., 1992). In another hospital based-study of patients with hand eczema, participants with past or present atopic disease showed a positive patch test reaction in a significantly lower proportion than non-atopic individuals. Furthermore, of all participants with a history of atopy, 22% had developed allergic contact dermatitis, while the corresponding figure for non-atopics was 45% (Rystedt, 1985). The reason for the apparently lower prevalence of allergic contact dermatitis in atopics might be an abnormal function of T-lymphocytes in atopic patients (Menne et al., 1987; Hanifin & Chan, 1995). However, a population-based study in Norwegian school children indicated a higher rate of contact allergies in atopic compared

to non-atopic participants (Dotterud & Falk, 1994; Dotterud & Falk, 1995).

5.10 Diet

It has been hypothesized that changes in food habits are related to the increased prevalence of atopic diseases. Seaton et al. (1994) suggested that the increase in atopic disorders in the United Kingdom may in part be a result of a change in diet. Between 1961 and 1985 the average weekly consumption of fresh fruit, green vegetables, potatoes, fresh fish and red meat was reduced in Britain. The authors argue that a reduced dietary intake of natural antioxidants is related to a higher susceptibility to oxidant attack and airway inflammation. The food groups that were consumed less frequently between 1961 and 1985 are main sources of antioxidants, such as vitamin C and β-carotene, ubiquinone, and cofactors for antioxidant defence mechanisms, such as selenium, zinc and copper.

The relationship between certain food groups and the risk of asthma was recently studied by Hodge et al. (1996). In this study, diet was assessed in 574 children by a detailed food frequency questionnaire including 200 food items, which was related to airway disease defined by respiratory symptoms or airway responsiveness to exercise. Children who ate fresh, oily fish had a significantly reduced risk of current asthma. No other food groups or nutrients were significantly associated with either an increased or reduced risk of current asthma (Hodge et al., 1996). The results of a cross-sectional study in Leipzig, Germany, indicated that a change towards a higher consumption of margarine was positively associated with hay fever; in turn, changes in the consumption of butter showed an inverse association with hay fever and atopic sensitization (von Mutius et al., 1998).

Adverse reactions to food are a commonly encountered condition, especially in infancy and early childhood. The highest prevalence occurs between 1.5 and 3 years of age when in this age group as many as 25% have been reported to have adverse reactions to food (Björksten & Kjellman, 1987). However, only a minority of these reactions depend on immunological mechanisms (i.e., allergy) (Björksten & Kjellman, 1987). Epidemiological studies on the most important dietary factors in relation to atopic diseases are reviewed below.

5.10.1 Breast feeding

The prophylaxis of atopy has been sought by elimination diets and by other preventive measures (Saarinen & Kajosaari, 1995). The role of breast feeding and/or avoidance of formulas based on cows' milk in early infancy has been the focus of much controversy (Anonymous, 1982). Small amounts of protein ingested by the mother are secreted unchanged into breast milk. Potentially allergenic food ingested by the mother may thereby be transferred to the infant and cause sensitization. For this reason, maternal dietary restriction during lactation has been recommended (Hattevig et al., 1989). A prospective, long-term follow-up study from infancy to early adulthood indicated that breast feeding can protect against development of atopic disease (Saarinen & Kajosaari, 1995). Differences between the infant feeding groups were identified for atopic eczema, food allergy and respiratory allergy. Breast feeding for longer than 1 month without other milk supplements offers prophylaxis against food allergy at 3 years of age and also against respiratory allergy at age 17 years. Six months of breast feeding was required to prevent eczema during the first 3 years and possibly also to prevent substantial atopy in adolescence. Thus in this study, breast feeding seemed to confer long-term protection against allergic sensitization.

In a trial to examine the effect of different feeding patterns on the incidence of atopic disease in newborns with a family history of atopy, the incidence of atopic symptoms in the control group of infants was 27% for those with a single relative with atopy and 50% for infants with a biparental history of atopy (Bardare et al., 1993). Breast feeding effectively reduced the incidence of atopic symptoms when the mothers complied with prescribed dietary restrictions. This may indicate that dietary restrictions are especially important in infants with biparental history of atopy.

The stated reasons for discouraging the premature introduction of solid food include the possible risk of excessive weight gain, vulnerability of the gut to infection, and increased susceptibility to the development of allergic disease. The incidence of gastrointestinal illness, wheeze, and nappy dermatitis was not found to be related to early introduction of solid food feeding. There was a significant but rather small increase in respiratory illness at a particular age among infants given solids early. The incidence of eczema was increased in

those infants who received solids at 8–12 weeks of age (Forsyth et al., 1993).

Risk of atopy has been associated with a high cord-blood IgE (Kjellman & Croner, 1984). Therefore, many studies have focused on investigating those infants with high cord-blood IgE because development of an atopic disorder is more likely in these children. A dual approach of allergen avoidance, focusing on foods (breast milk and extensively hydrolysed formulas) and aero-allergens (treatment of bed- and living-room with acaricides), in comparison with controls who did not undergo any intervention, was beneficial in selected high-risk infants (Hide et al., 1994). Avoidance of potent food allergens in early life may increase the threshold for sensitization in those high-risk infants. Whether sensitization has been avoided or merely deferred has yet to be proved. Reduced exposure of infants to allergens in food and in house dust lowered the frequency of allergic disorders in the first year of life for high-risk children, pre-natally randomized to a prophylactic or control group (Arshad et al., 1992). A similar result was found in an outpatient follow-up of 777 infants with a very low birth weight. Comparison after random assignment to early diet of human milk versus cows' milk-based pre-term or term formula revealed an increased risk of atopy for early exposure to cows' milk (Lucas et al., 1990).

5.10.2 Sodium

Asthma mortality is related geographically to sales of table salt and both epidemiological and experimental evidence suggest that a high dietary sodium intake may increase airway responsiveness. Studies have shown that a low-sodium diet contributes to a decrease in symptoms in children with severe asthma. Further investigations have presented ecological, observational and experimental evidence supporting a relation between salt intake and airway responsiveness (Tribe et al., 1994). Regional data from England and Wales showed a strong correlation between table salt purchases and asthma mortality for adult men and children of both sexes, but not for adult women. Asthma mortality in women was found to be more closely related to other factors (Burney, 1987).

As part of a wider survey on asthma, 138 men living in two Hampshire villages in England underwent a bronchial histamine challenge test and had their 24-h urinary excretion of sodium

measured. Bronchial reactivity was strongly related to 24-h excretion of sodium, suggesting that a high-sodium diet may enhance bronchial reactivity (Burney et al., 1986). A study on the effect of dietary sodium intake on the airway response to histamine supports the hypothesis that a high-sodium diet increases bronchial reactivity in men but not in women and suggests that moderate restriction of sodium intake in asthmatic men would reduce bronchial reactivity (Burney et al., 1989b).

A controlled cross-over study of 14 asthmatics found that high salt intake worsened forced expiratory volume in one second (FEV_1), peak expiratory flow rate (PEF) and symptoms (Medici & Vetter, 1991). This was interpreted as indicating a generalized dysfunction of cellular sodium regulation and providing an explanation for the salt-sensitivity of the asthmatics. In contrast, another study (Britton et al., 1994) found no support for the hypothesis that a high dietary sodium intake is a risk factor for airway hyperreactivity or atopic disease in the general adult population. No relationship either between Na^+ and K^+ intake assessed by a 7-day recall and bronchial hyperresponsiveness or chronic respiratory symptoms was found in a sample of 205 subjects (Zoia et al., 1995).

5.10.3 Selenium

There seems to be an association between reduced serum selenium concentration and lowered activity of the selenium-dependent enzyme glutathione peroxide (GSH-Px) (Hasselmark et al., 1993). The aim of a double-blind randomized study in 24 patients suffering from asthma was to investigate whether selenium (Se) supplementation in asthmatic patients increases GSH-Px activity and possibly brings about clinical improvement in the Se-supplemented group as compared to the placebo group. In the Se-supplemented group there were significant increases in serum Se and platelet GSH-Px activity after intervention, while no significant changes in these parameters could be observed in the placebo group. Although there were no significant changes in lung function measures in the Se-supplemented or placebo group, a statistically significant clinical improvement was observed in the Se-supplemented group. There are several possible mechanisms whereby a reduced selenium status with associated lower GSH-Px activity may contribute to the pathogenesis of asthma. Further research into the role of selenium and GSH-Px is required (Beasley et al., 1991).

Selenium concentrations and GSH-Px activity were lower in 56 asthmatic subjects compared to 59 control subjects (Flatt et al., 1990). In both the asthmatic and control groups, the mean whole blood selenium concentrations and GSH-Px activity were generally higher in those who reported having eczema or rhinitis or had positive responses to skin-prick tests. These findings are consistent with the hypothesis that low selenium concentrations may play a role in the pathogenesis of asthma in New Zealand.

5.10.4 Vitamins and antioxidants

Data from the Nurses Health Study (Troisi et al., 1995), a prospective investigation of major chronic diseases, suggests that antioxidant supplementation and intake of various fats during adulthood are not important determinants of asthma, although vitamin E from diet may have a modest protective effect. An effect of vitamin E on the inflammatory process seems plausible. The relation between lung function and dietary intake of the antioxidant vitamins C and E in the general population was investigated in a cross-sectional survey of a random sample of adults of Nottingham, England (Britton et al., 1995). After adjustment for gender, height, skin-prick test result and smoking, lung function parameters were significantly and independently related to the mean daily intake of vitamin C. These data support the hypothesis that lung function in the general population is related to antioxidant intake and that these vitamins may play a role in protecting against the development of chronic obstructive pulmonary disease. The generalizability of these study results, however, may be limited due to a low participation rate of less than 60%. In the Zutphen study, fruit intake was inversely related to the incidence of chronic nonspecific lung diseases (Miedema et al., 1993). No association was observed with intake of several antioxidants. The second US National Health and Nutrition Examination Survey (NHANES) reported an inverse association between wheezing and serum vitamin C and the serum zinc/copper ratio. These data suggest that several dietary constituents may influence the occurrence of respiratory symptoms in adults, independently of cigarette smoking (Schwartz & Weiss, 1990). The relationship between ventilatory function and winter fresh fruit consumption was studied in a random sample of British adults (Strachan et al., 1991). These findings suggest that antioxidant and other actions of vitamin C may protect against pulmonary emphysema and may reduce bronchorestrictor responses to environmental pollutants.

5.11 Number of siblings and crowding

Factors playing a role in the prevalence of allergic disease in industrialized countries might be associated with the generally improved standard of living (Williams et al., 1994b). Along with this change goes a smaller family size and lower number of children in families. Studies have observed a decrease in the prevalence of allergic rhinitis, eczema (Strachan, 1989, 1996)) and asthma symptoms (Shaw et al., 1994) with an increase in the number of older siblings. In addition, it was shown that the prevalence of atopic sensitization decreases with an increasing number of siblings (von Mutius et al., 1994a), and a strong inverse relation was found between atopic sensitization and the number of persons per room in households (Braback et al., 1995). Viral or bacterial cross-infections, especially in early life, which may occur more frequently in larger families, have been discussed to have a protective role against atopic disease by preventing proliferation of Th2-lymphocytes (Romagnani, 1992b; Holt, 1994; Martinez, 1994). Helminth infestations have also been suggested to have a protective effect against allergy development (Williams, 1992) but studies in populations with a high prevalence of asthma indicated no such beneficial effect (Mantle & Pepys, 1974; Martinez, 1994).

5.12 Indoor environment

One of the most important changes in lifestyle during the past decades in industrialized countries is the change in indoor climate mainly due to modern building and furnishing materials, better insulation, wall-to-wall carpeting, increased indoor temperature and less ventilation. As a consequence of these factors and behavioural changes, such as indoor pet keeping, many pollutants and allergens can accumulate within dwellings. This may lead to higher sensitization rates to indoor allergens and consequently to an increased incidence of allergic diseases like asthma. This is especially relevant because people in industrialized countries spend up to 90% of their time indoors (Platts-Mills, 1994; Berglund et al., 1994). A variety of indoor and outdoor sources contribute to the indoor pollution burden and might play a role in the development or exacerbation of allergic disease. Important indoor sources are respirable particles from stoves and tobacco smoke; combustion products from cookers, ovens and heaters; formaldehyde from foam insulation, chipboards, furniture and fabrics; volatile organic compounds (VOCs) and other chemicals from paints,

sprays fabrics, and combustion; biological material from animal sources such as dust mites, cats, etc., or from fungi, bacteria and pollen (Angle, 1988; Karol, 1991). Ventilation, humidity and temperature of dwellings also have an important influence on indoor concentrations of many pollutants and allergens (Brunekreef et al., 1989; Beggs & Curson, 1995). For many indoor factors, there is still a lack of good epidemiological studies.

5.12.1 Tobacco smoke

Passive exposure to tobacco smoke is an important risk factor for childhood asthma and wheezing, particularly when the child's mother smokes. Many studies have reported a higher risk of asthma or bronchial hyperresponsiveness among children exposed to tobacco smoke (Ware et al., 1984; Dekker et al., 1991; Martinez et al., 1992; Forastiere et al., 1992) and younger children are particularly prone to these side effects (Arshad et al., 1993; Stoddard & Miller, 1995; Platts-Mills et al., 1995). Children of smoking parents have also increased reactivity to allergens as assessed by skin-prick test (Martinez et al., 1988; Braback et al., 1995). Furthermore, children whose mothers smoked during pregnancy show increased IgE-levels in cord blood and increased risk of infant allergy (Magnusson, 1986). Therefore, the increasing prevalence of smoking in women of childbearing age may have contributed to the increase in atopic disease in children (Burney et al., 1990).

Although a number of studies in adults have shown an association between active smoking and asthma, others have failed to find such an association. Thus, the evidence is not conclusive and the effect may be only small (Platts-Mills et al., 1995). Active smoking can increase total IgE serum concentration and ex-smokers show a decline in serum IgE concentration after cessation of smoking (Burrows et al., 1981). Studies on occupational allergies showed that the frequency of IgE sensitization and asthma is higher in cigarette smokers (Anonymous 1985; Venables et al., 1985b). Little is known about the role of active and passive smoking on allergic rhinitis, and studies show, if any, only small effects (Ng & Tan, 1994a,b; Strachan, 1995; Tsunoda et al., 1995).

In summary, smoking is known as a respiratory irritant and as an important source for indoor pollution. However, although many studies suggested that smoking plays a role in the etiology of asthma,

there is no clear evidence from the available data to indict smoking as the driving force for the observed increases in asthma morbidity and mortality (Weiss et al., 1993). Nevertheless, even if the relative effect of active and passive smoking may be small, it can still have a major impact if large parts of populations are exposed (attributable risk) (Stoddard & Miller, 1995).

5.12.2 Pets

Many animals like cats, dogs, rodents, rabbits and cage birds live within or in close proximity to homes. About one third to one half of houses in the USA have a mammalian pet (Colloff et al., 1992; Ledford, 1994). There is strong evidence that exposure to a number of animal allergens can lead to primary sensitization and an increased risk of developing allergic disease (Colloff et al., 1992). The most important pet allergens are those from cats and dogs (Sears et al., 1989; von Mutius et al., 1994b; Ledford, 1994; Strachan & Carey, 1995). Even after permanent removal of a cat from the home it may take several months before the concentration of allergens in domestic dust falls (Wood et al., 1992; Colloff et al., 1992; Munir et al., 1994b). Pet owners visiting a home with no pets can bring in pet allergens on their clothes, and rub it off during their visit, resulting in a considerable amount of pet allergens in the visited flat (Munir et al., 1993; Munir et al., 1994a,b).

5.12.3 Biocontaminants

5.12.3.1 House dust mites and insects

House dust mites are an important source of indoor allergens. Most frequently detected species are *Dermatophagoides pteronyssinus, Dermatophagoides farina, Euroglyphus maynei* and *Blomia tropicalis* (Colloff et al., 1992; Ledford, 1994). The mites have a narrow optimal temperature range for growth from 18 °C to 27 °C (Ledford, 1994). Because dust mites depend on ambient humidity they grow poorly in dry or high altitude climates. Mite allergens can be measured directly by ELISA. Indirect measurements of mite allergens rely on guanine quantification in house dust. However, this method has been shown to be of limited clinical value since a considerable amount of guanine may originate from non-house-dust mite sources (Hallas et al., 1993). Sensitization to dust mites seems to play an important role in the relation between damp homes and childhood respiratory symptoms (Verhoeff et al., 1995). High mite allergen exposure has been reported

to increase the risk of sensitization in atopic children (Lau et al., 1989) and exposure in early childhood has been shown to be a determinant of subsequent asthma (Sporik et al., 1990). Sensitization to *Dermatophagoides pteronyssinus* and other allergens was addressed in a study performed shortly after the fall of the Berlin wall on 9- to 11-year-old children in eastern and western Germany. Skin-prick testing showed sensitization rates of 10.3% to *Dermatophagoides pteronyssinus* (wheal reaction ≥3mm) in western Germany and of 4.2% in eastern Germany, indicating that environmental and lifestyle factors may have an influence on sensitization to common allergens (von Mutius, 1994b).

Cockroaches are also a substantial indoor allergen source. Sensitization has been shown to be a risk factor for emergency room visits in asthmatics (Pollart et al., 1989). Exposure to high levels of cockroach allergens and allergy against cockroaches was found to explain much of the asthma-related health problems in inner-city children from the USA (Rosenstreich et al., 1997). Exposure to cockroaches has also been associated with allergic rhinitis (Ng & Tan, 1994b). Few data on sensitization to cockroaches exist for central Europe (Colloff et al., 1992), but the available data suggest that the prevalence of sensitization to cockroaches is low (Mosimann et al., 1992; Munir et al., 1994a). Other allergens from insects include moths, crickets, midges, locusts, beetles and various flies (Ledford, 1994).

5.12.3.2 *Moulds*

The most common indoor moulds responsible for allergies are *Aspergillus* species, *Cladosporium*, and *Penicillium* (Ledford, 1994). Moulds are responsive to temperature, humidity and substrate moisture level (Beggs & Curson, 1995). Damp housing has frequently been shown to be a risk factor for respiratory symptoms in children and adults (Brunekreef et al., 1989; Dekker et al., 1991; Brunekreef, 1992) and may play a role in the sensitization to mould allergens (Verhoeff et al., 1995). However, it has been suggested that the observed association between damp housing and childhood asthma may be partly due to parental over-reporting (Strachan, 1988). Other possible indoor sources of fungal allergens include air conditioning equipment, humidifiers, degrading organic materials, and soil used for indoor plants; these sources can also be important for allergens from bacteria or protozoa (Dekker et al., 1991; Ledford, 1994).

5.12.4 Other indoor factors

A large variety of everyday rubber products (e.g., balloons, baby pacifiers, sports equipment, adhesives, etc.) contain natural latex, which may be a source of sensitization and a factor in allergic disease expression. Airborne leaf parts of the popular indoor plant *Ficus benjamina* (weeping fig) also contain latex particles (Axelsson et al., 1990; Bircher et al., 1993). It is not yet known whether natural latex, which is a well known occupational allergen (Vandenplas, 1995) (see also section 5.8.3) has any impact on allergic disease on a population level. Contrary to expectation, non-feather bedding, especially foam pillows, have been suggested to be a possible determinant for symptoms of asthma in adolescents (Strachan & Carey, 1995).

5.13 Indoor and outdoor environmental factors

5.13.1 Nitrogen dioxide

Nitrogen dioxide (NO_2) is a strong oxidant. Indoor sources are cigarette smoke, gas and oil heaters or cookers, which can result in a high indoor concentration (Angle, 1988; Wardlaw, 1993). Main outdoor sources are combustion of fossil fuels in motor vehicles and power generation (Wardlaw, 1993). Combustion processes also generate a mixture of NO_2 and nitric oxide (NO), and formation of indoor nitrous acid (HNO_2) has also been demonstrated and associated with adverse respiratory effects (Samet et al., 1993). Experimental exposure and several epidemiological studies are inconclusive concerning the effect of NO_2 on lung function (Ware et al., 1984; Angle, 1988; Neas et al., 1991; Gorski & Tarkowski, 1992; Samet et al., 1993). The current understanding of NO_2 is that it can decrease pulmonary function in asthmatics, but its overall role in the development of allergic disease is not clear (Angle, 1988).

5.13.2 Sulfur dioxide, acid aerosols and particulate matter

The sources of these major outdoor air pollutants are mainly combustion of fossil fuels, like coal and oil, wood, and certain industrial processes. Sulfur dioxide (SO_2) in water forms sulfurous acid (Gorski & Tarkowski, 1992). The indoor concentration of SO_2 is raised if unvented combustion devices for kerosene are used or when cigarettes are smoked (Angle, 1988). Several studies on asthmatics have shown that SO_2 can provoke bronchoconstriction and asthma-like

symptoms even at low levels, especially during exercise and mouth breathing (Linn et al., 1983a,b). Animal studies indicated an enhancing effect of SO_2 on sensitization to allergens (Riedel et al., 1988).

Acid aerosols are comprised mainly of sulfuric acid and ammonium bisulfate (Wardlaw, 1993), which has also been shown to cause an increase in respiratory symptoms and a decrease in lung function (Koenig et al., 1983; Hackney et al., 1989). Acid aerosols can also comprise nitric acid, hydrochloric acid and hydroxymethan-sulfonic acid. Outdoor airborne acidity is associated with daily respiratory symptoms in asthmatics (Ostro et al., 1991).

Particulate matter (e.g., dust, dirt and smoke) is a major source of air pollution and a complex and varying mixture of substances. Sources are motor vehicle emissions, such as exhaust fumes and abraded tyre fragments (Williams et al., 1995b), factory and utility smokestacks, residential wood burning, construction activity, mining, agricultural tilling, open burning, wind blown dust, fires, etc. Several studies have suggested an association between particulate matter (PM) exposure and asthmatic symptoms (Dockery et al., 1989; Xu & Wang, 1993; Dockery & Pope, 1994; Abbey et al., 1995; Brunekreef et al., 1995). Particles of a diameter <10 μm (PM_{10}) are especially important for respiratory disease because they are readily inhaled deep into the lungs (CDC, 1994). Among them fine particles of a diameter <2.5 μm and ultrafine particles (<0.1 μm) may be most important (Peters et al., 1997). Animal studies indicated that diesel exhaust particles may increase the risk of sensitization against allergens (Muranaka et al., 1986; Takafuji et al., 1987; Fujimaki et al., 1994).

5.13.3 Volatile organic compounds, formaldehyde and other chemicals

Volatile organic compounds (VOCs) comprise a broad spectrum of substances including benzene, toluene, xylenes and aldehydes (Angle, 1988; Becher et al., 1996). VOCs are emitted by a large number of materials and only few subgroups have been investigated for their potential role in allergy. The total indoor amount of VOCs and several sub-types like toluene and terpenes have been associated with asthmatic symptoms (Norback et al., 1995). Outdoor sources of VOCs have also been found to be related to increased rates of chronic respiratory symptoms characteristic of reactive airways (Ware et al., 1993). Another important VOC is formaldehyde, which is ubiquitous

in the human environment. Important indoor sources are smoking, particle-boards, foam insulation and textiles (Imbus, 1985; Koenig, 1988). Formaldehyde exposure has been related to asthma and other allergies (Imbus, 1985; Angle, 1988; Wjst et al., 1994; Norback et al., 1995), but evidence that formaldehyde exposure can cause allergic diseases in the airways is limited (Becher et al., 1996). Other chemicals such as ethylenediamine and isocyanate may also contribute to the allergic disease burden in the general community (Ledford, 1994), but population-based data showing an association between allergies and indoor contamination by these pollutants are not available. The increasing use and diversity of household cleaning materials during the past decades have also been implicated in the expression of allergic diseases (Williams, 1992).

5.14 Outdoor air pollution

A link between outdoor air pollution and the increased prevalence of respiratory allergies such as asthma and allergic rhinitis has been suspected for some time. However, whether atmospheric air pollution can cause respiratory and other allergies is still not clear (Wardlaw, 1993). Contamination of the air with plant pollen is a major natural source of air pollution. Important causes of outdoor air pollution are burning fuels such as coal, oil and wood, smelting of ores, and other industrial processes. Natural sources like volcanoes play only a small role. Motor vehicle tail-pipe emissions are a major contributor of several pollutants, such as diesel particles, oxides of nitrogen (NO_X), carbon monoxide and other airborne particles (Bascom, 1996).

The increase of allergic respiratory diseases coincided with a decrease in many outdoor air pollutants like SO_2 and total suspended particles (TSP) (Weiss et al., 1993), whereas air pollution from motor vehicle emissions (e.g., O_3, NO_X) increased during the same period (Newman Taylor, 1995). Motor vehicles are also a major source of fine suspended particles (see section 2.5.3). Most outdoor air pollutants provoke more or less severe adverse effects in asthmatics, and exposure to multiple pollutants may cause synergistic effects (Koenig et al., 1990). A number of experimental animal studies indicate an association between allergy and air pollution (Osebold et al., 1980; Biagini et al., 1986; Muranaka et al., 1986; Takafuji et al., 1987; Riedel et al., 1988; Takafuji et al., 1989; Suzuki et al., 1993; Fujimaki et al., 1994). It is also assumed that pollutants can enhance the allergenicity of common allergens like pollen (Ishizaki et al., 1987;

Behrendt et al., 1991; Gorski & Tarkowski, 1992). However, the observation that the sensitization rate to common allergens in cities in eastern Germany with previously high levels of traditional air pollutants such as SO_2 or particulate matter was low in comparison to that of western Germany cities argues against the hypothesis that traditional air pollution from industrial production and household coal burning is the major factor driving the changes in morbidity patterns of allergic disease (von Mutius et al., 1992; von Mutius et al., 1994b). Similarly, a Swiss study reported effects of moderate average air pollution concentration from PM_{10}, NO_2 and SO_2 on respiratory symptoms, such as chronic or nocturnal dry cough and bronchitis, but not between air pollution and asthmatic and allergic symptoms or diseases in children (Braun-Fahrländer et al., 1997). These results are in close agreement with findings from the US Six Cities Study (Dockery et al., 1989). In addition, the findings of the ISAAC study do not provide support for an association between air pollution and childhood wheezing; for example, countries with low degrees of ambient air pollution such as New Zealand were among those with the highest prevalence of asthma symptoms (ISAAC Steering Committee, 1998). Likewise, recent results from the Pollution Effects on Asthmatic Children in Europe (PEACE) project found only little overall adverse effect of ambient air pollutants (e.g., PM_{10}, black smoke, SO_2 and NO_2) on respiratory health in children (PEACE, 1998).

5.14.1 Pollen and dust

Natural sources of air contamination are plant pollen from grass, ragweed, trees, etc. (Schutz-Kiss et al., 1995), moulds (Dawson & Mitchell, 1990), and natural particulate matter such as dust or dirt (CDC, 1994) which can cause symptoms of asthma and allergic rhinitis. Their role as a causal factor for the development of these diseases, however, is not clear. Air pollution due to natural factors like pollen or dust cannot easily explain the observed increase in allergic diseases in many regions because humans have always had intense outdoor contact with these substances. Several asthma outbreaks in populations have been shown to be related to man-made airborne allergen pollution. Examples are castor bean dust in USA, South Africa and Brazil (Anto, 1995), and soybean dust which was released during unloading of soybeans in the city harbour of Barcelona (Anto et al., 1989).

5.14.2 Ozone

A major source of ozone (O_3) is motor vehicle exhaust. O_3 is a main constituent of photochemical smog, and its formation requires NO_X and reactive organic compounds as precursors and ultraviolet radiation (Gorski & Tarkowski, 1992; Wardlaw, 1993; Beggs & Curson, 1995). It is the most ubiquitous air pollutant in the USA (Koenig, 1995). Although O_3 is mainly an outdoor pollutant, it is also present in low concentrations in the indoor environment (Koren, 1995). Many studies have shown that O_3 has an aggravating effect in asthmatics and can reduce lung function (Krzyzanowski et al., 1992; Gorski & Tarkowski, 1992; Wardlaw, 1993; Koenig, 1995; Koren & Bromberg, 1995). Unlike SO_2 there seems to be no marked difference in acute responses in asthmatics and non-asthmatics (Koenig et al., 1988). Animal models suggest that O_3, like other air pollutants, may increase sensitization to allergens (Osebold et al., 1980; Sears et al., 1989). Ambient levels of O_3 may also have a synergistic effect with pollen in the causation of allergic rhinitis (Bascom et al., 1990). However, only a few epidemiological investigations have been performed on a population level to evaluate the effect of ambient O_3 on asthma and allergies (e.g., Braun-Fahrländer et al., 1997) and further investigations are needed to study the effect of O_3 on these disorders under real-life exposure conditions (Magnussen et al., 1998).

5.14.3 Motor vehicle emissions

Motor vehicle traffic has increased dramatically in many countries (Utell et al., 1994) and it has been speculated that this increase may play a role in the observed changes of the prevalence of allergies. A number of occupational studies have shown an association between exposure to motor vehicle exhausts and adverse effects on respiratory symptoms and lung function (Gamble et al., 1987; Evans et al., 1988a; Ulfvarson & Alexandersson, 1990; Wade & Newman, 1993; Raaschou Nielsen et al., 1995). Others, however, have failed to find such an association (Speizer & Ferris, 1973; Tollerud et al., 1983; Ames et al., 1984). An association between allergic sensitization and components of motor vehicle exhaust fumes has been shown in various animal studies (Osebold et al., 1980; Muranaka et al., 1986; Takafuji et al., 1987; Riedel et al., 1988; Suzuki et al., 1993; Fujimaki et al., 1994; Lovik et al., 1997). A number of experimental studies suggested also an association between allergic disease and traffic pollution (Molfino et al., 1991; Braun Fahrländer et al., 1994; Devalia et al., 1994). Several epidemiological studies observed a relationship

between exposure to motor vehicle traffic at residence and morbidity from respiratory and allergic disorders in adults (Yokoyama et al., 1985; Ishizaki et al., 1987; Nitta et al., 1993) and in children (Wjst et al., 1993; Edwards et al., 1994; Weiland et al., 1994; Keil et al., 1996; Oosterlee et al., 1996; Duhme et al., 1996; Brunekreef et al., 1997; Duhme et al., 1998a). Air pollution from motor vehicle traffic derives also from mechanical abrasion of tyres, which contain potentially allergenic latex particles (Williams et al., 1995b). However, evidence that exposure to motor vehicle traffic can cause asthma or allergies is not conclusive.

5.15 Conclusions

Asthma and allergic disorders represent a substantial burden not only on the affected individuals but also on health care resources in many countries. The costs of asthma are partly due to uncontrolled disease, and are likely to rise as its prevalence and severity increase (Barnes et al., 1996). One approach to reduce costs would be to improve disease control. Another approach would be to reduce the prevalence of asthma by preventive measures, and this would be accompanied by a reduction in the costs of treatment and care (Peat, 1996). Environmental factors that have changed in the last decades appear to be largely responsible for the observed increase in the prevalence of asthma and allergic disease in many countries. The determinants of these changes need to be identified in order to design interventions that can reverse these trends. Such prevention strategies in the field of asthma and allergies can aim at high-risk groups or at populations as a whole (Rose, 1985). It is important to note that even if a preventive measure offers little to each individual it can bring large benefits to the community into which the preventive measure is introduced (prevention paradox) (Rose, 1985).

It is often impossible to blame a single culprit within a complex mixture of behaviours and exposures (such as indoor or outdoor air pollution) for observed adverse health outcomes, and the studied risk factor might also have different effects in the presence of other factors (Greenland, 1993). Because randomized assignment of individuals to certain exposures is impractical, if not unethical, in environmental epidemiology, researchers rely mainly on data from non-experimental studies with well-known inherent methodological shortcomings (Rothman, 1993). Depending on the hypotheses being studied, a number of epidemiological research strategies are available. The applications, strengths and weaknesses of different studies have been

described (e.g., Hennekens & Buring, 1987; Rothman, 1993; Morgenstern & Thomas, 1993). Interpretation of (epidemiological) study results has to consider random errors of estimation (due to chance) and systematic errors or bias. Important sources of bias (selection bias, information bias and confounding) may hamper the validity of observed results. Therefore, possible systematic errors should be considered already at the planning stage of any study and measures should be implemented to minimize or control bias. It is also crucial at the planning stage of a study to perform statistical power calculations to evaluate how many study participants are needed to assure a given probability of detecting a true effect of a given magnitude.

Epidemiological studies applying accurate exposure and disease measurements and taking into account important covariates, confounders and effect modifiers are needed (Hatch & Thomas, 1993; Prentice & Thomas, 1993). They can make an important contribution through regulatory decisions in public health in the difficult field of risk assessment for complex exposures.

Besides other measures, occupational sentinel health events (Mullan & Murthy, 1991) and structure–activity research (Jarvis et al., 1996; Graham et al., 1997) may be useful tools to evaluate the sensitizing capacity of a chemical substance in populations. Lists of well-known allergens categorized according to potency and degree of exposure are available (Kayser & Schlede, 1995), and criteria for classification of sensitizing substances in the environment have been defined. Furthermore, by comparing different populations with different grades of exposures, major disease determinants can be uncovered that would otherwise remain undetected, if the suspected risk factors show only little variation within a single population (Rose, 1985). For comparison reasons, data should be collected and analysed in a standardized way wherever possible. Using such strategies, major health determinants have been successfully described and studied in other fields, such as cardiovascular diseases and cancer. It is an essential prerequisite of such internationally conducted studies to obtain the health outcome and exposure data in a standardized way. New epidemiological initiatives investigating determinants of asthma and allergies need to incorporate these principles, as is the case with the ongoing International Study of Asthma and Allergies in Childhood (ISAAC) (Asher et al., 1995; ISAAC Steering Committee, 1998).

6. HAZARD IDENTIFICATION: DEMONSTRATION OF ALLERGENICITY

6.1 Hazard and risk; allergy and toxicity

The conventional scheme for chemical risk assessment for human health protection follows the sequence:

a) Hazard identification; what is the potential of the substance to cause harm (sensitization and provocation of an allergic reaction), and what is the dose–response relationship?

b) Exposure assessment

c) Risk assessment: what is the likelihood of eliciting an allergic reaction in humans at the relevant level of exposure; are there any groups of increased susceptibility?

d) Risk characterization: non-scientific consideration of the risk weighed against the benefit of using the substance resulting in the decision to ban or limit exposure (NAS, 1983, 1993).

Hazard identification, therefore, comprises procedures to determine the potential of a substance to induce allergy or elicit allergic reactions and the relationship between those properties and the circumstances of exposure.

The prediction may be based on theoretical considerations of chemical structure, possibly on *in vitro* experimental results, on *in vivo* animal data, and on prior observations in humans. If it depends on the results of laboratory studies and not on clinical observations, the prediction, as is common to toxicity tests, must take account of species differences in metabolism, responsivity, dose (exposure), and the adequacy of validation of the experimental system.

Testing allergenic potential requires study of selected immunological effects and differs from conventional toxicity testing in the nature and content of its procedures, which are focused on responses of the immune system and not on general screening for changes in all body systems. In both types of testing, however, there will be some form of relation between dose (exposure) and effect, as

the capacity of a substance to produce effects, its potency, will be represented by the dose (exposure) required to produce sensitization (or toxicity). A strong sensitizer will require only a small dose, whereas a less potent compound will require a higher dose, or multiple exposures. Unlike conventional toxicity, further exposure of a sensitized animal (or man) will elicit a harmful allergic reaction after a much smaller dose than that required for sensitization, although there will still be a graduation of the severity and nature of the hypersensitivity reaction, for example ranging from slight bronchoconstriction to fatal bronchospasm or anaphylaxis after respiratory challenge.

An additional important difference between conventional toxicity and allergy is that allergic sensitization (the induced state of hyperreactivity to a substance) normally persists for a long time, even for life, whereas for many toxic responses a state of lasting responsiveness is not induced. It is possible for different types of hypersensitization and provocation to be effective in the same organ but it is also possible for the route of sensitization and response to subsequent challenge to differ, e.g., sensitization via the skin and subsequent asthma on inhalation exposure.

6.1.1 Testing allergic potential and toxicity testing

Much testing to identify toxic hazard is done during industrial development of substances for purposes ranging from new medicines or consumer products to industrial intermediates or pesticides. There are well-developed regimes of accepted procedures done under controlled circumstances applicable to each intended use, and the results are used for regulatory purposes to control risk. Most of these procedures are not directed at revealing effects involving the immune system, although indirect indications may sometimes be obtained that can arouse suspicion that the immune system may have been affected.

Certain specialized procedures are also conducted, based on consideration of the way in which humans may be exposed (e.g., in the skin or by inhalation), which experience has shown can reveal certain types of sensitizing and allergenic potential. The latter types of test are the most important in the present context. The procedures and their value and limitations are discussed here.

Laboratory tests, especially *in vivo* procedures, should be done in such a way as to minimize the need for experimental animals and scarce human and technical resources, and every attempt is made to extract as much information as possible from the work that is done. That includes considerable attention paid to quality assurance of tests.

Accordingly, the testing of a new substance will follow a sequence, moving from theoretical to practical procedures. Only those techniques focused on the immune system are noted here, but it must be realized that product development and occupational safety needs require many other studies, too.

6.1.2 Databases and prior experience

A preliminary search should always be made for any information about experimental or clinical findings about the immunological consequences of exposure to the substance. This may include special consideration of any groups considered to be particularly susceptible, for example, because of pre-existing disease.

6.2 Validation and quality assurance

Obviously, the ideal situation would be that predictive tests yield easy-to-interpret outcomes and no false negatives or false positives, and that the tests will always give the same results, regardless of when or where they are carried out. In order to achieve this goal, validation of tests is required. Validation should occur at two levels: first at the level of technical quality and second at the level of specificity and sensitivity of the assay. It is important that potential methods are carefully evaluated for interlaboratory reproducibility and transferability, and for their ability to predict an *in vivo* end-point. Owing to the complexity of the immune system, it is quite likely that predictive assays for the capacity of chemicals to induce skin, respiratory or food allergy, or autoimmunity will not always function adequately to show the absence or presence of such activity (Kammüller, 1996). Therefore, the outcome of these tests should always be evaluated with great care.

6.3 Structure–activity relationships

Structure–activity models are directed towards a fuller understanding of the relationship between chemical structure and physicochemical properties and skin-sensitizing activity, with the objective of deriving ideally quantitative structure–activity relationships (QSAR). In this context, parameters that appear to be of particular importance are protein reactivity and lipophilicity associated with the capacity to penetrate into the viable epidermis (Basketter & Roberts, 1990; Barratt et al., 1994a). The correlation of the protein reactivity of chemicals with their skin-sensitization potential is well established (e.g., Dupuis & Benezra, 1982), and it is accepted that if a chemical is capable of reacting with a protein, either directly or after appropriate (bio)-chemical transformation, then it has the potential to be a contact allergen, assuming of course that it can locate in the appropriate epidermal compartment.

It is not within the scope of this monograph to give a comprehensive description of all the available models. A common feature of many of these models is that their development was based upon the mechanism of sensitization, i.e., the absorption of the chemical sensitizer through the skin, followed by its covalent modification of a skin-associated protein. Each of the existing structure–activity relationship (SAR) models proposes structural alerts, i.e., moieties associated with sensitizing activity. In all cases, the structural alerts comprise electrophilic moieties, or moieties that can be metabolized into electrophilic fragments (proelectrophiles). For example, Benezra et al. (1985) developed a hierarchical index of structures believed to be associated with allergic contact dermatitis that contained amines, ketones, metals, nitrogen-containing heterocycles, and oxygen-containing heterocycles, among others. Barratt et al. (1994a,b) developed a list of structural alerts based on the requirement for protein reactivity that included alkylating, acylating, and arylating agents, electrophiles, thiol exchange compounds, and free radical generators. Many of the existing SAR systems have incorporated physicochemical considerations (Roberts & Basketter, 1990a,b; Basketter et al., 1992; Ashby et al., 1995). The mathematical model described by Roberts & Basketter (1990a,b) for alkyl transfer agents has incorporated both a rate constant for reaction of a chemical with a nucleophile, and a lipophilicity factor (log P). Each of these models has been successful in predicting the activity of moderate to strong

contact sensitizers. Barratt et al. (1994b) reported the greatest success in predicting active sensitizing potential (98%). A smaller database using similar alerts (Payne & Walsh, 1994) had a much poorer positive predictive ability of 57%. The classification model of allergic contact dermatitis used by Hostynek et al. (1996) made use of several parameters and multiple regression to predict 79% of the active sensitizers and 88% of the inactive chemicals. The relative alkylation index (RAI) is useful only in the prediction of a homologous series of chemicals (Roberts & Basketter, 1990a,b). The model used by Benezra et al. (1985) did not specify whether a validation was attempted, therefore predictive ability is not known.

6.3.1 Case-Multicase system

The Case-Multicase system is not dependent upon a particular mechanism of sensitization and has shown ability to predict activity of weak sensitizers (Graham et al., 1996). The model operates by fragmenting chemicals in the database into substructures containing two or more heavy (non-hydrogen) atoms. It then identifies those fragments that are statistically associated with active chemicals and uses such fragments as structural alerts for prediction of test chemicals.

The database for this model was derived from reports of animal and human studies and consists of more than 1000 chemicals (Graham et al., 1996). The model identified 49 structural alerts of allergic contact dermatitis. The major ones were: (a) a nitrogen double-bonded to a carbon or a nitrogen; (b) substituted aromatic structures; (c) thiol- and disulfide-containing fragments; and (d) electrophilic moieties. The model has been evaluated by testing its ability to predict correctly the activity of chemicals for which there is evidence of sensitization ability. The concordance between predictions by the model and established evidence of sensitization was 90% (Graham et al., 1996).

6.3.2 DEREK skin sensitization rulebase

A historical database (Cronin & Basketter, 1994) containing results of about 300 guinea-pig maximization tests (Magnusson & Kligman, 1970a,b), carried out over a number of years according to a single protocol on defined single substances, was used to derive a set of structural alerts for skin sensitization. The approach employed was to group the substances, where possible, according to their most likely

mechanism of reaction with skin proteins. Where no mechanism could be clearly identified, structural alerts were derived for groups of chemicals with similar functional groups. This process initially resulted in the production of around 40 structure–activity rules (Barratt et al., 1994a), now increased to over 50. These were incorporated into the expert system Deductive Estimation of Risk from Existing Knowledge (DEREK) (Sanderson & Earnshaw, 1991; Ridings et al., 1996). DEREK contains both a controlling programme and a chemical rulebase. The chemical rulebase consists of descriptions of molecular structural alerts, which correlate with specific toxicological end-points.

Whilst details of the biology of skin sensitization are only partly understood, it is now widely accepted that the ability to react with a nucleophile, either directly or after appropriate metabolism, is a prerequisite for the large majority of skin sensitizers. However, the potential of a chemical to act as a contact allergen is further modulated by its ability to penetrate the stratum corneum and partition into the epidermal compartment of skin; this is apparent from a number of QSAR studies (Roberts & Basketter, 1990a,b; Basketter et al., 1992) in which skin-sensitization potential was found to depend crucially on physicochemical parameters such as the log octanol/water partition coefficient (log P_{ow}). These parameters have also been found to be equally important determinants of percutaneous absorption (Flynn, 1990), with higher log P_{ow} values, i.e., greater lipophilicity, broadly leading to greater permeability. In QSAR studies of skin permeability, the *in vitro* human skin permeability coefficient has also been shown to decrease with increasing relative molecular mass (Flynn, 1990) or molecular volume (Barratt, 1995). The skin-sensitization potential of a series of substituted phenyl benzoates was found to depend on log P_{ow} and relative molecular volume in the same way (Barratt et al., 1994c). The logical consequence is that two chemicals may contain the same structural alert (i.e., be reactive, presumably by the same mechanism), but one will be a skin sensitizer because it can penetrate the skin whilst the other will not be a skin sensitizer because its skin permeability is too low, e.g., *N*-methyl-*N*-nitrosourea and streptozotocin (Ashby et al., 1995).

6.3.3 *SAR for respiratory hypersensitivity*

An initial SAR model for respiratory-sensitizing chemicals has been described (Karol et al., 1996). The model is based on the

Case-Multicase system and the database was derived from a critical review of the published clinical literature. Criteria for inclusion of published data included a decrement in pulmonary function resulting from inhalation challenge with a non-irritating concentration of the chemical.

In all, 39 respiratory chemical allergens were identified from the literature search, all being obtained from human studies. Among the chemicals were diisocyanates, acid anhydrides, antibiotics and dyes. Since the model requires a data set of inactive chemicals, and such chemicals could not be found in the literature, chemicals that were inactive as dermal sensitizers were assumed to be inactive as respiratory sensitizers as well, and were added to the respiratory model as "inactive" chemicals.

The model identified structural alerts including the isocyanate functionality, amines and aromatic fragments. When respiratory sensitizers were compared with dermal sensitizers for both structural alerts and physicochemical characteristics, differences were noted. Among the physicochemical properties, respiratory chemicals had higher mean relative molecular mass and greater water solubility when compared with dermal sensitizers (Karol et al., 1996). However, the discrimination of dermal and respiratory sensitizers remains problematic.

6.4 Predictive testing *in vivo*

6.4.1 Testing for skin allergy

6.4.1.1 Testing in guinea-pigs

The guinea-pig was for many years the animal of choice for experimental studies of contact sensitization, and several test methods were developed in this species. The Draize test was developed over 50 years ago (Draize et al., 1944) and was widely used, but this is no longer the case and it has been superseded. Currently, the best known and most widely applied are the Buehler test (Buehler, 1965), the guinea-pig maximization test (Magnusson & Kligman, 1970a,b), and the guinea-pig optimization test (Maurer et al., 1975), and have formed the basis of hazard assessment for many years. Both the Magnusson and the Buehler test are recommended according to an OECD

guideline (OECD, 1992). While these tests differ with respect to procedural details, they are in principle similar. Guinea-pigs are exposed to the test material or to the relevant vehicle. In the Buehler test, both induction and challenge exposures are done topically; in this test, false negatives are frequently observed. The test was improved by occluded application of the test compound. In the guinea-pig maximization test, induction is produced by intradermal and occluded epidermal exposure, and in the optimization test induction is done by intradermal exposure and challenge by intradermal and occluded epidermal exposure. Adjuvant is employed also to augment the induction of the immune responses. For induction, concentrations of up to 5% or a maximum non-irritant concentration are used for intradermal injections, and up to 25 % for epidermal application. Some time after induction exposure, test and control animals are challenged at a distant site with a sub-irritant concentration of the chemical, which is generally lower than the concentration used for induction. Challenge-induced inflammatory reactions, measured as a function of erythema and/or oedema, are recorded 24 and 48 h later. Classification of sensitizing activity is based usually upon the percentage of test animals that display macroscopically detectable challenge reactions. Any compound inducing at least 30% positive animals in an adjuvant test is labelled as a sensitizer; in the case of a non-adjuvant test, 15% is sufficient for classification as a sensitizer.

Of the available guinea-pig test methods, the guinea-pig maximization test is generally selected when the aim is to identify the weakest of skin sensitizers. However, the method is not well suited to the estimation of relative sensitizing potency, because of the requirement for the use of intradermal injections of test material and Freund's complete adjuvant (FCA) (Basketter et al., 1996). The Buehler test is easier to use because the mode of application is epicutaneous occlusive treatment for both induction and elicitation (Chan et al., 1983).

Although guinea-pig test methods, such as the Buehler test and the guinea-pig maximization test, have been in use for more than 25 years, there has not been extensive examination of their sensitivity and specificity in comparison to what is known about human skin sensitization. Both the tests, when conducted properly, offer sufficient sensitivity to detect many known human skin sensitizers (Wahlberg &

Boman, 1985; Basketter et al., 1996). However, there has been very little assessment on the specificity of these methods.

It should be noted that these guinea-pig methods are not well suited to the identification of protein allergens. In general, the maximization of exposure involved in these skin sensitization tests will result simply in a large immune response. This will mask any differential allergic response.

6.4.1.2 *Testing in mice*

Increased understanding of the cellular and molecular mechanisms associated with contact allergy have derived largely from experimental investigations in the mouse. Two different types of tests to predict the capacity of chemicals to induce skin allergy have been developed. One is the mouse ear swelling test (MEST), which, like the guinea-pig methods described above, is based upon the evaluation of challenge-induced reactions in previously sensitized animals (Gad et al., 1986). In this test, mice sensitized by a comparatively rigorous regime (the intradermal injection of adjuvant followed by the daily application of the test material, for 4 consecutive days, to tape-stripped skin) are challenged on one ear with the test compound and on the contralateral ear with vehicle alone. Sensitizing potential is evaluated by consideration of both the degree of oedema (ear swelling) induced and the percentage of animals displaying a reaction (Thorne et al., 1991).

The second test developed in mice is the local lymph node assay (Kimber et al., 1989). In contrast to the mouse ear swelling test and guinea-pig assays, activity here is measured as a function of events occurring during the induction, rather than elicitation, phase of contact sensitization. Mice are treated daily, for 3 consecutive days, on the dorsum of both ears with the test material or with an equal volume of vehicle alone. Proliferative activity in draining lymph nodes (measured by the incorporation *in situ* of radiolabelled thymidine) is evaluated 5 days following the initiation of exposure.

The local lymph node assay has been the subject of extensive comparisons with guinea-pig methods (Kimber et al., 1994; Basketter et al., 1996), and offers significant advantages compared with the available guinea-pig test methods. Important among these is the fact

that there is an objective read-out. The assay has been evaluated by collaborative trials, and an OECD (Organisation for Economic Co-operation and Development) test guideline issued in 1992 states that the local lymph node assay (or the MEST) can now be used as a first stage in an assessment of skin-sensitizing activity (OECD, 1992). If a positive result is seen, then a test substance may be designated a potential sensitizer and it may not be necessary to conduct a further guinea-pig test. However, if a negative result is seen, a guinea-pig test must be conducted subsequently.

6.4.1.3 *Predictive testing for skin allergy in humans*

There are various skin test procedures for the diagnosis of several types of contact dermatitis (see also chapter 4). Basically, predictive tests in humans for skin allergy are similar to diagnostic tests for contact sensitization, but the aims are different. For diagnostic tests, the aim is determining sensitization to chemicals to which there was a prior exposure, and avoiding new sensitizations because of the procedure. For predictive testing in humans the aim is to show the sensitizing capacity in individuals that have not been exposed to the chemical previously.

Predictive testing in humans generally requires multiple occlusive patches for induction of sensitization (10 patches, 48 h each, same site) followed by a 2-week rest period and then challenge (48 h) with a patch at a new skin site (Marzulli & Maibach, 1973, 1996). There are a number of variations in these procedures, including the use of provocative chemical agents such as sodium lauryl sulfate (Kligman, 1966c), special skin preparation such as stripping (Spier & Sixt, 1955) or freezing (Epstein et al., 1963), special patches (Magnusson & Hersle, 1965), high concentrations at induction (Marzulli & Maibach, 1974), and 25–200 test subjects (Draize et al., 1944; Kligman, 1966b). It is not entirely clear, however, how useful these variations are and what the limitations are under the use conditions because validation has not kept pace with the use of the different approaches. Furthermore, predictive tests are often performed on a single chemical entity, whereas ultimate use may occur as part of a multicomponent formulation in a marketed product, where the vehicle and associated ingredients may influence the outcome. It is essential that the test has sufficient statistical power to provide appropriate protection for the population at risk. This is illustrated by the mathematical

considerations of Henderson & Riley (1945) in their classical paper on extrapolating data from a small test population to large numbers of users. Briefly stated, there may be no skin reactions in a test population of 200 random subjects, yet as many as 15 of every 1000 of the general population (95% confidence), or up to 22 of every 1000 may react (99% confidence). If the test group is reduced to 100 subjects, up to 30 of every 1000 of the general population may react (95% confidence). Conversely, when 1 of 200 subjects in a test population becomes sensitized, a test population of 10 000 subjects might show from 1 to 275 sensitized, with 95% confidence.

Prospective tests of skin sensitization using human volunteers should always be conducted in accordance with ethical principles.

6.4.2 Testing for respiratory allergy

Most of the animal models that are used for studying specific respiratory hypersensitivity were developed using allergens with high relative molecular mass, notably proteins. Very few animal models have been developed as predictive tests for hazard identification and risk assessment in the area of chemical-induced respiratory allergy. The majority of these models are based upon antibody-mediated events. The models differ with regard to the following aspects: the animal species utilized, the route of administration of the agent, the protocol for both induction and elicitation of responses, type of response measured, and judgment of significant response.

6.4.2.1 *Guinea-pig model*

The guinea-pig has been used for decades for the study of anaphylactic shock and pulmonary hypersensitivity (Sarlo & Karol, 1994). The guinea-pig is similar to humans in that the lung is a major shock organ for anaphylactic responses to antigens. The guinea-pig responds to histamine and can experience both immediate-onset and late-onset responses. Airway hyperreactivity and eosinophil influx and inflammation can also be demonstrated in this animal species. Mechanistic studies have been hampered by the lack of reagents needed to identify cells and mediators in respiratory allergy. In addition, the major anaphylactic antibody is IgG1a, whereas it is IgE in other rodent species and in humans. The *in vivo* passive cutaneous anaphylaxis (PCA) assay was used to measure IgG1a antibody

responses, but ELISA methods have now been developed that have eliminated some of the variability seen with the PCA.

The guinea-pig inhalation model focuses on identifying chemical sensitizers by measurement of the response, or elicitation phase, of sensitization. In contrast with the mouse IgE test, the model does not depend upon a preconceived mechanism of sensitization. Rather, it functions by reproducing the characteristics that typify the hypersensitivity reactions, i.e., the physiological response of the airways and the pulmonary inflammation. Measurement of specific antibody formation provides ancillary evidence of the response. The method has been successfully used to distinguish low relative molecular mass contact sensitizers from respiratory sensitizers. The model utilizes inhalation as the route of exposure for both the sensitization phase and the elicitation phase of the response. A variation of the method is the use of intratracheal administration of the agent (Sarlo & Karol, 1994). The model has the capacity to assess immediate-onset responses (IAR), as well as late-onset responses (LAR). The latter is possible since minimal restraint of animals is used. A dynamic air supply and passive detection devices allow continuous 24-h monitoring of respiratory function of animals. The ability to detect late-onset responses makes the model particularly appropriate for evaluation of chemical allergy, where late-onset responses are a frequent occurrence.

The advantages of the guinea-pig model for chemical sensitization include: use of inhalation as the relevant route of exposure; generation of atmospheres of reactive chemicals; measurement of physiological responses including immediate-onset responses, late-onset responses, fever and hyperreactive airways; measurement of specific antibody production; and histopathological evaluation of pulmonary tissue. Disadvantages of the model are the cost, the time involved, the need for specialized facilities, and the employment of guinea-pigs. The latter is a disadvantage in that IgGl rather than IgE is the major class of cytophilic hypersensitivity antibody in guinea-pigs.

Variations of the guinea-pig model have been developed to optimize the response, to monitor additional respiratory parameters, or to simplify the procedure (Briatico-Vangosa et al., 1994). Such variations include single or repeated intradermal administration of free chemical, elicitation with a multivalent chemical-adducted protein, and

measurement of flow-volume loops, respiratory minute volume, inspiratory and expiratory time, and peak respiratory flow rates. Using chemical-protein adducts for elicitation avoids the possible development of airway hyperreactivity due to chemical irritation (analogous to the reactive airways syndrome in humans).

Using the guinea-pig model, differences were readily apparent between sensitization to allergens of high relative molecular mass (HRMM) versus those of low relative molecular mass (LRMM). Responses to ovalbumin and to bacterial subtilisin (HRMM allergens) consisted of severe immediate-onset responses in 90–100% of animals and late-onset responses in 50% of animals (Thorne & Karol, 1989). By contrast, sensitization to diphenylmethane-4,4-diisocyanate (MDI), a LRMM allergen, consisted predominantly of late-onset responses (Karol & Thorne, 1988). This finding reflects the human experience where late-onset responses are the most frequently observed responses to LRMM allergens.

The model has been validated in two ways. Firstly, it has been established in several laboratories (Pauluhn & Eben, 1991; Sarlo & Clark, 1992; Stadler & Loveless, 1992; Warren et al., 1993; Sarlo & Karol, 1994) and responses of animals to inhaled toluene diisocyanate have been reproduced. This confirms the robustness of the model. Secondly, the model has been found to distinguish pulmonary from dermal chemical sensitizers, and from non-sensitizers. For example, inhalation of toluene diisocyanate (Karol, 1983) and diphenylmethane-4,4-diisocyanate (Karol & Thorne, 1988) by animals resulted in pulmonary sensitization, whereas similar exposure to formaldehyde (Lee et al., 1984) and hydrogenated diphenylmethane-4,4-diisocyanate (Karol & Thorne, 1988), two recognized contact sensitizers, resulted in dermal sensitivity. Further validation with additional classes of chemicals is needed to generate confidence that information will be applicable to human disease.

It should be emphasized that the measurement of respiratory responses induced by chemical respiratory allergens is technically demanding, and consequently conflicting results have been reported. For example, it has proven difficult to induce robust respiratory responses in animals sensitized to the potent human respiratory allergen diphenylmethane-4,4-diisocyanate. Pauluhn & Mohr (1994) reported that a proportion of animals sensitized to diphenylmethane-

4,4-diisocyanate by inhalation responded to challenge with inhaled diphenylmethane-4,4-diisocyanate, while those sensitized by intradermal injection did not. The converse finding was reported by Rattray et al. (1994).

Although the guinea-pig can be used for testing the capacity of chemicals to induce respiratory allergy, it needs further validation in terms of predictive value. However, it should be noted that it can also be used to test proteins for their ability to stimulate the production of anaphylactic antibody (Blaikie et al., 1995; Sarlo et al., 1997). Such information may be of value in assessing the relative allergenic potency of proteins.

6.4.2.2 *Mouse IgE model*

A mouse inhalation model to study airway responses to sensitizing chemicals has been developed (Garssen et al., 1991), but has not yet been used with a wide range of chemicals associated with respiratory allergy. The mouse IgE test currently represents the furthest developed systematic approach to the prediction of respiratory allergy in the mouse. It does not evaluate actual airway responses, but is based on the notion of the nature of immune responses elicited in mice by chemical allergens and of the qualitative differences in immune responses provoked by contact and respiratory sensitizers as they are generally found.

Topical administration to mice of chemical respiratory allergens stimulated a substantial increase in the serum concentration of total IgE, a response not seen with contact allergens considered to lack the ability to cause sensitization of the respiratory tract (Dearman & Kimber, 1991, 1992). Observations suggested that it might be possible to identify chemical respiratory sensitizers as a function of induced changes in serum IgE concentration; the advantage of this approach being that measurement of a serum protein, rather than of hapten-specific antibody, is required. This forms the basis of the mouse IgE test.

Investigations have suggested that the mouse IgE test may provide a useful method for the prospective identification of chemical respiratory allergens, a conclusion that is supported by recent studies in an independent laboratory (Potter & Wederbrand, 1995). It must be

emphasized, however, that to date the assay has been evaluated only with a limited number of chemicals, most of the analyses have been performed in a single laboratory, and the mechanistic basis of the model has not been established.

Respiratory allergic responses, associated with increased reactivity of airways, were observed in mice that were topically sensitized to and intranasally challenged with picryl chloride, a Th1-type immune-response-inducing chemical (Garssen et al., 1991). In addition, it has been shown that IgE-deficient mice undergo anaphylaxis (Oettigen et al., 1994). Other investigators have noted a decrease, rather than an increase, in serum IgE following exposure of mice to toluene diisocyanate (TDI) (Satoh et al., 1995). For this reason, actual testing of lung function *in vivo* after sensitization and challenge with chemicals known to sensitize, yet unable to produce IgE responses, as can be done in mice (Garssen et al., 1991) as well as in the guinea-pig (section 6.4.2.1), seems prudent. Moreover, standardization of the methodology is necessary, including dosages appropriate for testing, optimal time for repeated administration of the chemical and for obtaining sera, and clarification of the meaning of "elevated" IgE titre. Validation of the method is still needed.

6.4.2.3 *Rat model*

A rat bioassay has been developed by Pauluhn (1996). Nose-only exposure for 1 or 2 weeks, using non-irritant concentrations as judged in short-term pilot experiments, is carried out, upon which lung functions are tested and biochemical and morphological signs of effects in the airways are determined. The model needs further validation to evaluate whether it is suitable for the prediction of respiratory sensitization. A rat model to study respiratory syndromes, including the IgE-mediated allergic responses, in addition to anaemia and haemolysis in workers exposed to trimellitic anhydride (Zeiss et al., 1977), was used by Leach et al. (1987). In this model, animals are subjected to single or multiple exposures at several concentrations of trimellitic anhydride dust and at selected time points are challenged with a single exposure of trimellitic anhydride dust. The lungs are evaluated for haemorrhage and serum is tested for IgG antibody specific for trimellitic anhydride. The model has also been applied for other types of anhydrides.

6.4.2.4 *Predictive testing for respiratory allergy in humans*

For obvious reasons, predictive testing for respiratory sensitization is not done in humans. Occasionally, case reports may serve as an adequate hazard identification but not as a risk estimate, because data on route and extent of exposure, and on the "population at risk" are usually lacking. In the absence of case reports, it is not possible to conclude that there exists no potential for sensitization.

6.4.2.5 *Cytokine fingerprinting*

Contact and respiratory chemical allergens provoke in mice qualitatively different immune responses suggestive of divergent Th-cell activation and characterized by different patterns of cytokine production. Chronic exposure of mice, over a 13-day period, to trimellitic anhydride was found to result in the production of high levels of mitogen-inducible IL-4 and another Th2-cell cytokine interleukin 10 (IL-10) by draining lymph node cells, but only low levels of IFN-γ. In contrast, treatment of mice under the same conditions of exposure with oxazolone (a potent contact allergen) caused the production by draining lymph node cells of only comparatively low levels of IL-4 and IL-10, but high concentrations of IFN-γ (Dearman et al., 1995). Similar selective cytokine secretion profiles have been recorded following exposure of mice to other contact and respiratory chemical allergens (Dearman, 1996). These data raise the question of whether it might be possible to monitor the sensitizing properties of chemicals as a function of induced cytokine production profiles. Evidence suggests that this is the case, and the value of cytokine fingerprinting in the routine identification and classification of chemical allergens is being explored currently.

6.5 Testing for food allergy

Despite the availability of several methods to study antigenic and allergenic properties of protein products, current testing possibilities to investigate the allergenicity of food proteins at a pre-market stage are very poor. Validated methods with a high sensitivity have not yet been developed. However, some strategies can be followed to obtain additional relevant information apart from the information obtained from the currently applied assays; for instance, taking account of the role of the gastrointestinal tract physiology.

The major adverse reactions to food constituents that involve the immune system are to proteins; non-proteins may cause food intolerance that does not involve the immune system, and hence are non-allergic reactions that do not fall within the scope of this monograph.

Most proteins encountered by the immune system can produce immune responses. To determine the antigenicity of proteins, several assays are operational. However, these assays are based on parenteral application of the test proteins to laboratory animals. For food allergy research, three rodent species have frequently been used: the mouse, the guinea-pig and the rat. In many studies, sensitization was performed parenterally or passively and effects of enteral challenges were subsequently studied (Bloch & Walker, 1981; Freier et al., 1985; Granato & Piguet, 1986; Pahud et al., 1988; Miller & Nicklin, 1988; Turner et al., 1988, 1990; Curtis et al., 1990). In addition, effects of challenges have frequently been investigated in *in vitro* studies with intestinal tissue or with, for instance, ligated gut (Roberts et al., 1981; Baird et al., 1984; Lake et al., 1984; Catto-Smith et al., 1989a,b). The guinea-pig is the most regular test species. In general, any protein that may be recognized as an antigen (foreign protein) will induce a humoral immune response upon injection and will most likely give a positive testing result in rodent assays. Although the information from antigenicity assays may be of major relevance, in that they will provide information on the quality and vigour of the response, it must always be recognized that such assays only provide information in the species examined. Whether a protein has a high or low potency of inducing food allergic reactions in (susceptible) humans cannot be concluded or predicted based only on the results of parenteral antigenicity assays.

In addition to *in vivo* antigenicity assays, several (combinations of) physicochemical and immunochemical analyses are used routinely to detect antigenicity.

Determination of allergenic proteins or fragments that are able to cause activation of mast cells and basophils is possible using *in vitro* mast cell or basophil degranulation tests. For these assays, mast cells or basophils are loaded with antigen-specific cytophilic antibodies using serum from sensitized humans or test animals. The cells are subsequently incubated with the antigen or test product, and

degranulation of the cells can then be determined. A well-validated *in vivo* counterpart for the detection of mast cell activation is the Passive Cutaneous Anaphylaxis (PCA) test, in which sera from sensitized animals are injected subcutaneously in unsensitized animals. Possible cytophilic antibodies present in the sera are bound by the receptors on the mast cells in the skin, and bridging of the antigen-specific antibodies on the mast cells by injected antigen induces mast cell activation — the resulting reaction is detectable by various methods. In the Active Systemic Anaphylaxis (ASA) test, which is also a validated anaphylaxis test, actively sensitized animals are injected with the test substance intravenously and several parameters are recorded to determine the systemic anaphylactic response as a measure for the degree of sensitization.

In the evaluation of the potential allergenicity of food products, clinical assays such as skin-prick tests or challenge procedures may also be used. For instance, these assays are applicable in the evaluation of the residual allergenicity of hypoallergenic products, in the evaluation of cross-allergenicity, or in the evaluation of the possible allergenicity of food products derived from biotechnologically derived crops in which a gene from a known allergenic source species has been introduced. However, the use of patients in such assays for non-diagnostic purposes requires careful ethical consideration. Since many of the questions may also be addressed by performing *in vitro* assays such as immunochemical analyses, the use of sera from patients is always preferable to intentional exposure of humans.

6.6 *In vitro* approaches

Another approach for the detection of potential sensitizing capacity that would not rely on *in vivo* animal or human testing is directed towards the construction of *in vitro* experimental models that reflect accurately some pivotal event during skin sensitization. Accurate modelling of the immune system *in vitro* is not possible without considerable difficulty. Nevertheless advances have been made.

Wass & Belin (1990) and Gauggel et al. (1993) used biochemical techniques to examine the ability of low relative molecular mass chemicals to act as haptens by combining them with a model protein or a polypeptide. Gauggel et al. (1993) showed that their test correctly

identified 12 out of 14 known human allergenic haptens and 23 out of 24 non-allergenic low relative molecular mass chemicals. Neither method can detect sensitizers that must be metabolized to form a hapten.

6.7 Testing for autoimmunity

Although there are currently no predictive assays developed and validated to identify in the early phases of toxicity testing the potential of chemicals to induce autoimmune responses, it should be noted that assays to identify contact sensitizers (Kimber et al., 1994; Vial & Descotes, 1994) might be helpful to identify systemic sensitizers. Clinical signs of systemic immune-mediated side-effects usually become manifest only during advanced clinical development of drugs. The conditions used in routine preclinical toxicological screening are obviously not optimal for the detection of the immune-dysregulating potential of drugs and chemicals (e.g., small animal number, use of outbred animal strains, dynamics of disease development versus snapshot determinations, lack of predictive parameters). An economically and practically relevant question concerning screening studies is whether actual evidence of an agent's ability to induce manifest hypersensitivity or autoimmune disease should be and can be obtained, or whether (preferably short-term) assays not measuring the actual clinical end-points can be sufficiently predictive in this respect.

In vitro tests for biological effects of sensitizers or chemicals able to induce autoimmunity are at present in their infancy. It should be emphasized that there are two major limitations: (a) it is so far impossible to completely reproduce *in vitro* the complex micro-environment in which immune responses are initiated *in vivo*; and (b) some immune reactions are elicited not by native xenobiotics but by their metabolic products generated *in vivo*.

6.7.1 Popliteal lymph node assay

At present this appears to be the only method available to examine the ability of chemicals to induce an autoimmune response, but the usefulness of the results in risk assessment remains to be demonstrated. Based on the hypothesis that chemicals may elicit autoimmune disorders by a mechanism resembling graft-versus-host reactions, an existing graft-versus-host assay, the popliteal lymph node

assay, has been modified to study chemical-induced immune reactions (Bloksma et al., 1995). Using the popliteal lymph node assay, many drugs known to occasionally induce immune-mediated systemic side-effects in humans were shown to trigger significant reactions in mice and rats (Gleichmann, 1981; Gleichmann et al., 1983, 1989; Hurtenbach et al., 1987; Kammüller & Seinen, 1988; Stiller-Winkler et al., 1988; Kammüller, 1989a; Thomas et al., 1989, 1991; de Backer et al., 1990; Verdier et al., 1990; Katsutani & Shionoya, 1992; Krzystyniak et al., 1992; Bloksma et al., 1995). Effects observed were very similar to those induced during a local graft-versus-host reaction in the popliteal lymph node assay (de Bakker et al., 1990). Immunogenetic studies in mice with diphenylhydantoin (Bloksma et al., 1988) and D-penicillamine (Hurtenbach et al., 1987) have indicated that the extent of popliteal lymph node enlargement is controlled by major histocompatibility complex (MHC (H-2)) as well as non-major histocompatibility complex genes. Furthermore, the popliteal lymph node assay was able to discriminate between structurally closely related compounds, for example chemical congeners of D-penicillamine (Hurtenbach et al., 1987), diphenylhydantoin (Kammüller & Seinen, 1988) and zimeldine (Thomas et al., 1989, 1991). Thus, the direct popliteal lymph node assay seems to be a versatile tool for recognizing T-cell-activating drugs and chemicals, including autoimmunogenic chemicals, but it also produces false-negative results (Bloksma et al., 1995). With the adoptive transfer popliteal lymph node assay, sensitized cells are used as probes to detect the formation *in vivo* of immunogenic metabolites of low relative molecular mass chemicals (Kubicka-Muranyi et al., 1993; Bloksma et al., 1995). However, further mechanistic studies and interlaboratory validation is required before either variant of the assay can be recommended for routine use in the preclinical toxicity screening (Verdier et al., 1997).

6.7.2 Animal models of autoimmune disease

Three basic types of animal models may be employed to identify the potential of drugs or chemicals to induce systemic hypersensitivity or autoimmune responses: (a) genetically predisposed animals; (b) autoimmunization; and (c) organic or chemically induced (Table 26). In each type of model the development and severity of symptoms is multifactorial, in that the disease state can be influenced by age, hormonal and/or environmental factors. In addition there is a

Table 26. Experimental models for autoimmune diseases[a]

Autoimmune disease	Classification		
	Genetically predisposed strains[b]	Autoimmunization[c]	Biological/chemical induction
Autoimmune thyroiditis (Hashimoto's and Graves')	Murphy-Roths lymphoma (MRL) mouse, BioBreeding (BB) rat, Obese strain (OS) chicken	Thyroglobulin — EAT (mouse, rat)	
Insulin-dependent Diabetes mellitus	Non-obese diabetic (NOD) mouse, BioBreeding (BB) rat, Diseases resistant BioBreeding (DRBB) rat, Brown Norway rat		Streptozotocin (STZ) (mouse)
Myasthenia gravis		Acetylcholine receptor — EAMG (mouse, rat)	Penicillamine (mouse, rat)
Multiple sclerosis		Myelin basic protein EAE (mouse, rat, chicken)	
Rheumatoid arthritis	Murphy-Roths lymphoma (MRL)/lpr mouse, Severe combined immunodeficient (SCID) mouse, Human leukocyte antigen B27 (HLA B27) transgenic rat	CFA + Type II collagen (mouse, rat, monkey) CFA + TB hsp (mouse, rat)	Streptococcal cell-wall (rat)
Systemic lupus erythematosus	Murphy-Roths lymphoma MRL +/+ mouse, Murphy-Roths lymphoma MRL/lpr mouse, Murphy-Roths lymphoma (MRL)-mp-lpr/lpr mouse, New Zealand White (NZW) 2410 mouse, New Zealand Black (NZB) mouse/ New Zealand White (NZW) mouse	CFA + antiDNA antibodies (mouse, rat)	Mercury (mouse, rat, monkey) Penicillamine (mouse, rat) Procainamide (mouse, rat)
Systemic sclerosis (Scleroderma)	Tight skin (TSK) mouse,		

[a] Adapted from Cohen & Miller (1994); Farine (1997); Bigazzi (1997)
[b] lpr = lymphoproliferation
[c] CFA = Complete Freunds Adjuvant

tendency for more than one autoimmune disorder to occur in a number of individual models. Nevertheless, a number of syndromes similar to that observed in humans can be mimicked in animal models.

The genetically predisposed models, whether naturally occurring, transgenic or knockout based, tend to be the most reliable and therefore have been more commonly employed in autoimmunity research (Lo, 1996). In this type of model, mild to severe syndromes spontaneously develop, usually due to specific MHC allele mutations encoding class II molecules and often inducing function abnormalities of the $CD4^+$ Th-cell (Theofilopoulos, 1995a,b). The most common genetic models include several MRL mouse variants for autoimmune thyroiditis, arthritis and lupus, several variants of New Zealand Brown mice (NZB), New Zealand White mice (NZW) and BXSB mice for lupus, and the non-obese diabetic (NOD) mouse and BioBreeding (BB) rat for insulin-dependent diabetes mellitus (Cohen & Miller, 1994).

Autoimmunization with purified self-antigens can elicit a specific autoimmune response, particular when adjuvants are administered in conjunction with self-proteins. A frequently used model of this type, experimental autoimmune encephalomyelitis, is induced by immunization of rodents with myelin basic protein. The resulting pathology is a $CD4^+$ T-cell-mediated autoimmune disease characterized by central nervous system perivascular lymphocyte infiltration and destruction of the myelin nerve sheath with resultant paralysis, similar to that observed in patients with multiple sclerosis (Constantinescu et al., 1998). Additional models included immunization with thyroglobulin to simulate Hashimoto's thyroiditis, and the injection of type II collagen to induce rheumatoid arthritis (Wick et al., 1974; Durie et al., 1994).

In experimental models, foreign substances are used to induce the autoimmune disease state. These include chemicals, drugs and biological substances such as bacterial or viral antigens. Brown Norway (BN) rats injected with non-toxic amounts of mercuric chloride which produce no signs of overt toxicity develop an immunologically mediated disease characterized by a T-cell-dependent polyclonal B-cell activation (Pelletier et al., 1994). These animals demonstrate increases in serum IgE and the production of autoantibodies to a number of proteins including DNA, laminin,

collagen IV and other components of the glomerular basement membrane. Proteinuria and nephrotic syndrome similar to that observed in humans are also observed. The disease is characterized by a glomerulonephropathy evolving in two phases: (a) a linear anti-GBM antibody deposition along the glomerular capillary pattern, and (b) a change to a granular pattern of immunofluorescence with the appearance of immune complex deposits (Pelletier et al., 1994).

6.8 Clues from general toxicity tests

The conditions used in conventional systemic toxicological screening are not designed for the detection of the potential of drugs and chemicals to induce allergy or autoimmunity, as general toxicity screening does not include specialized tests required for this purpose. In more recent guidelines for general toxicity screening, attention is given to the immune system, especially with the purpose of detecting direct toxicity to components of the immune system. This pertains for instance to the updated OECD guideline for 28-day oral toxicity testing (Koeter, 1995). According to this guideline, lymphoid organs are weighed and lymphoid tissues are examined microscopically. In addition to morphological examinations of lymphoid organs, a selection of non-lymphoid tissues (e.g., blood vessels, renal glomeruli, synovial membranes, thyroid, skin, liver and lungs) should be investigated. Tissue damage, protein (immune) complex deposits, and/or inflammatory cell infiltrates in these tissues may indicate the induction of hypersensitivity or autoimmune phenomena.

It is common practice in toxicological pathology to rely most on statistically significant differences in incidence between test groups and controls. However, individual variability can be high, especially regarding hypersensitivity and autoimmune phenomena (Holt & Sedgwick, 1987; Kammüller et al., 1989). Therefore, in studies with a limited number of animals per group, a change in only a single or a few test animals may have considerable biological relevance. This is particularly true in outbred animals.

In addition to morphological examinations in routine toxicological studies, measurements of some immunologically relevant serum parameters can provide important information about antibody-mediated responses. Parameters may comprise levels of total immunoglobulins and of various immunoglobulin (sub)classes, immune

complexes, and some commonly observed autoantibodies, e.g., anti-nuclear antibodies (ANAs), anti-histone, and anti-single-stranded deoxyribonucleic acid (denatured; ssDNA) autoantibodies. Some of these autoantibodies proved to be useful in the diagnosis of procainamide-induced systemic lupus erythematosus in humans as shown by Rubin et al. (1995). Measurement of these parameters at suitably chosen intervals, especially during subchronic and chronic exposure, may obviate the snapshot nature of the histopathological examinations and will give a reflection of cellular and humoral immune function in time. With regard to autoantibody measurements it is important to measure both total immunoglobulin classes and subclasses. This can be illustrated by murine models of spontaneous systemic lupus erythematosus in which a switch from IgM to IgG autoantibodies could be associated with development of overt disease. Also, data on drug-induced systemic lupus erythematosus in humans point in this direction. Anti-nuclear antibodies in patients with procainamide-induced systemic lupus erythematosus appeared to be of the IgG class (in particular IgG1 and IgG3), whereas IgM anti-nuclear antibodies are predominant in asymptomatic users of the drug.

It has been suggested that parameters of the immune system are included in conventional chronic toxicity testing and also in reproductive toxicity testing and evaluation, since the developing immune system may be particularly vulnerable to immune dysregulating effects (Hollady & Luster, 1996). For example, a newly developed immunomodulating agent was found to induce thyroiditis and significant antithyroglobulin autoantibodies in a 6-month and a 2-year rat study (Verdier et al., 1997).

7. RISK ASSESSMENT

7.1 Introduction

Risk assessment for human health protection is the final stage in evaluating the likelihood that potential adverse health effects will manifest themselves in humans.

7.2 Risk assessment of allergy

Effective risk assessment requires an appreciation of the potential of a chemical or drug to induce an allergic response, or elicit an allergic reaction, and its potency. Potency here is considered as the amount of chemical necessary to induce an allergic response or to elicit an allergic reaction. Risk assessment demands also an understanding of the conditions of human exposure, i.e., the extent, duration and route(s) of likely exposure.

With the assembly of the available data completed, risk assessors must decide whether sufficient data are available to proceed. It may be necessary to produce a preliminary or provisional risk assessment irrespective of the availability of full documentation. However, at this stage, following comparison of what is available with minimal data sets, consideration is normally given to further searching, further requests for information to industry, and the use of modelling or default values to fill data gaps. A decision is then made on whether to proceed.

Risk assessment of allergy is complicated by the fact that there exist two related, but nevertheless independent considerations. Allergic disease almost always occurs in two phases. During the first phase the previously non-sensitized individual is exposed to sufficient allergen in such a way that an allergic response is induced which results in sensitization. This is the induction phase. An allergic reaction will be elicited if this now sensitized individual is exposed again to the same allergen under conditions where a secondary, more aggressive allergic response is provoked. This is known as the elicitation phase. It is important to appreciate that these phases usually differ with respect to the exposure conditions required. The induction of allergic sensitization in a previously naive individual almost invariably requires exposure to concentrations of the allergen that are greater than those

necessary to elicit a response in a sensitized subject. Allergic reactions can be provoked in sensitized individuals with small amounts of the inducing allergen that are without effect in non-sensitized populations. It is possible also that sensitization may be achieved from exposure via a route that is different from that needed to elicit a response. Thus, there is some evidence that dermal exposure to certain chemical allergens may stimulate the quality of immune response necessary to cause sensitization of the respiratory tract. The elicitation of pulmonary responses will subsequently be provoked following inhalation exposure of the now sensitized individual to the relevant chemical.

Taken together it is clear that risk assessment for chemically induced allergic disease has two components: (a) the likelihood that a chemical will induce sensitization in a previously unsensitized individual; and (b) the conditions under which a chemical will provoke allergic reactions in those who are already sensitized.

Information on risk, plus suggestions about any group of increased susceptibility, is used for risk evaluation, that is the process of deciding for medical, ethical, economic and legal reasons whether the identified risk will be "acceptable" and under what conditions, e.g., restricted availability of the substance in special circumstances or wider availability with a warning label and advice about protective measures. The decision may also be made to ban the use of a material deemed to carry too large a risk in relation to anticipated benefit from its use. These decisions reflect socioeconomic and political factors as well as medical and scientific conclusions, and so lie outside the purpose of this monograph.

7.3 Factors in risk assessment of allergy

Risk assessment requires answers to the following questions:

- *Who will be exposed?*

How many people: Is there any information to suggest particular susceptibility (or resistance) to allergic hypersensitivity, e.g., genetic, nutritional or other factors?

- *Circumstances of the exposure*

  Is it an initial sensitizing exposure or a subsequent provocative exposure that may elicit an allergic reaction?

- *Extent and duration of exposure*

  This defines the "load" of the allergen. Most of the available evidence suggests that it is the peak concentration of exposure rather than the cumulative delivered dose that is critical in determining the extent to which sensitization will develop. The same principle probably applies also to the elicitation of allergic reactions in sensitized individuals.

- *Nature of exposure*

  Exposure to chemical allergens occurs frequently in the context of mixtures and formulations. An important consideration is whether other components of the mixture will influence the ability of the chemical allergen to induce sensitization or elicit a reaction.

- *Route*

  If it is the first exposure, is it by a route that may sensitize people, usually via inhalation or skin contact, or by ingestion, which is much less likely to do so? If an individual is already sensitized, is it via the route by which he has already been sensitized, in which case an allergic response may occur? Or, will it be by another route, which may not necessarily evoke an immunological response?

- *Evidence of hazard*

  What is the nature and quality, i.e., the "strength", of the laboratory information showing the sensitizing potential of the substance? Has it come from laboratory tests of proven value, from chance observations during some other type of experiment, or is it based on prediction from structure–activity relationships? In each of these, are the data qualitative or quantitative?

- *Prior evidence of risk*

  Are there already results from humans, either as tests in a clinical laboratory setting, or following exposure in the real world of work or home, showing that people have been sensitized and how, and the consequences of subsequent exposure by any route?

Taking all this information together, each aspect of which has already been discussed in this monograph, should suggest the totality of the *risk* under a given set of circumstances, in other words, the likelihood that a given exposure to a substance will sensitize people, or evoke an allergic response in them, perhaps the proportion affected, and the nature of that response (including clinical feature of the disorder and their severity). That represents the ideal. In practice, the available information may be incomplete, and skilled judgement is required to extrapolate from whatever is known to suggest what may happen in practice.

7.4 Information aspects

The three commonest deficiencies in knowledge are discussed below.

7.4.1 No information about hazard

For a novel substance, a novel use, or because hypersensitivity may not previously have been recognised, there will be no information from humans. In that case, prediction can only be based upon the chemical structure of the substance, the results of appropriate laboratory tests, and assumptions about exposure of people.

Such an extrapolation based solely on laboratory-defined hazard is the usual situation faced by the toxicologist, occupational health specialist and regulator in dealing with a new substance.

As discussed earlier in this monograph, many of the laboratory procedures available to identify hazard are well proven and some are valuable in assessing relative potency for skin sensitizers. Their quantitative predictivity, sensitivity and specificity are difficult to state, but they probably give about 80% correct negative results, i.e.,

sensitization is unlikely (Kimber & Maurer, 1996) and about 60% correct positive predictions, i.e., skin sensitization is likely to occur.

For respiratory tract sensitization, far fewer results are available, but a positive finding in any of the types of experiment considered here should be regarded as a powerful indicator of this type of hazard. The strength of a negative finding is less certain, because there is little published information.

True hypersensitivity to oral antigens is still poorly understood, and the more common but more fickle "food intolerance" remains clouded by uncertainties. For a novel substance in a food, it is not yet possible to predict the likelihood that it will cause true allergy or intolerance if consumed. Known allergens and the other materials that sometimes cause disorders when eaten can be indicated by reference to published clinical information, which should also point both to the likely frequency of the harmful response, and whether it is affected by ethnic or by other physiological factors, the methods of preparation and cooking, and the matrix in which a putative food allergen is presented, because the complex nature of the mixtures that are foods does affect the risk of sensitization and elicitation of reactions to materials in the diet.

The best predictor at present of the risk of true immunologically mediated hypersensitivity to a food component is probably demonstration of the presence of antibodies to known potent allergens, such as peanut (groundnut) protein.

Predicting the risk of autoimmunity, too, can only be done by demonstrating the presence of a known antigen in an appropriate matrix, or, with less certainty, by demonstrating a very close structural analogy to a known cause of an autoimmune response.

7.4.2 Scanty or no information about exposure

It is sometimes difficult to envisage exactly how a substance will be used in the workplace or at home, or how it may be disseminated via the environmental media. In both of these instances the prediction of risk must carry some uncertainty.

The problem of predicting exposure is well shown by the demonstration of an increased incidence of asthmatic attacks in the population of the city of Barcelona during large-scale transhipment of soy beans in the docks. That appears to have released a sufficient quantity of an allergen into the air for it to be disseminated and to expose the entire population under certain meteorological conditions, resulting in allergic asthma in so many people as to have a serious effect on the health of the community.

7.4.3 *Unreliable or scanty information about risk*

This is a particular problem because of the propensity of clinicians to report unusual or unique examples of disorders and not to describe negative findings. It may be difficult, therefore, to gain a balanced or critical view of the allergenic effects of substances from much of the clinical literature. It can provide an indication of suspicion, but large, carefully constructed series of patients or cautious surveillance results are rare.

At a different level, but well able to provide helpful information, are national occupational health statistics on appropriate industrial diseases, particularly those for which a pension has been awarded. For many reasons those series are unlikely to provide unbiased information about incidence or prevalence, because of the inevitable bias when legal liability may be involved, but they can act as broad indicators of risk, and even of the allergenic potential of particular substances (Drever, 1995).

Databases of consumer products used by the general population are very limited and may be close to capricious in their content, but they should be consulted when possible.

Overall, therefore, the risk assessor has a very difficult task in the general field of allergic hypersensitivity. At present, laboratory tests may indicate the potential for a hazard, and searching consideration may suggest many of the features about exposure. From that a risk to humans may be predicted in qualitative terms, at least for the common route of domestic and occupational exposure of the skin. The prediction of respiratory tract allergy remains less certain, and the likelihood of effects following ingestion, or of an autoimmune disorder, can very rarely be predicted.

Many predictive test methods serve simply to identify the inherent potential of a chemical to induce allergy but provide no indication of the potency with which it will do so. One problem is that some methods do not incorporate a dose–response analysis. The other issue is that some tests measure activity as the frequency of responses rather than the severity of responses. The need is to have available information on potency defined as the quantity of chemical necessary to induce sensitization (or to elicit a reaction). Some newer test methods are beginning to address this issue and are providing information about allergic potency relative to index allergens. Such comparisons have been of value in setting safe occupational exposure levels and minimizing the risk of allergic disease.

As in any form of toxic reaction, "dose" is important, in that initial sensitization requires at least a certain minimum exposure (concentration of allergen, its local availability at the site of administration, and the duration of contact). In someone already sensitized, the likelihood of producing a clinical disorder and its severity are also related to dose, although, by definition, the quantity of allergen required to produce an effect is very much smaller than that associated with a conventional toxic action. This aspect of the extent or intensity of dose (=exposure) is more important at the practical level of preventing sensitization or protecting the sensitized individual, i.e., risk management, but it should not ignored at the risk assessment stage.

A further difficulty experienced by the risk assessor in this area is that of individual susceptibility. Genotype does play some role in the propensity of people to develop clinical disorders due to hypersensitivity, as shown, for example, by the familial nature of atopy. There is also good clinical evidence, however, to show that the inheritance of eczematous skin hypersensitivity, or of asthmatic hypersensitivity to common domestic allergens, involves many powerful factors in addition to genetic constitution. Although there is no *a priori* reason to believe that humans lack "immune response" genes for hypersensitivity as pragmatically defined in animals, their importance remains uncertain. Some chemically related autoimmune diseases though are associated with certain "immune response" genotypes. For example, HLA DR3 and DR3/B8 haplotypes are associated with vinyl chloride disease (Black et al., 1983).

Skin sensitization risk assessment is not a highly prescriptive process that should always be followed in the same way (Calvin, 1992). On the contrary, what is necessary is that it is carried out thoroughly to the point where the risk has been adequately assessed. In some circumstances, this point may be reached quite quickly and with minimal expenditure of time and effort. In other cases, substantial and sustained effort is required. For example, in the situation where exposure to the contact allergen is essentially zero, then even for highly potent contact allergens, there is no need to go further with a risk assessment because allergic contact dermatitis will not occur. Furthermore, if the exposure is sufficiently low, it may not be necessary to know precisely the potency of a contact allergen. Simply the knowledge that it is not a very strong allergen may be sufficient to permit a proper conclusion of the risk assessment. Another situation where risk assessment may be relatively simple is the replacement of an ingredient with another of the same or similar type (e.g., an alternative supplier of a raw material). In such a case, and where the risk is already known to be very low, all that may be necessary is to confirm that the specification of the new source of raw material is the same. Alternatively, data that provide evidence that the relative sensitization potential of the old and new materials is similar may suffice. In contrast, even where the intrinsic sensitization potential is very low, if skin contact is sufficiently intense and prolonged, then sensitization may occur. An example of this is the situation where medicines are applied continuously to skin, often damaged and/or inflamed skin, under occlusion. A prime example is found with stasis ulcers, where a variety of medicines and chemicals with negligible sensitization potential, such as cetostearyl alcohol and paraben esters, quite frequently cause allergic contact dermatitis.

The comments above on skin sensitization all presuppose a good knowledge of the relative sensitizing potency of the substance under consideration. Such data may be obtained by examination of the skin sensitization potential of the substance in guinea-pig methods referred to earlier in this monograph. Critical to this type of analysis is data from the same method at the same testing institution with suitable benchmark substances. These may well need to be selected on a case by case basis in the light of the risk assessment that needs to be made. Alternatively, use made be made of the local lymph node assay, the results of which can be interpreted to yield an objective estimate of relative sensitizing potency (Kimber & Basketter, 1997).

To determine what represents a safe threshold for a sensitizer is a complex matter (Basketter et al., 1997). The key point is that the threshold depends on the method used to determine it. Thresholds in animal models my differ substantially from those for humans even in situations where the pattern of exposure is similar. However, where it is possible to match the type of exposure in the test in humans to that which is expected in practice, then such data may be interpreted directly to humans (Johansen et al., 1996b).

7.5 Conclusions

The scientific, practical and clinical uncertainties that affect allergic hypersensitization make the task of the risk assessor particularly difficult. In practice, it may be reasonable in such assessments to favour the fail-safe approach by emphasising the "precautionary principle", namely that if there is even a suspicion of a risk, exposure should be minimized and preferably be entirely prevented. That will have a considerable influence on the development of new substances, on devising new uses for existing materials, and on instituting controls over exposure in the home and safe working practices, because an assessment that suggests a risk of allergic sensitization must lead at once to an appropriate decision about risk management.

8. TERMINOLOGY

Adhesion molecules. Molecules, belonging mainly to the immunoglobulin or integrin superfamily of molecules (e.g., LFA-1, ICAM-1), expressed on the membrane of various cells of the immune system. Interactions with each other as receptors and corresponding ligands facilitate cooperation (cross-talk) of cells, signal transduction and information transfer between cells.

Adjuvant. A material that enhances immune response to substances in a non-antigen-specific manner.

Allergen. An antigen that provokes allergy.

Allergic contact dermatitis. An inflammatory skin disease resulting from allergic sensitization.

Allergy. Hypersensitivity caused by exposure to an exogenous antigen (allergen) resulting in a marked increase in reactivity and responsiveness to that antigen on subsequent exposure, resulting in adverse health effects.

Allogenic. Term describing genetically different phenotypes in different (non-inbred) individuals of the same species.

Anaphylaxis/anaphylactic reaction. A local or systemic immediate hypersensitivity reaction initiated by mediators released after immunological stimulation. Symptoms can be a drop in blood pressure related to vascular permeability and vascular dilatation, and obstruction of airways related to smooth muscle contraction/bronchoconstriction.

Anergy. Lack of immune responsiveness (usually defined as lack of response to common recall antigens).

Antibody. An immunoglobulin produced by activated B-cells and plasma cells after exposure to an antigen with specificity for the inducing antigen.

Antibody-dependent cell-mediated cytotoxicity (ADCC). Lysis of various target cells coated with antibody by Fc receptor-bearing killer cells, including large granular lymphocytes (NK cells), neutrophils, eosinophils and mononuclear phagocytes.

Antigen. Any compound recognized by antigen-receptor-bearing lymphocytes. Antigens induce immune responses or tolerance. Antigens inducing immune responses only with the help of T-cells are T-dependent antigens, while those that do not need T-help are T-independent antigens. All immunogens are antigens but not all antigens are necessarily immunogens (see also **immunogens**).

Antigen-presenting cells. Cells expressing MHC gene products, with the capacity to process and present antigen. Macrophages, dendritic cells, B-lymphocytes and Langerhans cells are termed professional or constitutive antigen-presenting cells. However, other cells (such as endothelial cells) can acquire the ability to present antigen in certain pathological conditions.

Antigen processing and presentation. Protein antigens are processed (cleaved by enzymes) in various compartments of antigen-presenting cells. The immunogenic peptides interact with the binding sites of MHC class II products (exogenous antigens) or with those in MHC class I products (endogenous antigens, including viruses). The processed antigen-MHC complex is recognized by the antigen receptor complex of T-lymphocytes.

Antigenic determinant. A single antigenic site (epitope) usually exposed on the surface of a complex antigen. Epitopes are recognized by antigen-receptors on T- or B-cells (T-cell epitopes or B-cell epitopes).

Anti-nuclear antibody. Antibody directed to nuclear antigen. These antibodies can have various specificities (e.g., to single- or double-stranded DNA, or to histone proteins). These antibodies are frequently observed in patients with rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic lupus erythematosus, and mixed connective tissue disease. Also called anti-nuclear factor (ANF).

Arthus reaction (Gell and Coombs Type III reaction). Inflammatory response, generally evoked in skin, that is induced by immune complexes formed after injection of antigen in an individual containing antibodies.

Asthma. A respiratory disorder characterized by variable air flow limitation. Most cases are associated with bronchial hyperresponsiveness and chronic inflammatory changes in the airways.

Atopic dermatitis. Inflammation of the skin in atopic individuals. The term is broader than atopic eczema (see also atopic eczema).

Atopic eczema. Chronic skin disease, often localized on flexural surfaces, in individuals with propensity to develop IgE-mediated allergy. The term describes eczema occurring in atopic individuals and does not imply mechanisms (see also eczema).

Atopy. A genetic predisposition toward development of IgE-mediated immediate hypersensitivity reactions against common environmental antigens.

Autoimmune disease. A disease involving immune responses against self antigens, resulting in pathological change.

Autoimmunity. Responses against self (autologous) antigens.

B-lymphocytes. Bone-marrow-derived lymphocytes, expressing an antigen-receptor complex composed of membrane-bound immunoglobulin (mIg) and associated molecular chains. B-cell receptors interact with epitopes directly (no MHC restriction). Activated B-lymphocytes produce antibody and are efficient antigen-presenting cells. They are the precursors of plasma cells.

Basophil. A circulating granular leukocyte having prominent cytoplasmic granules when stained with dyes that indicate a basic pH. The granules contain histamine and sulfated mucopolysaccharides. After binding of antigen to membrane-bound IgE via FcεRI receptors, they release histamine, platelet activating factor (PAF) and leukotrienes, and other inflammatory mediators.

Bronchoalveolar lavage. Harvesting of cells and fluid from the lung, commonly by bronchoscopy and lavage.

Bronchoprovocation. Use of inhaled triggers (cold air, histamine, methacholine, allergen, etc.) to assess the responsivity of the airways.

Carrier. An immunogenic macromolecule (usually protein) to which a hapten is attached, allowing the hapten to be immunogenic.

CD. A molecular marker on a cell surface that may be used operationally to define phenotype, origin and activation state of the cell.

CD3. A molecule composed of five polypeptide chains associated with the heterodimer T-cell receptor (TCR), forming the T-cell receptor complex (TCR/ CD3); CD3 transduces the activating signals when antigen binds to the TCR.

CD4. A cell surface antigen belonging to the immunoglobulin superfamily of molecules. Marker of T helper cells. As an adhesion molecule, it interacts with the non-polymorphic part of MHC class II gene product.

CD8. A cell surface molecule belonging to the immunoglobulin superfamily of molecules. Marker of suppressor and cytotoxic T-cells. As an adhesion molecule, it interacts with the MHC class I gene product.

CD16. Low-affinity Fcγ receptor (FcγRIII) expressed mainly on NK cells, granulocytes and macrophages, mediating ADCC.

CD23. Low-affinity Fcε receptor induced by IL-4 and expressed on activated B-cells and macrophages.

Cell-mediated or cellular response. A specific immune response in which T-lymphocytes mediate the effects, either through the release of cytokines or through cytotoxicity.

Class I MHC gene products. Antigens encoded by the MHC class I genes are expressed on all nucleated cells. They present antigen-derived peptides of endogenous origin.

Class II MHC gene products. Antigens encoded by the MHC class II genes are expressed on antigen-presenting cells. They present antigen-derived peptides of endogenous origin.

Clonal anergy. A form of self tolerance developing as a consequence of negative selection during the thymic selection processes. Clones of thymocytes whose antigen receptors (TCR) bind with high affinity to self antigens in association with MHC molecules are inactivated.

Complement system. A group of serum proteins with the capacity to interact with each other when activated. The chain reaction of the activated complement components results in formation of a lytic complex and several biologically active peptides of low relative molecular mass (anaphylatoxins). The system can be activated by antigen-antibody complexes (classical pathway) and by other components, e.g., bacteria (alternative pathway). As an effector mechanism of the humoral immune response, the activated complement system facilitates opsonization, phagocytosis and lysis of cellular antigens.

Contact sensitivity. A state of immunological sensitization in which an eczematous epidermal reaction may occur when a hapten is applied to the skin of a sensitized individual. (see allergic contact dermatitis).

Contact urticaria. Urticaria provoked by contact with inducing agents (see urticaria).

Cross-reactivity. The ability of an antibody or a T-cell, specific for one antigen, to react with a second antigen; a measure of relatedness between two antigenic substances, and/or polyspecificity of the antibody molecule (e.g., some rheumatoid factors), or of the T-cell receptor.

Cytokines. Group of substances (biologically active peptides), mainly synthesized by lymphocytes (**lymphokines**) or monocytes/ macrophages (**monokines**), that modulate the function of cells in immunological reactions; cytokines include interleukins. Some cytokines (pleotrophic cytokines) have a broad spectrum of biological actions, including: neuromodulation, growth factor activity and proinflammatory activity (see also interleukins).

Cytotoxic T-cell (cytolytic T-cell)(CTL). A subpopulation of T-cells with the capacity to lyse target cells displaying a determinant in association with MHC gene products, recognized by its antigen receptor complex (TCR/CD3).

Delayed-type hypersensitivity (DTH) (Gell and Coombs Type IV reaction). A form of T-cell-mediated immunity in which the ultimate effector cell is the activated mononuclear phagocyte (macrophage); the response of DTH appears fully over 24 to 48 h. Previous exposure is required. Examples include response to *Mycobacterium tuberculosis* (tuberculin test) and contact dermatitis.

Dendritic cell. A cell type characterized by extended cytoplasmic protrusions and a high expression of adhesion molecules and Class II MHC gene products effecting antigen presentation to specific lymphocytes (see also Langerhans' cell).

Dermatitis. Inflammatory skin disease showing redness, swelling, infiltration, scaling and sometimes vesicles and blisters.

Desensitization. Generally transient state of specific non-reactivity in previously sensitized individual, resulting from repeated antigen exposures.

Eczema. A dermatitis characterized by non-contagious inflammation of skin with typical clinical (itch, erythema, papules, seropapules, vesicles, squames, crusts, lichenification) and dermatohistological (spongiosis, acanthosis, parakeratosis, lymphocytic infiltration) findings. Often due to sensitization.

Elicitation. Production of a cell-mediated or antibody-mediated allergic response by exposure of a sensitized individual to an allergen.

Endocytosis. The uptake by a cell of a substance from the environment by invagination of its plasma membrane; it includes both phagocytosis mediated by receptors and pinocytosis.

Enzyme-linked immunosorbent assay (ELISA). An assay in which an enzyme is linked to an antibody and a labelled substance is used to measure the activity of bound enzyme and, hence, the amount of bound antibody. With a fixed amount of immobilized antigen, the

amount of labelled antibody bound decreases as the concentration of unlabelled antigen is increased, allowing quantification of unlabelled antigen (**competitive ELISA**). With a fixed amount of one immobilized antibody, the binding of a second, labelled antibody increases as the concentration of antigen increases, allowing quantification of antigen (**sandwich ELISA**).

Eosinophil. A circulating granular leukocyte having prominent granules that stain specifically by eosin and containing numerous lysosomes. Eosinophils are important effector cells in immune reactions to antigens that induce high levels of IgE antibodies (e.g., parasites). Eosinophils are also abundant at sites of immediate hypersensitivity reactions.

Epidemiology. The study of the distribution and determinants of health-related states or events in specified populations, and the application of this knowledge to manage health problems.

Epitope. A single antigenic determinant.

Fc receptors. Receptors expressed on a wide range of cells, interacting with the Fc portion of immunoglobulins belonging to various isotypes. Membrane-bound Fc receptors mediate different effector functions (endocytosis, antibody-dependenT-cellular cytotoxicity (ADCC)) and induce mediator release. Both the membrane-bound and soluble forms of Fc receptors regulate antibody production of B-cells.

Forced expiratory volume in 1 second (FEV_1). Physiological measurement of the volume of air expired in one second with a maximal respiratory effort.

Forced ventilatory capacity (FVC). The physiological measurement of lung volume associated with a complete respiratory effort.

Glomerulonephropathy. Disease of the glomeruli, which may show either thickening of the basement membrane — membranous glomerulopathy associated with IgG deposits — due to the accretion of proteins, or "minimal change glomerulopathy", in which there is functional damage but little structural change by light microscopy.

Hapten. A non-immunogenic compound of low relative molecular mass which becomes immunogenic after conjugation with a carrier protein or cell and in this form induces immune responses. Antibodies, but not T-cells, can bind the hapten alone in the absence of carrier.

Helper T-cell. A functional subpopulation of T-cells (expressing CD4 antigen) that help to generate cytotoxic T-cells and cooperate with B-cells in the production of an antibody response. Helper T-cells recognize antigen in association with MHC class II gene products. Depending on their capacity to produce various cytokines one can functionally differentiate Th1 (IL-2 and IFN gamma producing) and Th2 (IL-3, IL-4 and IL-6 producing) cells.

Human leukocyte antigen (HLA). The major human histocompatibility complex situated on chromosome 6. Human HLA-A, -B and -C (resembling mouse H-2K, D and L) are class I MHC molecules, whereas HLA DP, -DQ and -DR (resembling mouse I-A and I-E) are class II MHC molecules.

Humoral immune response. An immune response in which specific antibodies induce the effector functions (such as phagocytosis and activation of the complement system).

Hyperreactivity. An abnormally increased response to a stimulus.

Hypersensitivity. Abnormally increased immunologically mediated response to a stimulus. Sometimes used loosely for any increased response (see also hypersusceptibility, hyperreactivity)

Hypersensitivity pneumonitis (HPS) / extrinsic allergic alveolitis (Gell and Coombes Type III reaction). An immune-mediated inflammatory disease of the lung parenchyma caused by exposure to an inhaled chemical allergen or organic dust.

Hypersusceptibility. Adverse effects in an individual occurring under exposure conditions that result in no effects in the great majority of the population or an individual exhibiting exaggerated effects in comparison with the great majority of those showing some adverse effects.

IgE-binding Fc receptors. The high-affinity IgE-binding FcεR type I is expressed on mast-cells and basophils. Interacts with IgE antibodies with high affinity. The cross-linking of these receptors results in release of mediators (such as histamine). The receptor is composed of alpha, beta and gamma chains; the alpha chain contains the IgE binding site, while the gamma chain is responsible for signal transfer. The low-affinity IgE binding Fc receptor (CD23) is expressed on B-cells, its soluble (truncated) form is generated by proteolytic cleavage and regulates IgE production of B-cells.

Immediate-type hypersensitivity (Gell and Coombs Type I reaction). A form of antibody mediated immunity that takes place in minutes to hours after the administration of antigen. Previous exposure is required. An example is allergic rhinitis to pollen antigen.

Immunodeficiency. Defects in one or more components of the immune system resulting in inability to eliminate or neutralize non-self antigens. Congenital or primary immunodeficiencies are genetic or due to developmental disorders (such as congenital thymic aplasia). Acquired or secondary immunodeficiencies develop as a consequence of malnutrition, malignancies, immunosuppressive compounds, radiation or infection of immunocompetent T-cells with human immunodeficiency virus (HIV). Defects of the nonspecific defence system may also result in immunodeficiency.

Immunogen. A substance capable of eliciting a specific immune response manifested by the formation of specific antibodies and/or specifically committed lymphocytes. To induce an antibody response an immunogen must possess structurally and functionally distinct determinants for activation of B-cells and T-cells.

Immunoglobulin (Ig). Immunity-conferring portion of the plasma- or serum-gammaglobulins. Various isotypes (classes and subclasses) of immunoglobulins have a common core structure of two identical light (L) and two identical heavy (H) polypeptide chains, which contain repeating homologous units folded in common globular motifs (Ig domains). The amino acid sequences of the N-terminal domains are variable (V domains), in contrast to the more conserved constant regions (C domains). The V domains contain the complementarity-determining regions (CDRS) forming the antigen-binding sites,

whereas the C domains trigger several effector functions of the immune system (see also antibody).

Immunoglobulin gene superfamily. Genes encoding proteins containing one or more Ig domains (homology units) that are homologous to either Ig V or C domains. Cell surface and soluble molecules mediating recognition, adhesion or binding functions in and outside the immune system, derived from the same precursor, belong to this family of molecules (e.g., Ig, TCR, MHC Class I and II, CD4, CD8, FcγR, NCAM, PDGFR).

Incidence (epidemiological). The number of new cases of disease in a defined population during a specified period of time.

Interleukin. Immunoregulatory proteins, also designated as lymphokines, monokines or cytokines. General features are: low relative molecular mass (<80 000) and frequently glycosylated; regulate immune cell function and inflammation by binding to specific cell surface receptors; transient and local production; act in paracrine, autocrine or endocrine manner, with stimulatory or blocking effect on growth/differentiation; very potent, function at picomolar concentrations. Interleukins represent an extensive series of mediators with a wide range of overlapping functions. Other mediators in this series are *c*-kit ligand, interferons, tumour necrosis factor, transforming growth factor, and a family of low relative molecular mass mediators, called chemokines.

Intolerance. Non-immunologically mediated adverse reactions. In food intolerances these may be due to pharmacological properties of food constituents, metabolic disorders or responses of unknown etiology.

Langerhans' cells. Bone-marrow-derived epidermal cells with a dendritic morphology, expressing CD1 marker in humans and containing the cytoplasmic organelle, called the Birbeck granule. They express Class II MHC antigen and are capable of antigen presentation (see also dendritic cells).

Lymphocyte. Bone-marrow-derived cell with little cytoplasm, with the ability to migrate and exchange between the circulation and tissues, to home to sites of antigen exposure, and to be held back at

these sites. The only cells that specifically recognize and respond to antigens (mainly with the help of accessory cells). Lymphocytes consist of various subsets differing in their function and products (e.g., B-lymphocytes, helper-T-cells, cytolytic T-cells).

Macrophage. Mononuclear cells derived from monocytes residing in tissues. Activated by different stimuli they may appear in various forms such as epitheloid cells and multinucleate giant cells. Macrophages found in different organs and connective tissues have been named according the specific locations, e.g., as microglia, alveolar macrophages or Kupffer cells. Macrophages may function as antigen-presenting cells, effector cells of cell-mediated immunity, and phagocytes eliminating opsonized antigens.

Major basic protein. A small basic arginine-rich peptide (pI 10.9, relative molecular mass of 13 800) in the granules of eosinophils that kills helminths and protozoa.

Major histocompatibility complex (MHC). A cluster of genes encoding cell surface antigens that are polymorphic within a species and have a crucial function in signalling between lymphocytes and cells expressing antigen and in recognition of self.

Mast cell. Tissue bound mononuclear granular cells with staining affinity for basic dyes at low pH. The specific granules contain mediators of allergic inflammation, e.g., histamine. Upon stimulation with antigen via membrane-bound IgE antibodies, they release preformed and newly generated mediators. Two types of mast cells exist. Tryptase-containing T-mast cells are mainly associated with mucosal epithelial ceils. Chymase-containing TC mast cells are long-living connective tissue cells.

Mitogen. A substance that causes cells to synthesize DNA and proliferate without acting as an antigen.

Monocyte. Bone-marrow-derived mononuclear phagocytic leukocyte, with bean-shaped nucleus and fine granular cytoplasm containing lysosomes, phagocytic vacuoles and cytoskeletal filaments. Once transported to tissues they develop into macrophages.

Natural killer (NK) cell. A subset of lymphocytes found in blood and some lymphoid tissues, derived from the bone marrow and appearing as large granular lymphocytes (LGL). NK cells possess the capacity to kill certain tumour cells or virus-infected normal cells. The killing is not induced by specific antigen and is not restricted by MHC molecules.

Nephropathy. Disease of the kidney that may involve either or both the glomeruli (specialized structures where blood is filtered) and the renal tubules (connected structures where the composition of the filtrate is greatly modified in accordance with the physiological needs of the body).

Nephrotic syndrome. A clinical disease in which damage to glomeruli has caused leaky filtration, resulting in major loss of protein from the body.

Neutrophil (polymorphonuclear leukocyte). Granular leukocytes having a nucleus with three to five lobes and fine cytoplasmic granules stainable by neutral dyes. The cells have properties of chemotaxis, adherence to immune complexes, and phagocytosis. The cells are involved in a variety of inflammatory processes including late-phase allergic reactions.

Occupational asthma. Asthma caused by a sensitizing agent present in the workplace, usually after a period of asymptomatic exposure.

Opsonization. Coating of antigens with antibody and/or complement components. The interaction of opsonized complexes with Fc- or complement-receptors facilitates their uptake by the receptor-bearing phagocytic cells.

Oral tolerance. Orally induced and immune-mediated non-responsiveness.

Peak expiratory flow rate (PEFR). A physiological measure of the maximum air flow.

Plasma cell. A terminally differentiated B-lymphocyte with little or no capacity for mitotic division, that can synthesize and secrete antibody. Plasma cells have eccentric nuclei, abundant cytoplasm and

distinct perinuclear haloes. The cytoplasm contains dense rough endoplasmic reticulum and a large Golgi complex.

Platelet activating factor. Low relative molecular mass phospholipid generated from alkyl phospholipids in mast cells, basophilic and neutrophilic granulocytes, and monocytes-macrophages, which mediates microthrombus formation of platelets in hypersensitivity reactions.

Prevalence (epidemiology). The number of cases of disease occurring in a given population at a designated time.

Prevention of allergy. *Primary prevention* is the control of the exposures inducing allergy. *Secondary prevention* is the control of exposure of sensitized individuals.

Pseudo-allergy. Non-immunological "hypersensitivity" with clinical symptoms and signs mimicking those of allergic diseases.

Radioallergosorbent test (RAST). A solid-phase radioimmunoassay for detecting IgE antibody specific for a particular antigen.

Rate (epidemiology). The frequency with which an event occurs in a defined population.

Reactive airways dysfunction syndrome (RADS). A syndrome characterized by reversible airflow limitation and complicating bronchial hyperresponsiveness induced by acute exposure to high concentrations of non-sensitizing irritant gases at work.

Sensitization. Induction of specialized immunological memory in an individual by exposure to an allergen.

Stem cell. Pluripotent cells, representing 0.01% of bone marrow cells, having the capacity for self renewal, and committed to differentiate along particular lineages, e.g., erythroid, megakaryocytic, granulocytic, monocytic and lymphocytic. Cytokines stimulate the proliferation and maturation of distinct precursors.

Suppressor T-lymphoctye. A subpopulation of T-lymphocytes that inhibits the activation phase of immune responses. They are CD8+, and their growth and differentiation may be regulated by CD4+ cells.

Tolerance. Persistent condition of specific immunological unresponsiveness, resulting from previous non-sensitizing exposure to the antigen.

Urticaria. Transient eruption of skin characterized by erythematous or oedematous swelling (wheal) of the dermis or subcutaneous tissue.

9. CONCLUSIONS

Allergy is an important, world-wide, health problem. It affects a substantial proportion of the population and all age groups. For reasons that are poorly understood, the overall frequency of allergy is increasing.

The various and complex interactions between chemicals, drugs and proteins, the immune system and the target organ(s) that lead to the manifestation of allergic hypersensitivity and autoimmunity are reviewed in this monograph. Extensive research into these topics continues, so new developments are anticipated. The multiplicity of endogenous and exogenous factors that have an impact upon allergy are considered. Exogenous factors, including allergens themselves, as well as infection, air pollution and life style (e.g., tobacco smoking) are all of importance. In addition, genetic predisposition to a particular allergic disorder is an important determinant of reactivity.

The epidemiology of allergy demonstrates the widespread nature of this group of disorders and, as a consequence, highlights the need for proper attention to the identification of allergic hazards and their assessment in the implementation of appropriate risk management strategies.

Methods of hazard identification for skin sensitizers are well established. They have still to be standardized for respiratory sensitizers and are not yet available for other types of allergen. Techniques to measure the potency of skin sensitizers are being applied for the purpose of risk assessment.

Once allergic hazards have been well characterized, risk assessment, risk management and risk communication are critical elements to reduce the incidence of allergic disorders. Risk assessment requires that the hazard, of known potency, is evaluated in the light of the nature and extent of exposure. Risk management measures, such as control of exposure and product labelling, must be implemented when the risk assessment indicates the need.

More is being learned of the physicochemical and immunological features of food allergens, which may eventually be of predictive value.

The complexity of the mechanisms of allergic disorders makes it difficult at present to suggest *in vitro* predictive methods of general applicability, although application of structure–activity relationships deserves further consideration.

10. RECOMMENDATIONS FOR PROTECTION OF HUMAN HEALTH

a) Effective strategies to prevent allergy should be employed, based on good information about allergens in products and the environment. Control of exposure should be the basis for preventing or minimizing the occurrence of allergic disease.

b) There is urgent need to determine the cause of the increased frequency of allergy.

c) Methods of surveillance should be instituted to define the frequency of allergies of different types.

d) The measurement of exposure of individuals and of populations may be difficult, but adequate assessment is essential to any analysis of the association between exposure and effect. The specific nature of immune responses represents a unique type of biomarker in studying past exposure, for example, by the use of skin patch or prick testing, or assay of IgE antibodies to detect sensitization by specific allergens.

e) Worker surveillance systems, the quality of medical examination of workers and education of workers exposed to chemicals should be improved in order to reveal occupational allergic diseases at an early stage. Relatively simple notification schemes for occupational disorders and post-marketing surveillance of medicines provide economic screening and alerting systems for allergic diseases. Monitoring these disorders in the workplace is particularly valuable because exposure there is likely to be greater than anywhere else.

f) The efficacy and value of primary and secondary prevention and intervention strategies should be assessed at intervals using validated epidemiological techniques.

g) For allergic contact dermatitis, the available *in vivo* predictive models are of proven value for antigens of low relative molecular mass. The need is to discover how best they can be used to show the potency of allergens.

h) For allergic disorders of the respiratory tract, available *in vivo* test methods for substances of low relative molecular mass are promising,

but their predictive value and specificity need to be substantiated. For protein allergens, some animal test methods are being developed, but they require further evaluation using substances of known allergenic potential in humans.

i) It is important that information about the presence of allergens in products (e.g., food) be readily available, for instance in databases and on product labels, so that regulators and individuals can adopt appropriate precautions.

j) The main method for avoiding occupational chemically induced autoimmune disease is the control of exposure. Pre-exposure assessment of those exposed to chemicals of immunomodulating potential should be considered in order to document any pre-existing features of connective tissue diseases. Strict adherence to guidelines to avoid or minimize exposure is advised, including the use of good occupational hygiene practices. Other risk factors such as smoking should be minimized and regular occupational medical examinations should be considered.

k) There is a need for investigation of the quantitative relationships between immune responses induced by chemicals and the severity of allergic reactions.

l) Determination of the minimal exposure and the duration of it required to cause sensitization or elicit an allergic response is important in controlling allergic disease.

m) It is important to devise standard strategies for the clinical investigation and diagnosis of allergy and to apply them internationally to examine the causes and incidence of allergic disorders. This will require the production and availability of standardized extracts of biological allergens of controlled potency, as well as regular consideration of the components of standard series of allergens used in testing.

n) Public health authorities, health professionals and government agencies should consider how to estimate the human and economic costs to individuals and society of allergic diseases.

o) Public health authorities, health professionals, the public and especially the workforce would benefit from better information about the occurrence, causes, clinical manifestations and consequences of different types of allergy.

11. FURTHER RESEARCH

a) The adjuvant effect of environmental factors, such as pollutants, particulates, tobacco smoke and UV radiation, on the induction and elicitation of allergic responses to other chemicals should be investigated. The influence of other forms of toxicity, including direct immunotoxicity, should also be studied.

b) The influence of formulation and mixtures on the induction and elicitation of chemical allergy needs to be explored.

c) The relevance of route of exposure to chemicals to the development of allergic disease, particularly allergic sensitization of the respiratory tract, needs to be investigated.

d) The relevance of route of exposure to the development of tolerance should be explored.

e) The role of IgE antibody and other immune effector mechanisms in the development of chemical respiratory allergy needs to be clarified.

f) Understanding of the cellular mechanisms responsible for the induction of allergy, such as how allergens are processed and presented to T-cells, is necessary to facilitate the development of predictive *in vitro* tests.

g) Improved, reliable, sensitive, specific and robust biomarkers of exposure to allergens are needed. Ideally, they should be non-invasive and suitable for field use. Similarly, better techniques for dosimetry of allergens are needed.

h) The basis for the difference in the response of asthmatics and non-asthmatics to airborne pollutants needs exploration.

i) It is important to understand the relative contributions of life-style, nutrition, pollution and change in the pattern of childhood infection and immunization to the development of allergy.

j) Mechanisms of sensitization to food allergens need further research. In addition, more needs to be known about the occurrence and clinical importance of cross-reactivity of complex allergens, especially those of natural origin, such as pollen and food components.

k) It is necessary to identify the properties that control the allergenic characteristics and potency of proteins, including the reasons why they induce transient or persistent food allergy.

l) In the case of autoimmune disease, predictive methods need to be developed and validated.

REFERENCES

Aalberse RC, Koshte V, & Clemens JG (1981) Immunoglobulin E antibodies that cross react with vegetable foods, pollen and hymenoptera venom. J Allergy Clin Immunol, **68**: 356–364.

Abbey DE, Hwang BL, Burchette RJ, Vancuren T, & Mills PK (1995) Estimated long-term ambient concentrations of PM10 and development of respiratory symptoms in a nonsmoking population. Arch Environ Health, **50**(2): 139–152.

Aberg N, Engstrom I, & Lindberg U (1989) Allergic diseases in Swedish school children. Acta Paediatr Scand, **78**: 246–252.

Abramson S, Miller RG, & Phillips RA (1977) The identification in adult bone marrow of pluripotent and restricted stem cells of the myeloid and lymphoid systems. J Exp Med, **145**: 1567–1579.

Adams LE & Hess EV (1991) Drug-related Lupus: Incidence, mechanisms and clinical implications. Drug Saf, **6**: 431–449.

Adams RB, Planchon SM, & Roche JK (1993) Interferon-gamma modulation of epithelial barrier function: time course, reversibility, and site of cytokine binding. J Immunol, **150**: 2356–2363.

Adinoff AD, Telez P, & Clark RAF (1988) Atopic dermatitis and aeroallergen contact sensitivity. J Allergy Clin Immunol, **81**: 736–742.

Alanko K, Keskinen H, Björkstén F, & Ojanen S (1978) Immediate-type hypersensitivity to reactive dyes. Clin Allergy, **8**: 25–31.

Alarcon-Segovia D (1985) Scleroderma. In: Rose NR & Mackay IR ed. The autoimmune diseases. New York, London, San Diego, Academic Press, pp 119–143.

Allison AC (1989) Theories of self tolerance and autoimmunity. In: Kammüller M, Bloksma N, & Seinen W ed. Autoimmunity and toxicology: Immune disregulation induced by drugs and chemicals. Amsterdam, Oxford, New York, Elsevier Science Publishers, pp 67–115.

Amagi M, Ishii K, Hashimoto T, Gamou S, Shimizu N, & Nishikawa T (1995) Conformational epitopes of pemphigus antigens (Dsg1 and Dsg3) are calcium dependent and glycosylation dependent. J Invest Dermatol, **105**: 243–247.

American Medical Association (1987a) Council of Scientific Affairs, American Medical Association, Report I, Part I, of the Allergy Panel (1987): *in vivo* diagnostic testing and immunotherapy for allergy. J Am Med Assoc, **258**: 1363–1367.

American Medical Association (1987b) Council of Scientific Affairs, American Medical Association, Report I, Part II, of the Allergy Panel (1987): *in vitro* testing for allergy. J Am Med Assoc, **258**: 1639–1643.

Ames RG, Hall DS, & Reger RB (1984) Chronic respiratory effects of exposure to diesel emissions in coal mines. Arch Environ Health, **39**: 389–394.

Amin S, Lahti A, & Maibach HI (1996) Contact urticaria and the contact urticaria syndrome. In: Marzulli FN & Maibach HI ed. Dermatotoxicology, 5th ed. London, Taylor and Francis, pp 485–505.

Andersen KE & Maibach HI (1985) Guinea pig sensitization assays. In: Andersen KE & Maibach HI ed. Contact allergy: Predictive tests in guinea pigs. Basel, Karger, pp 263–290.

Andersen KE & Rycroft RJG (1991) Recommended patch test concentrations for preservatives, biocides and antimicrobials. Contact Dermatitis, **25**: 1–18.

Andersen KE, Burrows D, & White IR (1995) Allergens from the standard series. In: Rycroft RJG, Menné T, Frosch PJ, & Benezra C ed. Textbook of contact dermatitis, 2nd ed. Berlin, Heidelberg, New York, Springer Verlag, pp 416–457.

Anderson HR (1974) The epidemiological and allergic features of asthma in the New Guinea Highlands. Clin Allergy, **4**: 171–183.

Anderson HR (1989) Increase in hospital admissions for childhood asthma: trends in referral, severity, and readmissions from 1970 to 1985 in a health region of the United Kingdom. Thorax, **44**: 614–619.

Anderson JA (1991) The clinical spectrum of food allergy in adults. Clin Exp Allergy, **21**(suppl 1): 304–314.

Anderson SD & Smith CM (1991) Osmotic challenges in the assessment of bronchial hyperresponsiveness. Am Rev Respir Dis, **143**(3/2): S43–S46.

Anderson LB, Dreyfuss EM, Logan U, Johnstone E, & Glaser J (1970) Melon and banana sensitivity coincident with ragweed pollinosis. J Allergy Clin. Immunol, **45**: 310–319.

Anderson HR, Bailey PA, Cooper JS, Palmer JC, & West S (1983) Morbidity and school absence caused by asthma and wheezing illness. Arch Dis Child, **58**: 777–784.

Anderson HR, Pottier AC, & Strachan DP (1992) Asthma from birth to age 23: incidence and relation to prior and concurrent atopic disease. Thorax, **47**: 537–542.

Anderson HR, Butland BK, & Strachan DP (1994) Trends in prevalence and severity of childhood asthma. Br Med J, **308**: 1600–1604.

Anderson C, Hehr A, Robbins R, Hasan R, Athar M, Mukhtar H, & Elmets CA (1995) Metabolic requirements for induction of contact sensitivity to immunotoxic polyaromatic hydrocarbons. J Immunol, **155**(7): 3530–3537.

Angelini G (1995) Allergens related to specific exposures: Topical drugs. In: Rycroft RJG, Menné T, Frosch PJ, & Benezra C ed. Textbook of contact dermatitis, 2nd ed. Berlin, Heidelberg, New York, Springer Verlag, pp 477–498.

Angle CR (1988) Indoor air pollutants. Adv Pediatr, **35**: 239–280.

Anonymous (1982) Breast feeding and eczema/asthma. Lancet, 1(8277): 910–911.

Anonymous (1985) Smoking, occupation, and allergic lung disease. Lancet, **1**(8435): 965.

Anto JM (1995) Asthma outbreaks: an opportunity for research? Thorax, **50**(3): 220–222.

Anto JM, Sunyer J, Rodriguez Roisin R, Suarez Cervera M, & Vazquez L (1989) Community outbreaks of asthma associated with inhalation of soybean dust. Toxicoepidemiological Committee. New Engl J Med, **320**: 1097–1102.

Armentia A, Martin Santos JM, Quintero A, Fernandez A, Barber D, Alonso E, & Gil I (1990) Bakers' asthma: Prevalence and evaluation of immunotherapy with a wheat flour extract. Ann Allergy, **65**(4): 265–272.

Amon R & Teitelbaum D (1993) On the existence of suppressor cells. Int Arch Allergy Immunol, **100**: 2–7.

Arshad SH, Matthews S, Gant C, & Hide DW (1992) Effect of allergen avoidance on development of allergic disorders in infancy. Lancet, **339**: 1493–1497.

Arshad SH, Stevens M, & Hide DW (1993) The effect of genetic and environmental factors on the prevalence of allergic disorders at the age of two years. Clin Exp Allergy, **23**: 504–511.

Ashby J, Basketter DA, Paton D, & Kimber I (1995) Structure activity relationships in skin sensitizing sensitization using the murine local lymph node assay. Toxicology, **103**: 177–194.

Asher MI, Pattemore PK, Harrison AC, Mitchell EA, Rea HH, Stewart AW, & Woolcock AJ (1988) International comparison of the prevalence of asthma symptoms and bronchial hyperresponsiveness. Am Rev Respir Dis, **138**: 524–529.

Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Mitchell EA, Pearce N, Sibbald B, Stewart AW, Strachan D, Weiland SK, & Williams HC (1995) International study of asthma and allergies in childhood (ISAAC): Rationale and methods. Eur Respir J, **8**(3): 483–491.

Asherson GL, Zembala M, Perera MACC, Mayhew B, & Thomas WR (1977) Production of immunity and unresponsiveness in the mouse by feeding contact sensitizing agents and the role of suppressor cells in the Peyers's patches, mesenteric lymph nodes and other lymphoid tissue. Cell Immunol, **33**: 145–155.

Atkins FM, Steinberg SS, & Metcalfe DD (1985a) Evaluation of immediate adverse reactions to foods in adult patients: I. Correlation of demographic, laboratory, and prick skin test data with response to controlled oral food challenge. J Allergy Clin Immunol, **75**: 348–355.

Atkins FM, Steinberg SS, & Metcalfe DD (1985b) Evaluation of immediate adverse reactions to foods in adult patients: II. A detailed analysis of reaction patterns during oral food challenge. J Allergy Clin Immunol, **75**: 356–355.

Atkinson NF Jr & Platts-Mills TAE (1988) IgE and atopic disease. In: Immunological diseases, 4th ed. Boston, Massachusetts, Little Brown, vol 2, pp 1009–1026.

Avnstorp C (1992) Cement eczema: An epidemiological intervention study. Acta Dermatol Venereol, **179**(suppl): 1–22.

Axelsson IG, Johansson SG, Larsson PH, & Zetterstrom O (1990) Characterization of allergenic components in sap extract from the weeping fig (Ficus benjamina). Int Arch Allergy Appl Immunol, **91**(2): 130–135.

Bachert C, Hauser U, Prem B, Rudack C, & Ganzer U (1995) Proinflammatory cytokines in allergic rhinitis. Eur Arch Otorhinolaryngol, **252**(Suppl 1): 155–214.

Baird AW, Coombs RRA, McLaughlan P, & Cuthbert AW (1984) Immediate hypersensitivity reactions to cow milk proteins in isolated epithelium from ileum of milk-drinking guinea-pigs: Comparison with colonic epithelia. Int Arch Allergy Appl Immunol, **75**: 255–263.

Baker DB, Gann PH, Brooks SM, Gallagher J, & Bernstein IL (1990) Cross-sensitization study of platinum salts sensitization among precious metals refinery workers. Am J Ind Med, **18**: 653–664.

Bakke P, Gulsvikk A, & Eide GE (1990) Hay fever, eczema and urticaria in southwest Norway. Allergy, **45**: 515–522.

Ball B, Horstman D, Folinsbee L, Gerrity T, DeWitt P, Abdul-Salaam S, & Brown J (1993) Pulmonary responses in asthmatics performing light exercise in clean air(air) and 0.16 ppm ozone (O_3). Am Rev Respir Dis, **147**: A640 (abstract).

Barbee RA (1987) The epidemiology of asthma. Monogr Allergy, **21**: 21–41.

Barbee RA, Kaltenborn W, Lebowitz MD, & Burrows B (1987) Longitudinal changes in allergen skin test reactivity in a community population sample. J Allergy Clin Immunol, **79**(1): 16–24.

Bardare M, Vaccari A, Allievi E, Brunelli L, Coco F, de Gaspari GC, & Flauto U (1993) Influence of dietary manipulation on incidence of atopic disease in infants at risk. Ann Allergy, **71**(4): 366–371.

Barnes PJ, Baranjuk J, & Belvisi MG (1991) Neuropeptides in the respiratory tract. Am Rev Respir Dis, **144**: 1187–1198.

Barnes PJ, Jonsson B, & Klim JB (1996) The costs of asthma. Eur Respir J, **9**: 636–642.

Barratt MD (1995) Quantitative structure activity relationships for skin permeability. Toxicol *in vitro*, **9**: 27–37.

Barratt MD, Basketter DA, Chamberlain M, Payne MP, Admans GD, & Langowski JJ (1994a) Development of an expert system rule base for identifying contact allergens. Toxicol *in vitro*, **8**: 837–839.

Barratt MD, Basketter DA, Chamberlain M, Admans GD, & Langowski JJ (1994b) An expert system rule base for identifying contact allergens. Toxicol *in vitro*, **8**: 1053–1060.

Barratt MD, Basketter DA, & Roberts DW (1994c) Skin sensitization structure-activity relationships for phenyl benzoates. Toxicol *in vitro*, **8**: 823–826.

Barrett JT (1988) Textbook of immunology, 5th ed. St. Louis, Missouri, C.V. Mosby & Co.

Barrett SP, Toh BH, Alderuccio F, van Driel I, & Gleeson PA (1995) Organ-specific autoimmunity induced by adult thymectomy and cyclophosphamide-induced lymphopenia. Eur J Immunol, **25**: 238–244.

Barry DM, Burr ML, & Limb ES (1991) Prevalence of asthma among 12 year old children in New Zealand and South Wales: a comparative survey. Thorax, **46**: 405–409.

Bascom R (1996) Environmental factors and respiratory hypersensitivity: the Americas. Toxicol Lett, **86**(2–3): 115–130.

Bascom R, Naclerio RM, Fitzgerald TK, Kagey Sobotka A, & Proud D (1990) Effect of ozone inhalation on the response to nasal challenge with antigen of allergic subjects. Am Rev Respir Dis, **142**(3): 594–601.

Basketter DA & Roberts DW (1990) Structure activity relationships in contact allergy. Int J Cosmet Sci, **12**: 81–90.

Basketter DA, Roberts DW, Cronin M, & Scholes EW (1992) The value of the local lymph node assay in quantitative structure-activity investigations. Contact Dermatitis, **27**: 137–142.

Basketter DA, Gerberich GF, Kimber I, & Loveless SE (1996) The local lymph node assay — a valuable alternative to currently accepted skin sensitisation tests. Food Chem Toxicol, **34**: 651–660.

Basketter DA, Cookman G, Gerberich GF, Hamaide N, & Potokar M (1997) Skin sensitisation thresholds: Determination in predictive models. Food Chem Toxicol, **35**: 417–425.

Bates DV & Sizto R (1987) Air pollution and hospital admissions in southern Ontario: The acid summer haze effect. Environ Res, **43**: 317–331.

Baur V & Fruhmann G (1981) Specific IgE antibodies in patients with isocyanate asthma. Chest, **80**(suppl): 73S–76S.

Baur X, Dewair M, & Fruhmann G (1984) Detection of immunologically sensitised isocyanate workers by RAST and intracutaneous skin tests. J Allergy Clin Immunol, **73**: 610–618.

Baur X, Marek W, Ammon J, Czuppon AB, Marczynski B, Raulf-Heimsoth M, Roemmelt H, & Fruhman G (1994) Respiratory and other hazards of isocyanates. Int Arch Occup Environ Health, **66**: 141–152.

Beasley R, Roche WR, Roberts JA, & Holgate ST (1989) Cellular events in the bronchi in mild asthma and after bronchial provocation. Am Rev Respir Dis, **139**: 806–817.

Beasley R, Thomson C, & Pearce N (1991) Selenium, glutathione peroxidase and asthma. Clin Exp Allergy, **21**(2): 157–159.

Becher R, Hongslo JK, Jantunen J, & Dybing E (1996) Environmental chemicals relevant for respiratory hypersensitivity: the indoor environment. Toxicol Lett, **86**: 155–162.

Beeson PB (1994) age and sex associations of 40 autoimmune diseases. Am J Med, **96**: 457–462.

Beezhold DH, Sussman GL, Liss GM, & Chang N-S (1996) Latex allergy can induce clinical reactions to specific foods. Clin Exp Allergy, **26**: 416–422.

Beggs PJ & Curson PH (1995) An integrated environmental asthma model. Arch Environ Health, **50**(2): 87–94.

Behrendt H, Friedrich KH, Kainka-Stänicke E, Darsow U, Becker WM, & Tomingas R (1991) New trends in allergy. In: Ring J & Pryzbilla B ed. Allergens and pollutants in the air: A complex interaction. Berlin, Heidelberg, New York, Springer Verlag, pp 467–478.

Behrendt H, Becker W, Friedrichs K, Darsow U, & Tomingas R (1992) Interaction between aeroallergens and airborne particulate matter. Int Arch Allergy Immunol, **99**: 425–428.

Behrendt H, Krämer U, Dolgner R, Hinrichs J, Willer H, Hagenbeck H, & Schlipköter W (1993) Elevated levels of total IgE in East German children: Atopy, parasites or pollutants? Allergo J, **2**: 31–40.

Behrendt H, Friedrich KH, Krämer U, Hitzfeld B, Becker WM, & Ring J (1996) The role of indoor and outdoor air pollution in allergic diseases. In: Vos J, Younes M, & Smith E ed. Allergic hypersensitivities induced by chemicals. Boca Raton, Florida, CRC Press, pp 173–182.

Bekesi JG, Roboz JP, Fischbein A, & Mason P (1987) Immunotoxicology: Environmental contamination by polybrominated biphenyls and immune dysfunction among residents of the State of Michigan. Cancer Detect Prev, **1**(suppl): 29–37.

Bell RG (1996) IgE, allergies and helminth parasites: a new perspective on an old conundrum. Immunol Cell Biol, **74**: 337–345.

Bencko V, Vasilieva EV, & Symon K (1980) Immunological aspects of exposure to emissions from burning coal of high beryllium content. Environ Res, **22**(2): 439–449.

Benezra C, Sigman CC, Perry LR, Helmes CT, & Maibach HI (1985) A systematic search for structure-activity relationships of skin contact sensitizers: methodology. J Invest Dermatol, **85**: 351–356.

Benjamini E & Leskowitz S (1991) Immunology: A short course. New York, Wiley-Liss.

Bentley AM, Maestrelli P, Fabbri LM, Menz G, Storz Chr, Bradley B, Jeffrey PK, Durham SR, & Kay AB (1991) Immunohistology of the bronchial mucosa in occupational, intrinsic and extrinsic asthma. J Allergy Clin Immunol, **87**: 246 (abstract).

Berglund M, Braback L, Bylin G, Jonson JO, & Vahter M (1994) Personal NO_2 exposure monitoring shows high exposure among ice-skating schoolchildren. Arch Environ Health, **49**: 17–24.

Bernhisel-Broadbent J & Sampson HA (1989) Cross-allergenicity in the legume botanical family in children with food hypersensitivity. J Allergy Clin Immunol, **83**: 435–440.

Bernhisel-Broadbent J, Taylor S, & Sampson HA (1989) Cross-allergenicity in the legume botanical family in children with food hypersensitivity: II. Laboratory correlates. J Allergy Clin Immunol, **84**: 701–709.

Bernstein DI, Patterson R, & Zeiss CR (1982a) Clinical and immunologic evaluation of trimellitic anhydride- and phthalic anhydride-exposed workers using a questionnaire and comparative analysis of enzyme linked immunosorbent and radioimmunoassay studies. J Allergy Clin Immunol, **69**: 311–318.

Bernstein M, Day JH, & Welsh A (1982b) Double-blind food challenge in the diagnosis of food sensitivity in the adult. J Allergy Clin Immunol, **70**: 205–211.

Bernstein DI, Gallagher JA, D'Souza L, & Bernstein IL (1984) Heterogeneity of specific IgE responses in workers sensitised to acid anhydride compounds. J Allergy Clin Immunol, **74**: 794–801.

Biagini RE, Bernstein IL, Gallagher JS, Moorman WJ, Brooks S, & Gann PH (1985) The diversity of reaginic immune responses to platinum and palladium salts. J. Allergy Clin Immunol, **76**(6): 794–802.

Biagini RE, Moorman WJ, Lewis TR, & Bernstein IL (1986) Ozone enhancement of platinum asthma in a primate model. Annu Rev Respir Dis, **134**: 719–725.

Bieber TH & Ring J (1992) *In vivo* modulation of the high-affinity receptor for IgE (FcεRI) on human epidermal Langerhans cells. Int Arch Allergy Immunol, **99**: 204–207.

Bigazzi PE (1988) Autoimmunity induced by chemicals. J Toxicol Clin Toxicol, **26**: 125–156.

Bigazzi PE (1997) Autoimmunity caused by xenobiotics. Toxicology, **119**: 1–21.

Bignon JS, Aron Y, Ju LY, Kopferschmitt MC, Garnier R, Mapp C, Fabbri LM, Pauli G, Lockhart A, Charron D, & Swierczewski E (1994) HLA class II alleles in isocyanate-induced asthma. Am J Respir Crit Care Med, **149**: 71–75.

Bilyk N & Holt PG (1995) Cytokine modulation of the immunosuppressive phenotype of pulmonary alveolar macrophage populations. Immunology, **86**: 231–237.

Bindslev-Jensen C, Hansen TK, Norgaard A, Vestergaard H, & Lars K (1994a) New controversial techniques in the diagnosis of food hypersensitivity. In: Johansson SGO ed. Progress in allergy and clinical immunology. Toronto, Bern, Hans Huber, vol 3, pp 468–274.

Bindslev-Jensen C, Skov P, Madsen F, & Poulsen LK (1994b) Food allergy and food intolerance - what is the difference? Ann Allergy, **72**: 317–320.

Bircher AJ, Wüthrich B, Langauer S, & Schmid P (1993) [Ficus benjamina, a perennial inhalation allergen of increasing importance.] Schweiz Med Wochenschr, **123**(22): 1153–1159 (in German).

Björksten B & Kjellman NIM (1987) Perinatal factors influencing the development of allergy. Clin Rev Allergy, **5**: 339–347.

Black CM, Welsh KI, Walker AE, Bernstein RM, Catoggio LJ, McGregor AR, & Lloyd Jones JK (1983) Genetic susceptibility to scleroderma-like syndrome induced by vinyl chloride. Lancet, **1**: 53–55.

Blaikie L, Basketter DA, & Morrow T (1995) Experience with a guinea pig model for the assessment of respiratory allergens. Hum Exp Toxicol, **14**: 73.

Blanco C, Carrillo T, Castillo R, Quiralte J, & Cuevas M (1994) Avocado hypersensitivity. Allergy, **49**: 454–459.

Bloch KJ & Walker WA (1981) Effect of locally induced intestinal anaphylaxis on the uptake of a bystander antigen. J Allergy Clin Immunol, **67**: 312–316.

Bloksma N, Kammüller ME, Punt P, & Seinen W (1988) Strain-dependence of primary popliteal lymph node reactions to subcutaneous injection of diphenylhydantoin in mice. In: Kammüller ME ed. A toxicological approach to chemical-induced autoimmunity. Utrecht, The Netherlands, University of Utrecht, pp 79–92 (PhD Thesis).

Bloksma N, Kubicka-Muranyi M, Schuppe H-C, Gleichmann E, & Gleichmann H (1995) Predictive immunotoxicological test systems: Suitability of the popliteal lymph node assay in mice and rats. Crit Rev Toxicol, **25**: 369–396.

Bloom BR, Salgame P, & Diamons B (1992) Revisiting and revising suppressor T cells. Immunol Today, **13**(4): 131–136.

Bock SA (1987) Prospective appraisal of complaints of adverse reactions to foods in children during the first 3 years of life. Pediatrics, **79**(5): 683–688.

Bock SA, Sampson HA, Atkins FM, Zeiger RS, Lehrer S, Sachs M, Bush RK, & Metcalfe DD (1988) Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. J Allergy Clin Immunol, **82**: 986–997.

Boerrigter GH & Scheper RJ (1987) Local and systemic desensitization induced by repeated epicutaneous hapten application. J Invest Dermatol, **88**(1): 3–7.

Bohadana AB, Massin N, Wild P, Kolopp MN, & Toamain JP (1994) Respiratory symptoms and airway responsiveness in apparently healthy workers exposed to flour dust. Eur Respir J, **7**(6): 1070–1076.

Bonini S, Magrini L, Rotiroti G, Ronchetti MP, & Onotati P (1994) Genetic and environmental factors in the changing incidence of allergy. Allergy, **49**: 6–14.

Bonnet M, Angibaud G, Cantagrel A, Montastruc JL, & Janet M (1995) Myasthénie induite par la tiopronine au cours du traitement de la polyarthrite rhumatoïde. Rev Neurol, **151:** 67–68.

Borelli S (1981) [Dermatological indications for climatherapy in the high altitude of Davos (1560 m and higher.] In: Borelli S & Düngemann H ed. [Allergology and dermatology.] Frankfurt, Germany, IMP-Verlag, pp 564–660 (in German).

Borelli S & Schnyder UW (1962) [Constitutional dermatitis without atopy.] In: Miescher G & Storck H ed. [Dermatitis: I. Handbook of treatment.] Berlin, Heidelberg, New York, Springer Verlag, vol 1/1, pp 254–319 (in German).

Bos JD & Kapsenberg ML (1993) The skin immune system: progress in cutaneous biology. Immunol Today, **14**: 75–78.

Braback L, Breborowicz A, Julge K, Knutsson A, Riikjarv MA, Vasar M, & Bjorksten B (1995) Risk factors for respiratory symptoms and atopic sensitisation in the Baltic area. Arch Dis Child, **72**(6): 487–493.

Braun Fahrländer C, Kunzli N, Domenighetti G, Carell CF, & Ackermann Liebrich U (1994) Acute effects of ambient ozone on respiratory function of Swiss schoolchildren after a 10-minute heavy exercise. Pediatr Pulmonol, **17**: 169–177.

Braun-Fahrländer C, Vuille JC, Sennhauser FH, Neu U, Künzle T, Grize L, Gassner M, Minder C, Schindler C, Varonier HS, & Wüthrich B (1997) Respiratory health and long-term exposure to air pollutants in Swiss schoolchildren — SCARPOL Team: Swiss study on childhood allergy and respiratory symptoms with respect to air pollution, climate and pollen. Am J Respir Crit Care Med, **155**: 1042–1049.

Breathnach SM (1988) The Langerhans cell: Centenary review. Br J Dermatol, **119**(4): 463–469.

Briatico-Vangosa G, Braun CLJ, Cookman G, Hofmann T, Kimber I, Loveless SE, Morrow T, Pauluhn J, Sorensen T, & Niessen HJ (1994) Respiratory allergy: Hazard identification and risk assessment. Fundam Appl Toxicol, **23**: 145–158.

Britton WJ, Woolcock AJ, Peat JK, Sedgwick CJ, Lloyd DM, & Leeder SR (1986) Prevalence of bronchial hyperresponsiveness in children: the relationship between asthma and skin reactivity to allergens in two communities. Int J Epidemiol, **15(2)**: 202–209.

Britton J, Pavord I, Richards K, Knox A, Wisniewski A, Weiss S, & Tattersfield A (1994) Dietary sodium intake and the risk of airway hyperreactivity in a random adult population. Thorax, **49**(9): 875–880.

Britton JR, Pavord ID, Richards KA, Knox AJ, Wisniewski AF, Lewis SA, Tattersfield AE, & Weiss ST (1995) Dietary antioxidant vitamin intake and lung function in the general population. Am J Respir Crit Care Med, **151**(5): 1383–1387.

Broder I, Higgins MW, Mathews KP, & Keller JB (1974a) Epidemiology of asthma and allergic rhinitis in a total community, Tecumseh, Michigan. J Allergy Clin Immunol, **53**: 127–138.

Broder I, Higgins MW, Matthews KP, & Keeler JB (1974b) Epidemiology of asthma and allergic rhinitis in a total community, Tecumseh, Michigan: 3. Second survey of the community. J Allergy Clin Immunol, **53**: 127–138.

Brooks SM (1982) The evaluation of occupational airways disease in the laboratory and workplace. J Allergy Clin Immunol, **70**: 56–66.

Broughton A & Thrasher JD (1988) Antibodies and altered cell-mediated immunity in formaldehyde-exposed humans. Comments Toxicol, **2**: 155–174.

Bruijnzeel-Koomen C, Van Wichen DF, Toonstra J, Berrens L, & Bruijnzeel PL (1986) The presence of IgE molecules on epidermal Langerhans cells in patients with atopic dermatitis. Arch Dermatol Res, **278**(3): 199–205.

Bruijnzeel-Koomen C, Ortolani C, Aas K, Bindslev-Jensen C, Björkstén B, Moneret-Vautrin D, & Wüthrich B (1995) Position paper of the European Academy of Allergy and Clinical Immunology on adverse reactions to food: Adverse reactions to food. Allergy, **50**: 623–635.

Brunekreef B (1992) Damp housing and adult respiratory symptoms. Allergy, **47**(5): 498–502.

Brunekreef B, Dockery DW, Speizer FE, Ware JH, Spengler JD, & Ferris BG (1989) Home dampness and respiratory morbidity in children. Am Rev Respir Dis, **140**: 1363–1367.

Brunekreef B, Dockery DW, & Krzyzanowski M (1995) Epidemiologic studies on short-term effects of low levels of major ambient air pollution components. Environ Health Perspect; **103**(Suppl 2): 3–13.

Brunekreef B, Janssen NA, de Hartog J, Harssema H, Knape M, & van Vliet P (1997) Air pollution from truck traffic and lung function in children living near motorways. Epidemiology, **8**(3): 298–303.

Bruynzeel DP, von Blomberg-van der Flier M, Scheper RJ, van Ketel WG, & de Haan P (1985) Penicillin allergy and the relevance of epicutaneous tests. Dermatologica, **171**: 429–434.

Bryson JS, Caywood BE, & Kaplan AM (1991) Relationship of cyclosporine A-mediated inhibition of clonal deletion and development of syngeneic graft-versus-host disease. J Immunol, **147**: 391–397.

Buckley RH (1992) Primary immunodeficiency diseases. In: Wyngaarden J & Smith L ed. Cecil's textbook of medicine. Philadelphia, Pennsylvania, W.B. Saunders Company, pp 1446–1453.

Buehler EV (1965) Delayed contact hypersensitivity in the guinea pig. Arch Dermatol, **5**(91): 171–177.

Buist AS & Vollmer WM (1990) Reflections on the rise in asthma morbidity and mortality. J Am Med Assoc, **264**: 1719–1720.

Burge PS (1989) Diagnosis of occupational asthma. Clin Exp Allergy, **19**(6): 649–652.

Burlingame RW & Rubin RL (1991) Drug-induced anti-histone autoantibodies display two patterns of reactivity with substructures of chromatin. J Clin Invest, **88**: 680–690.

Burney P (1987) A diet rich in sodium may potentiate asthma: Epidemiologic evidence for a new hypothesis. Chest, **91**: 143S–148S.

Burney PG, Britton JR, Chinn S, Tattersfield AE, Platt HS, Papacosta AO, & Kelson MC (1986) Response to inhaled histamine and 24 hour sodium excretion. Br Med J Clin Res Ed, **292**: 1483–1486.

Burney PG, Laitinen LA, Perdrizet S, Huckauf H, Tattersfield AE, Chinn S, Poisson N, Heeren A, Britton JR, & Jones T (1989a) Validity and repeatability of the IUATLD (1984) bronchial symptoms questionnaire: An international comparison. Eur Respir J, **2**(10): 940–945.

Burney PG, Neild JE, Twort CH, Chinn S, Jones TD, Mitchell WD, Bateman C, & Cameron IR (1989b) Effect of changing dietary sodium on the airway response to histamine. Thorax, **44**: 36–41.

Burney PG, Chinn S & Rana RJ (1990) Has the prevalence of asthma increased in children? Evidence from the national study of health and growth 1973–86. Br Med J, **300**: 1306–1310.

Burney P, Chinn S, Jarvis D, Luczynska C, & Lai E (1996) Variations in the prevalence of respiratory symptoms, self-reported asthma attacks, and use of asthma medication in the European Community respiratory health survey (ECRHS). Eur Respir J, **9**: 687–695.

Burnett RT, Dales R, Krewski D, Vincent R, Dann T, & Brook JR (1995) Associations between ambient particulate sulphate and admissions to Ontario hospitals for cardiac and respiratory diseases. Am J Epidemiol, **142**: 15–22.

Burr ML, Butland BK, King S, & Vaughan Williams E (1989) Changes in asthma prevalence: Two surveys 15 years apart. Arch Dis Child 1989, **64**: 1452–1456.

Burrows B, Halonen M, Barbee RA, & Lebowitz MD (1981) The relationship of serum immunoglobulin E to cigarette smoking. Am Rev Respir Dis, **124**(5): 523–525.

Busse WW (1990) Respiratory infections: their role in airway responsiveness and the pathogenesis of asthma. J Allergy Clin Immunol, **85**: 671–683.

Butcher BT & Salvaggio JE (1986) Occupational asthma. J Allergy Clin Immunol, **78**(4): 547–556.

Butler JM, Chan SC, Stevens S, & Hanifin JM (1983) Increased leukocyte histamine release with elevated cyclic AMP-phosphodiesterase activity in atopic dermatitis. J Allergy Clin Immunol, **71**: 490–497.

Byers VS, Levin AS, Ozonoff DM, & Baldwin RW (1988) Association between clinical symptoms and lymphocyte abnormalities in a population with chronic domestic exposure to industrial solvent contaminated domestic water supply and a high incidence of leukaemia. Cancer Immunol Immunother, **27**: 77–81.

Calkhoven P, Aalbers M, Koshte L, Pos O, Oei HD, & Aalberse RC (1987) Cross-reactivity among birch pollen, vegetables and fruits as detected by IgE antibodies is due to at least three distinct cross-reactive structures. Allergy, **42**: 382–390.

Calvin G (1992) Risk management case history — detergents. In: Richardson ML ed. Risk management of chemicals. London, Royal Society of Chemistry, pp 120–136.

Carr RI, Etheridge PD, & Tilley D (1985) Failure of oral tolerance in NZB/W mice is antigen dependent and parallels antibody patterns in human systemic lupus erythematosus (SLE). Fed Proc, **44**: 1542 (abstract).

Cartier A, Thompson NC, Frith PA, Roberts R, & Hargreave FE (1982) Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway caliber. J Allergy Clin Immunol, **70**: 170–177.

Cartier A, Bernstein IL, Burge PS, Cohn JR, Fabbri LM, Hargreave FE, Malo JL, McKay RT, & Salvaggio JE (1989) Guidelines for bronchoprovocation on the investigation of occupational asthma: Report of the Subcommittee on Bronchoprovocation for Occupational Asthma. J Allergy Clin Immunol, **84**(5/2): 823–829.

Catto-Smith AG, Patrick MK, Hardin JA, & Gall DG (1989a) Intestinal anaphylaxis in the rat: Mediators responsible for the ion transport abnormalities. Agents Actions, **28**: 185–191.

Catto-Smith AG, Patrick MK, Scott RB, Davidson JS, & Gall DG (1989b) Gastric response to mucosal IgE-mediated reactions. Am J Physiol, **257**: G704 (abstract).

CDC (US Center for Disease Control) (1994) Populations at risk from particulate air pollution — United States, 1992. Morbid Mortal Wkly Rep, **43**: 290–293.

CDC (US Center for Disease Control) (1995) Asthma--United States, 1982–1992. Morbid Mortal Wkly Rep, **43**: 952–955.

Challacombe SJ (1983) Salivary antibodies and systemic tolerance in mice after oral immunization with bacterial antigens. Ann NY Acad Sci, **409**: 177–193.

Challacombe SJ & Tomasi TB (1987) Oral tolerance. In: Brostoff J & Challacombe SJ ed. Food allergy and intolerance, Section F: Model systems of antigen handling. Philadelphia, Pennsylvania, Ballière Tindall, pp 255–268.

Chambers CA & Allison JP (1997) Co-stimulation in T cell responses. Curr Opin Immunol, **9**: 396–404.

Chan PK, Baldwin RC, Parsons RD, Moss JN, Stiratelle R, Smith JM, & Hayes AW (1983) Kathon biocide: Manifestation of delayed contact dermatitis in guinea pigs is dependent on the concentrations for induction and challenge. J Invest Dermatol, **81**: 409–411.

Chan-Yeung M (1990) Occupational asthma. Chest, **98**(suppl 5): 148S–161S.

Chan-Yeung M (1995) Occupational Asthma. Environ Health Perspect, **103**(suppl 6): 249–252.

Chan-Yeung M & Malo JL (1995a) Occupational asthma. N Engl J Med, **333**(2): 107–112.

Chan Yeung M & Malo JL (1995b) Epidemiology of occupational asthma. In: Asthma and rhinitis. Oxford, London, Boston, Scientific Publications, pp 44–57.

Chan-Yeung M, Malo JL, Busse W, & Holgate ST ed. (1995) Asthma and rhinitis: Epidemiology of occupational asthma. Oxford, London, Boston, Blackwell Scientific Publications, pp 44–57.

Chand N, Harrison JE, Rooney S, Pillar J, Jakubicki R, Nolan K, Diamantis W, & Sofia RD (1992) Anti-IL-5 monoclonal antibody inhibits allergic late phase bronchial eosinophilia in guinea pigs: a therapeutic approach. Eur J Pharmacol, **211**: 121–123.

Chandor SB (1988) Autoimmune phenomena in lymphoid malignancies. Clin Lab Med, **8**: 373–384.

Chapman JR & Roberts DW (1984) Humoral immune dysfunction as a result of prenatal exposure to diphenylhydantoin: correlation with the occurrence of physical defects. Teratology, **30**: 107–117.

Charous BL (1994) The puzzle of latex allergy: some answers, still more questions. Ann Allergy, **73**(4): 277–281.

Chase MW (1946) Inhibition of experimental drug allergy by prior feeding of the sensitizing agent. Proc Soc Exp Biol Med, **61**: 257–259.

Chen Y, Kuchroo VK, Inobe J, Hafler DA, & Weiner HL (1994) Regulatory T cell clones induced by oral tolerance: Suppression of autoimmune encephalomyelitis. Science, **265**: 1237–1240.

Chua KY, Stewart GA, Thomas WR, Simpson RJ, Dilworth RJ, Plozza TM, & Turner KJ (1988) Sequence analysis of cDNA coding for a major house dust mite allergen, Der p 1. J Exp Med, **167**: 175–182.

Cirla AM (1994) Cobalt-related asthma: clinical and immunological aspects. Sci Total Environ, **150**(1–3): 85–94.

Cleland LG & Bell DA (1978) The occurrence of systemic lupus erythematosus in two kindreds in association with severe selective IgA deficiency. J Rheumatol, **5**: 288–293.

Clinton PM, Kemeny DM, Amlot P, Urbanek R, & Lessof MH (1986) Histamine release from peripheral blood leucocytes in egg-allergic patients. Clin Allergy, **16**: 345–354.

Coca AF & Cooke RA (1923) On the classification of the phenomena of hypersensitiveness. J Immunol, **8**: 163–182.

Cockcroft DW (1988) Allergens. In: Barnes PJ, Rodger IW, & Thomson NC ed. Asthma: Basic mechanisms and clinical management. New York, London, San Diego, Academic Press, pp 445–464.

Coenraads PJ & Smit J (1995) Epidemiology. In: Rycroft RJG, Menné T, Frosch PJ, & Benezra C ed. Textbook of contact dermatitis, 2nd ed. Berlin, Heidelberg, New York, Springer Verlag, pp 133–150.

Coffman RL, O'Hara J, Bond MW, Carty J, Zlotnik A, & Paul WE (1986) B- cell stimulatory factor-1 enhances the IgE response of lipopolysaccharide activated B-cells. J Immunol, **136**(12): 4538–4541.

Coffman RL, Lebman D, & Shrader B (1989) Transforming growth factor-beta specifically enhances IgA production by lipopolysaccharide-stimulated murine B-lymphocytes. J Exp Med, **170**: 1039–1044.

Cohen IR & Miller A ed. (1994) Autoimmune disease models. New York, London, San Diego, Academic Press.

Colloff MJ, Ayres J, Carswell F, Howarth PH, Merrett TG, Mitchell EB, Walshaw MJ, Warner JO, Warner JA, & Woodcock AA (1992) The control of allergens of dust mites and domestic pets: A position paper. Clin Exp Allergy, **22**(suppl 2): 1–28.

Conell JT (1969) Quantitative intranasal pollen challenges: III. The priming effect in allergic rhinitis. J Allergy, **43**: 33–44.

Constantinescu CS, Hilliard B, Fujioka T, Bhopale MK, Calida D, & Rostami AM (1998) Pathogenesis of neuroimmunologic diseases. Immunol Res, **17**: 217–227.

Cookson WOCM, Sharp PA, Faux JA, & Hopkin JM (1989) Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. Lancet, **1**: 1292–1295.

Cookson WOCM, Young RP, Sandford AJ, Moffatt MF, Shirakawa T, Sharp PA, Faux JA, Julier C, Le Souef PN, Lathrop GM, & Hopkin JM (1992) Maternal inheritance of atopic IgE responsiveness on chromosome 11q. Lancet, **340**: 381–384.

Cooper KD (1994) Atopic dermatitis: recent trends in pathogenesis and therapy. J Invest Dermatol, **102**: 128–137.

Cooper KD, Kang K, Chan SC, & Hanifin JM (1985) Phosphodiesterase inhibition by Ro20-1724 reduces hyper-IgE synthesis by atopic dermatitis cells *in vitro*. J Invest Dermatol, **804**: 477–482

Cormio L, Turjanmaa K, Talja M, Andersson LC, & Ruutu M (1993) Toxicity and immediate allergenicity of latex gloves. Clin Exp Allergy, **23**(7): 618–623.

Corrigan CJ & Kay AB (1992) T cells and eosinophils in the pathogenesis of asthma. Immunol Today, **13**: 501–507.

Cotterill JA (1991) Psychosomatic approaches in the treatment of atopic eczema. In: Ruzicka T, Ring J, & Przybilla B ed. Handbook of atopic eczema. Berlin, Heidelberg, New York, Springer Verlag, pp 459–465.

Crespo JF, Pascual C, Burks AW, Helms RM, & Esteban MM (1995) Frequency of food allergy in a pediatric population from Spain. Pediatr Allergy Immunol, **6**: 39–43.

Croner S & Kjellman NI (1990) Development of atopic disease in relation to family history and cord blood IgE levels. Pediatr Allergy Immunol, **1**: 14–20.

Cronin E (1991) Formaldehyde is a significant allergen in women with hand eczema. Contact Dermatitis, **25**: 276–283.

Cronin MTD & Basketter DA (1994) Multivariate QSAR analysis of a skin sensitization database: SAR and QSAR. Environ Res, **2**: 1–21.

Crowe SE & Perdue MH (1992) Gastrointestinal food hypersensitivity: Basic mechanisms of pathophysiology. Gastroenterology, **103**: 1075–1095.

Cullinan P, Lowson D, Nieuwenhuijsen MJ, Sandiford C, Tee RD, Venables KM, McDonald JC, & Newman Taylor AJ (1994) Work related symptoms, sensitisation, and estimated exposure in workers not previously exposed to flour. Occup Environ Med, **51**(9): 579–583.

Curtis GH, Patrick MK, Catto-Smith AG, & Gall DG (1990) Intestinal anaphylaxis in the rat: Effect of chronic antigen exposure. Gastroenterology, **98**: 1558–1566.

Czernielewski JM & Demarchez M (1987) Further evidence for the self-reproducing capacity of Langerhans cells in human skin. J Invest Dermatol, **88**: 17–20.

Dakin R (1982) Remarks on cutaneous affection, produced by certain poisonous vegetables. Am J Med Sci, **4**: 98–100.

Dally MB, Hunter JV, Hughes EG, Stewart M, & Newman Taylor AJ (1980) Hypersensitivity to platinum salts: a population study. Am Rev Respir Dis, **12**(4): 120 (abstract).

Dannaeus A & Inganäs M (1981) A follow-up study of children with food allergy: Clinical course in relation to serum IgE-antibody levels to milk, egg and fish. Clin Allergy, **11**: 533–539.

Darsow U, Vieluf D, & Ring J (1995) Atopy patch test with different vehicles and allergen concentrations: an approach to standardisation. J. Allergy Clin Immunol, **95**: 677–684.

Davies RJ, Butcher BR, O'Neil CE, & Salvaggio JE (1977) The *in vitro* effect of toluene diisocyanate on lymphocyte cyclic adenosine monophosphate production by isoproterenol, prostaglandin and histamine. J Allergy Clin Immunol, **60**: 223–229.

Dawson KP & Mitchell EA (1990) Asthma in New Zealand children. J Asthma, **27**: 291–297.

Daynes RA, Dudley DJ, & Araneo BA (1990) Regulation of murine lymphokine production *in vivo*. II. Dehydroepiandrosterone is a natural enhancer of interleukin synthesis by helper T cells. Eur J Immunol, **20**(49): 793–802.

Dearman RJ & Kimber I (1991) Differential stimulation of immune function by respiratory and contact chemical allergens. Immunology, **72**: 563–570.

Dearman RJ & Kimber I (1992) Divergent immune responses to respiratory and contact chemical allergens: antibody elicited by phthalic anhydride and oxazolone. Clin Exp Allergy, **22**: 241–250.

Dearman RJ, Hegarty JM, & Kimber I (1991) Inhalation exposure of mice to trimellitic anhydride induces both IgG and IgE anti-hapten antibody. Int Arch Allergy Appl Immunol, **95**: 70–76.

Dearman RJ, Basketter DA, & Kimber I (1995) Differential cytokine production following chronic exposure of mice to chemical respiratory and contact allergens. Immunology, **86**: 545–550.

Dearman RJ, Basketter DA, & Kimber I (1996) Characterization of chemical allergens as a function of divergent cytokine secretion profiles induced in mice. Toxicol Appl Pharmacol, **138**: 308–316.

De Bakker JM, Kammüller ME, Muller ESM, Lam AW, Seinen W, & Bloksma N (1990) Kinetics and morphology of chemically induced popliteal lymph node reactions compared with antigen-, mitogen-, and graft-versus-host-reaction-induced responses. Virchows Arch B Cell Pathol, **58**: 279–287.

Dekker C, Dales R, Bartlett S, Brunekreef B, & Zwanenburg H (1991) Childhood asthma and the indoor environment. Chest, **100**: 922–926.

De Martino M, Novembre E, & Cozza G (1988) Sensitivity to tomato and peanut allergens in children monosensitized to grass pollen. Allergy, **43**: 206–213.

Deo YM, Graziano RF, Repp R, & van de Winkel JGJ (1997) Clinical significance of IgG Fc receptors and Fcγ-directed immunotherapies. Immunol Today, **18**: 127–135.

Derynck R, Lindquist PB, Lee A, Wen D, Tamm T, Graycar JL, Rhee L, Mason AJ, Miller DA, Coffey RJ, Moses HL, & Chen EY (1988) A new type of transforming growth factor-ß. EMBO J, **7**: 3737–3743.

Devalia JL, Rusznak C, Herdman MJ, Trigg CJ, Tarraf H, & Davies RJ (1994) Effect of nitrogen dioxide and sulphur dioxide on airway response of mild asthmatic patients to allergen inhalation. Lancet, **344**: 1668–1671.

Devlin RB, McDonnell WF, Mann R, Becker S, House DE, Schreinemachers D, & Koren HS (1991) Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung. Am J Respir Cell Mol Biol, **4**: 72–81.

De Weck AL, Stadler BM, Urwyler A, Wehner HU, & Bühlmann RP (1993) Cellular allergen stimulation test (CAST) — A new dimension in allergy diagnostic. ACI News, **5**: 9.

De Zotti R, Larese F, Bovenzi M, Negro C, & Molinari S (1994) Allergic airway disease in Italian bakers and pastry makers. Occup Environ Med, **51**(8): 548–552.

Diaz-Sanchez D, Tsien A, Fleming J, & Saxon A (1997) Combined diesel exhaust particulate and ragweed allergen challenge markedly enhances human *in vivo* nasal ragweed-specific IgE and skews cytokine production to a T helper cell 2-type pattern. J Immunol, **158**: 2406–2413.

Diaz-Sanchez D, Tsien A, Fleming J, & Saxon A (1999) Effect of topical fluticasone propionate on the mucosal allergic response induced by ragweed allergen and diesel exhaust particle challenge. Clin Immunol, **90**(3): 313–322.

Diem JE, John RN, Hendrick DJ, Glindmeyer HW, & Dharmarajan V (1982) Five year longitudinal study of workers employed in a new toluene di-isocyanate manufacturing plant. Am Rev Respir Dis, **126**(3): 420–428.

Diller WF (1987) Facts and fallacies involved in the epidemiology of isocyanate asthma. Bull Eur Physiopathol Respir, **23**(6): 551–553.

Djukanovic R, Wilson J, Britten K, Wilson SJ, Walls AF, Roche WR, Howarth PH, & Holgate ST (1990) Quantitation of mast cells and eosinophils in the bronchial mucosa of symptomatic atopic individuals and healthy control subjects using immunohistochemistry. Am Rev Respir Dis, **142**: 863–871.

Dockery DW & Pope CA (1994) Acute respiratory effects of particulate air pollution. Annu Rev Public Health, **15**: 107–132.

Dockery DW, Speizer FE, Stram DO, Ware JH, Spengler JD, & Ferris BGJ (1989) Effects of inhalable particles on respiratory health of children. Am Rev Respir Dis, **139**: 587–594.

Dockery DW, Pope CA III, Xu X, Spengler JD, Ware JH, Fay ME, Ferris BG Jr, & Speizer FE (1993) An association between air pollution and mortality in six US cities. N Engl J Med, **329**: 1753–1759.

DOH (1998) Report of the Silicone Gel Breast Implants Independent Review Group. London, Department of Health, pp 1–36.

Domen PL, Muckerheide A, & Michael JG III (1987) Abrogation of oral tolerance. J Immunol, **139**: 3195–3198.

Dooms-Goossens A & Morren M (1992) Results of routine patch testing with corticosteroids in 2073 patients. Contact Dermatitis, **26**: 182–191.

Dorsch W & Ring J (1981) Induction of late cutaneous reactions by skin blister fluid from allergen-tested and normal skin. J Allergy Clin Immunol, **67**: 117–123.

Dotterud LK & Falk ES (1994) Metal allergy in north Norwegian schoolchildren and its relationship with ear piercing and atopy. Contact Dermatitis, **31**(5): 308–313.

Dotterud LK & Falk ES (1995) Contact allergy in relation to hand eczema and atopic diseases in north Norwegian schoolchildren. Acta-Paediatr, **84**(4): 402–406.

Dowse GK, Turner KJ, Stewart GA, Alpers MP, & Woolcock AJ (1985) The association between dermatophagoides mites and the increasing prevalence of asthma in village communities within the Papua New Guinea highlands. J Allergy Clin Immunol, **75**(1): 75–83.

Draize JH, Woodard G, & Calvery HD (1944) Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther, **83**: 377–390.

Dreborg S (1989) Skin tests used for standardization of allergenic preparation. In: Dreborg S ed. Skin tests used in type I allergy testing: Position paper prepared by the Subcommittee on Skin Tests of the European Academy of Allergology and Clinical Immunology. Allergy, **44**(suppl 10): 13–51.

Dreborg S & Foucard T (1983) Allergy to apple, carrot and potato in children with birch pollen allergy. Allergy, **38**: 167–172.

Drever F ed. (1995) Occupational health — Decennial supplement: The Registrar General's decennial supplement for England and Wales. London, Office of Population Censuses and Surveys, Health and Safety Executive, 374 pp (Series DS No. 10).

Druce HM (1993) Allergic and nonallergic rhinitis. In: Middleton E, Reed Cellis EF, Adkinson NF, Junginger JW, & Busse WW ed. Allergy: Principles and practice. St. Louis, Missouri, C.V. Mosby & Co., pp 1433–1453.

Druet P (1989) Contributions of immunological reactions to nephrotoxicity. Toxicol Lett, **46**: 55–64.

Dubost J-J, Souteyrand P, & Sauvezie B (1991) Drug-induced vasculitides. Clin Rheumatol, **5**: 119–138.

Dubroff LM & Reid RJ Jr (1980) Hydralazine-pyrimidine interactions may explain hydralazine induced lupus erythematosus. Science, **208**: 404–406.

Ducombs G & Schmidt R (1995) Plant and plant products. In: Rycroft RJG, Menné T, Frosch PJ, & Benezra C ed. Textbook of contact dermatitis, 2nd ed. Berlin, Heidelberg, New York, Springer Verlag, pp 589–634.

Ducombs G, Benezra C, Talaga P, Andersen KE, Burrows D, Camarasa JG, Dooms-Goossens A, Lachapelle J-M, Frosch P, Menné T, Rycroft R, White IR, & Wilkinson JD (1990) Patch testing with sesquiterpene lactone mix: A marker for contact allergy to Compositae and certain other plants — A multicenter study of the E.E.C.D.R.G., 1989. Contact Dermatitis, **22**: 249–253.

Duhme H, Weiland SK, Keil U, Kraemer B, Schmid M, Stender M, & Chambless L (1996) The association between self-reported symptoms of asthma and allergic rhinitis and self-reported traffic density on street of residence in adolescents. Epidemiology, **7**: 578–582.

Duhme H, Weiland SK, Rudolph P, Wienke A, Kramer A, & Keil U (1998a) Asthma and allergies among children in West and East Germany: a comparison between Münster and Greifswald using the ISAAC phase I protocol. Eur Respir J, **11**: 840–847.

Duhme H, Weiland SK, & Keil U (1998b) Epidemiological analyses of the relationship between environmental pollution and asthma. Toxicol Lett, **102–103**: 307–316.

Dupuis G & Benezra C (1982) Contact dermatitis to simple chemicals: A molecular approach. New York, Marcel Dekker.

Durham SR, Graneek BJ, Hawkins R, & Newman Taylor AJ (1987) The temporal relationship between increases in airway responsiveness to histamine and late asthmatic responses induced by occupational agents. J Allergy Clin Immunol, **79**: 398–406.

Durie FH, Fava RA, & Noelle RJ (1994) Collagen-induced arthritis as a model of rheumatoid arthritis. Clin Immunol Immunopathol, **73**(1): 11–18.

Dvoracek JE, Yunginger JW, Kern EB, Hyatt RE, & Gleich GJ (1984) Induction of nasal late-phase reaction by insufflation of ragweed pollen extract. J Allergy Clin Immunol, **73**: 363–368.

EAACI (European Academy of Allergology and Clinical Immunology) (1992) Subcommittee on Occupational Allergy — Guidelines for the diagnosis of occupational asthma. Clin Exp Allergy, **22**(1): 103–108.

Edman B (1988) The usefulness of detailed information to patients with contact allergy. Contact Dermatitis, **19**: 43–48.

Edwards J, Walters S, & Griffiths RK (1994) Hospital admissions for asthma in preschool children: Relationship to major roads in Birmingham, United Kingdom. Arch Environ Health, **49**: 223–227.

Edworthy SM, Martin L, Barr SG, Birdsell DC, Brant RF, & Fritzler MJ (1998) A clinical study of the relationship between silicone breast implants and connective tissue disease. J Rheumatol, **25**(2): 254–260.

Ehlers A, Stangier U, & Gieler U (1995) Treatment of atopic dermatitis: A comparison of psychological and dermatological approaches to relapse prevention. J Consult Clin Psychol, **63**(4): 624–635.

Eidson M, Philen RM, Sewell CM, Voorhees R, & Kilbourne EM (1990) L-tryptophan and eosinophilia-myalgia syndrome in New Mexico. Lancet; **335**(8690): 645–648.

Elsayed S & Apold J (1977) Allergenic structure of allergen M from cod: II. Allergenicity of the limited tryptic hydrolysis peptides of fragment TM2. Int Arch Allergy Appl Immunol, **54**(2): 171–175.

Emberlin J (1994) The effects of patterns in climate and pollen abundance on allergy. Allergy, **49**: 15–20.

Enders F, Przybilla B, Ring J, Burg G, & Braun-Falco O (1988) [Epicutaneous testing with standard batteries of allergens: Results from 12,026 patients.] Hautarzt, **39**: 779–786 (in German).

Enk AH & Katz SI (1995) Contact sensitivity as a model for T cell activation in skin. J Invest Dermatol, **105**: 80S–83S.

Epstein WL, Kligman AM, & Senecal IP (1963) Role of regional lymph nodes in contact sensitization. Arch Dermatol, **88**: 789–792.

Eriksson NE, Formgren H, & Svenonius E (1982) Food hypersensitivity in patients with pollen allergy in Sweden. Allergy, **37**(3): 437–443.

Ernst P, Demissie K, Joseph L, Locher U, & Becklake MR (1995) Socioeconomic status and indicators of asthma in children. Am J Respir Crit Care Med, **152**(2): 570–575.

Estlander T & Jolanki R (1988) How to protect the hands. Dermatol Clin, **6**: 105–114.

Estlander T, Keskinen H, Jolanki R, & Kanerva L (1992) Occupational dermatitis from exposure to polyurethane chemicals. Contact Dermatitis, **27**(3): 161–165.

Estlander T, Kanerva L, Tupasela O, Keskinen H, & Jolanki R (1993) Immediate and delayed allergy to nickel with contact urticaria, rhinitis, asthma and contact dermatitis. Clin Exp Allergy, **23**: 306–310.

ETFAD (1993) Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. Dermatology, **186**: 23–31.

Council of the European Communities (1976) Council directive of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products (76/768/EEC). Off J Eur Communities, **L-262**: 169–183.

Council of the European Communities (1994) European Parliament and Council directive 94/27/EC of 30 June 1994: Nickel. Off J Eur Communities, **L 18811–2**.

Evans R (1993) Epidemiology and natural history of asthma, allergic rhinitis and atopic dermatitis (eczema). In: Middleton E, Reed Cellis EF, Adkinson NF, Junginger JW, & Busse WW ed. Allergy: Principles and practice. St. Louis, Missouri, C.V. Mosby & Co., pp 1109–1136.

Evans RG, Webb K, Homan S, & Ayres SM (1988a) Cross-sectional and longitudinal changes in pulmonary function associated with automobile pollution among bridge and tunnel officers. Am J Ind Med, **14**: 25–36.

Evans RG, Webb KB, Knutsen AP, Roodman ST, Roberts DW, Bagby JR, Garrett WA, & Andrews JS (1988b) A medical follow-up of the health effects of long-term exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Arch Environ Health, **43**: 273–278.

Eynon BE & Parker DC (1992) Small B cells as antigen-presenting cells in the induction of tolerance to soluble protein antigens. J Exp Med, **175**: 131–138.

Fabbri LM, Boschetto P, Zocca E, Melani G, Pivirotto F, & Plebani M (1987) Bronchoalveolar neutrophilia during late asthmatic reactions induced by toluene di-isocyanate. Am Rev Respir Dis, **136**: 36–42.

Fairchild RL, Palmer E, & Moorhead JW (1993) Production of DNP-specific/Class I MHC-restricted suppressor molecules is linked to the expression of T cell receptor α- and ß-chain genes. J Immunol, **150**(1): 67–77.

Farine J-C (1997) Animal models in autoimmune disease in immunotoxicity assessment. Toxicology, **119**: 29–35.

Fauci A, Schnittman SM, & Poli G (1991) Immunopathologenic mechanisms in human immunodeficiency virus (HIV) infection. Ann Intern Med, **114**: 678–693.

Fawcett IW, Newman Taylor AJ, & Pepys J (1977) Asthma due to inhaled chemical agents - epoxy resin systems containing phthalic anhydride, trimellitic anhydride and triethylene tetramine. Clin Allergy, **7**: 1–14.

Fireman E, Ben Efraim S, Grief J, Alguetti A, Ayalon D, & Topilsky M (1988) Correlation between prostaglandin E2 production and suppressor activity of alveolar macrophages from patients with interstitial lung diseases. Immunol Lett, **18**(2): 159–165.

Flatt A, Pearce N, Thomson CD, Sears MR, Robinson MF, & Beasley R (1990) Reduced selenium in asthmatic subjects in New Zealand. Thorax, **45**: 95–99.

Fleming DM & Crombie DL (1987) Prevalence of asthma and hay fever in England and Wales. Br Med J Clin Res Ed, **294**(6567): 279–283.

Flens MJ, Zaman GJR, Van der Valk P, Izquierdo MA, Schroeijers AB, Scheffer GL, van der Groep P, De Haas M, Meijer CJLM, & Scheper RJ (1996) Tissue distribution of the multidrug resistance protein. Am J Pathol, **148**: 1237–1247.

Flindt-Hansen H & Isager H (1987) Scleroderma after occupational exposure to trichloroethylene and trichloroethane. Acta Dermatol Venereol, **67**: 263–264.

Flynn GL (1990) Physicochemical determinants of skin absorption. In: Gerrity TR & Henry CJ ed. Principles of route-to-route extrapolation for risk assessment. Amsterdam, Oxford, New York, Elsevier Science Publishers, pp 93–127.

Flyvholm M-A (1997) Formaldehyde exposure at the workplace and in the environment. Allergologie, **20**(5): 225–231.

Flyvholm M-A & Menné T (1992) Allergic contact dermatitis from formaldehyde: A case study focusing on sources of formaldehyde exposure. Contact Dermatitis, **27**: 27–37.

Flyvholm M-A, Menné T, & Maibach HI (1996) Skin allergy: Exposures and dose–response relationships. In: Vos JG, Younes M, & Smith E ed. Allergic hypersensitization induced by chemicals: Recommendations for prevention. Boca Raton, Florida, CRC Press, pp 261–285.

Flyvholm M-A, Hall BM, Agner T, Tiedemann E, Greenhill P, Vanderveken W, Freeberg FE, & Menné T (1997) Threshold for occluded formaldehyde patch test in formaldehyde-sensitive patients. Contact Dermatitis, **36**: 26–33.

Foerster J (1993) Autoimmune hemolytic anemias. In: Lee GR, Bithell TC, Foerster J, Athens JW, & Lukens JN ed. Wintrobe's clinical haematology, 9th ed. London, Lea and Febiger, pp 1170–1196.

Folinsbee LJ (1992) Human health effects of air pollution. Environ. Health Perspect, **100**: 45–56.

Forastiere F, Corbo GM, Michelozzi P, Pistelli R, Agabiti N, Brancato G, Ciappi G, & Perucci CA (1992) Effects of environment and passive smoking on the respiratory health of children. Int J Epidemiol, **21**(1): 66–73.

Forster H, Topping M, & Newman Taylor AJ (1988) Specific IgG and IgG_4 antibody to tetrachlorophthalic anhydride. Allergy Proc, **9**: 296.

Forsyth JS, Ogston SA, Clark A, Florey CD, & Howie PW (1993) Relation between early introduction of solid food to infants and their weight and illnesses during the first two years of life. Br Med J, **306**(6892): 1572–1576.

Foucard T (1991) Allergy and allergy-like symptoms in 1,050 medical students. Allergy, **46**: 20–26.

Fowler JF, Skinner SM, & Belsito DV (1992) Allergic contact dermatitis from formaldehyde resins in permanent press clothing: An underdiagnosed cause of generalized dermatitis. J Am Acad Dermatol, **27**: 962–968.

Freier S, Eran M, & Goldstein R (1985) The effect of immediate type gastrointestinal allergic reactions on brush border enzymes and gut morphology in the rat. Pediatr Res, **19**: 456–459.

Freiman DE & Hardy HL (1970) Beryllium disease: The relation of pulmonary pathology to clinical course and prognosis based on a study of 130 cases from the US Beryllium Case Registry. Hum Pathol, **1**: 25–44.

Friedmann PS (1990) The immunology of allergic contact dermatitis: The DNCB story. Adv Dermatol, **5**: 175–196.

Friedmann PS, Moss C, Shuster S, & Simpson JM (1983) Quantitative relationships between sensitizing dose of DNCB and reactivity in normal subjects. Clin Exp Immunol, **53**: 706–710.

Friedman SM, Posnett DN, Tumang JR, Cole BC, & Crow K (1991) A potential role for microbial superantigens in the pathogenesis of systemic autoimmune disease. Arthritis Rheum, **34**: 468–480.

Fudenberg HH (1966) Immunological deficiency, autoimmune disease, and lymphoma: observations, implications, and speculations. Arthritis Rheum, **9**: 464–472.

Fujimaki H, Nohara O, Ichinose T, Watanabe N, & Saito S (1994) IL-4 production in mediastinal lymph node cells in mice intratracheally instilled with diesel exhaust particulates and antigen. Toxicology, **92**: 261–268.

Fuller KA, Pearl D, & Whitacre CC (1990) Oral tolerance in experimental autoimmune encephalomyelitis: Serum and salivary antibody responses. J Neuroimmunol, **28**: 14–26.

Fye KH & Sack KE (1991) Rheumatic diseases. In: Stites DP & Terr AI ed. Basic and clinical immunology, 7th ed. Norwalk, San Mateo, Appleton & Lange, pp 438–463.

Gabriel SE, O'Fallon WM, Kurland LT, Berad CM, Woods JE, & Melton LJ (1994) Risk of connective-tissue diseases and other disorders after breast implantation. N Engl J Med, **330**(24): 1697–1702.

Gad SC, Dunn BJ, Dobbs DW, Reilly C, & Walsh RD (1986) Development and validation of an alternative dermal sensitization test : The mouse ear swelling test (MEST). Toxicol Appl Pharmacol, **84**: 93–114.

Gamble J, Jones W, & Minshall S (1987) Epidemiological-environmental study of diesel bus garage workers: chronic effects of diesel exhaust on the respiratory system. Environ Res, **44**: 6–17.

Gammelgaard B, Fullerton F, Avnstorp C, & Menné T (1992) Permeation of chromium salts through human skin *in vitro*. Contact Dermatitis, **27**: 302–310.

Gardner MLG (1988) Gastrointestinal absorption of intact proteins. Annu Rev Nutr, **8**: 329–350.

Garssen J, Nijkamp FP, Van der Vliet H, & Van Loveren H (1991) T-cell mediated induction of airway hyperreactivity in mice. Am Rev Resp Dis, **144**: 931–938.

Garssen J, Nijkamp FP, Van de Vliet H, & Van Loveren H (1994) A role for cellular immunity in the induction of airway hyperresposiveness induced by small molecular weight compounds. Toxicol Lett, **72**: 151–154.

Gauggel DL, Sarlo K, & Asquith TN (1993) A proposed screen for evaluating low-molecular-weight chemicals as potential respiratory allergens. J Appl Toxicol, **13**(5): 307–313.

Gautam SC, Chikkala NF, & Battisto JR (1985) Orally induced tolerance generates an efferently acting suppressor T cell and an acceptor T cell that together downregulate contact sensitivity. J Immunol, **135**: 2975–2983.

Geier J & Schnuch A (1996) No cross-sensitization between MCI/MI, benzisothiazolinone and octylisothiazolinone. Contact Dermatitis, **34**(2): 148–149.

Gell PGH & Coombs RRA (1963) Clinical aspects of immunology. Oxford, London, Boston, Blackwell Scientific Publications.

Gell PGH, Coombs RRA, & Lachman R (1975) Clinical aspects of immunology, 3rd ed. Oxford, London, Boston, Blackwell Scientific Publications.

Gergely J & Sarmay G (1996) FcγRII-mediated regulation of human B cells. Scand J Immunol, **44**: 1–10.

Gergen PJ, Mullally DI, & Evans R (1988) National survey of prevalence of asthma among children in the United States, 1976 to 1980. Pediatrics, **81**: 1–7.

Gerling I, Serreze DV, Christianson S, & Leiter E (1992) Intrathymic islet cell transplantation reduces beta-cell autoimmunity and prevents diabetes in NOD/Lt mice. Diabetes, **41**: 1672–1676.

Gilliam JN & Sontheimer RD (1982) Skin manifestations of SLE. Clin Rheum Dis, **8**: 207–218.

Glazier A, Tutschka PJ, Farmer ER, & Santos GW (1983) Graft-versus-host disease in cyclosporine A-treated rats after syngeneic and autologous bone marrow reconstitution. J Exp Med, **158**: 1–8.

Gleich GJ (1990) The eosinophil and bronchial asthma: Current understanding. J Allergy Clin Immunol, **85**(2): 422–436.

Gleichmann H (1981) Studies on the mechanism of drug sensitization: T-cell-dependent popliteal lymph node reaction to diphenylhydantoin. Clin Immunol Immunopathol, **18**: 203–211.

Gleichmann HIK, Pals ST, & Radaszkiewicz T (1983) T-cell-dependent B-cell proliferation and activation induced by administration of the drug diphenylhydantoin to mice. Hematol Oncol, **1**: 165–176.

Gleichmann E, Pals ST, Rolink AG, Radaszkiewicz T, & Gleichmann H (1984) Graft-versus-host reactions: clues to the etiopathology of a spectrum of immunological diseases. Immunol Today, **5**: 324–332.

Gleichmann E, Vohr H-W, Stringer C, Nuyens J, & Gleichmann H (1989) Testing the sensitization of T cells to chemicals: From murine graft-versus-host (GVH) reactions to chemical-induced GVH-like immunological diseases. In: Kammüller ME, Bloksma N, & Seinen W ed. Autoimmunity and toxicology. Amsterdam, Oxford, New York, Elsevier Science Publishers, pp 363–390.

Goettsch W, Garssen J, De Gruijl FR, & Van Loveren H (1993) UV-B and the immune system: a review with special emphasis on T cell-mediated immunity. Thymus, **21**: 93–114.

Goh C (1995) Non eczematous contact reactions. In: Rycroft RJG, Menné T, Frosch PJ, & Benezra C ed. Textbook of contact dermatitis, 2nd ed. Berlin, Heidelberg, New York, Springer Verlag, pp 221–233.

Goldman M, Druet P, & Gleichmann E (1991) T_H2 cells in systemic autoimmunity: insights from allogeneic diseases and chemically-induced autoimmunity. Immunol Today, **12**: 223–227.

Gorski P & Tarkowski M (1992) Non specific environmental factors and asthma development. Pol J Occup Med Environ Health, **5**: 227–236.

Goust JM (1993) Immediate hypersensitivity. Immunol Ser, **58**: 343–359.

Graham C, Gealy R, Macina OT, Karol MH, & Rosenkranz HS (1996) QSAR for allergic contact dermatitis. Quant Struct-Activ Relat, **15**: 1–7.

Graham C, Rosenkranz HS, & Karol MH (1997) Structure-activity model of chemicals that cause human respiratory sensitization. Regul Toxicol Pharmacol, **26**: 296–306.

Granato DA & Piguet PF (1986) A mouse monoclonal IgE antibody anti bovine milk beta-lactoglobulin allows studies of allergy in the gastrointestinal tract. Clin Exp Immunol, **63**(3): 703–710.

Graycar JL, Miller DA, Arrick BA, Lyons RM, Moses HL, & Derynck R (1989) Human transforming growth factor-ß3: recombinant expression, purification, and biologic activities in comparison with transforming growth factors ß1 and ß2. Mol Endocrinol, **3**: 1977–1986.

Greaves IA, Wegman DH, Smith TJ, & Spiegelman DL (1984) Respiratory effects of two types of solder flux used in the electronics industry. J Occup Med, **26**(2): 81–85.

Greenland S (1993) Basic problems in interaction assessment. Environ Health Perspect, **101**(suppl): 59–66.

Gryboski JD (1967) Gastrointestinal milk allergy in infants. Pediatrics, **40**: 354–362.

Guillet GG & Guillet MH (1992) Natural history of sensitizations in atopic dermatitis. Arch Dermatol, **128**: 187–192.

Gulbenkian AR, Egan RW, Fernandez X, Jones H, Kreutner W, Kung T, Payvandi F, Sullivan L, Zurcher JA, & Watnik AS (1992) Interleukin-5 modulates eosinophil accumulation in allergic guinea pig lung. Am Rev Respir Dis, **146**: 263–266.

Gut J, Christen U, Frey N, Koch V, & Stoffler D (1995) Molecular mimicry in halothane hepatitis: biochemical and structural characterization of lipoylated autoantigens. Toxicology, **97**: 199–224.

Haahtela T, Lindholm H, Bjorksten F, Koskenvuo K, & Laitinen LA (1990) Prevalence of asthma in Finnish young men. Br Med J, **301**: 266–268.

Hackney JD, Linn WS, & Avol EL (1989) Acid fog: Effects on respiratory function and symptoms in healthy and asthmatic volunteers. Environ Health Perspect, **79**: 159–162.

Haddad ZH, Kalra V, & Verma S (1979) IgE antibodies to peptic and peptic-tryptic digests of beta-lactoglobulin: significance in food hypersensitivity. Ann Allergy, **42**: 368–371.

Halken S, Høst A, Hansen LG, & Østerballe O (1992) Effect of an allergy prevention programme on incidence of atopic symptoms in infancy. Allergy, **47**: 545–553.

Halken S, Høst A, Nilsson L, & Taudorf E (1995) Passive smoking as a risk factor for development of obstructive respiratory disease and allergic sensitization. Allergy, **50**(2): 97–105.

Hallas TE, Yi X, & Schou C (1993) Does guanine concentration in house-dust samples reflect house-dust mite exposure? Allergy, **48**(5): 303–305.

Hananan D (1990) Transgenic mouse models of self-tolerance and autoreactivity by the immune system. Annu Rev Cell Biol, **6**: 493–537.

Hang L, Aguado MT, Dixon FJ, & Theofilopoulos AN (1985) Induction of severe autoimmune disease in normal mice by simultaneous action of multiple immunostimulators. J Exp Med, **161**: 423–428.

Hanifin JM (1983) Atopic dermatitis and other endogenous eczemas. Semin Dermatol, **2**: 1–44.

Hanifin JM (1987) Epidemiology of atopic dermatitis. Monogr Allergy, **21**: 116–131.

Hanifin JM (1993) Atopic dermatitis. In: Middleton E, Reed Cellis EF, Adkinson NF, Junginger JW, & Busse WW ed. Allergy: Principles and practice. St. Louis, Missouri, C.V. Mosby & Co., pp 1581–1604.

Hanifin JM & Chan SC (1995) Monocyte phosphodiesterase abnormalities and dysregulation of lymphocyte function in atopic dermatitis. J Invest Dermatol, **105**(suppl 1): 84S–88S.

Hanifin JM & Lobitz WC Jr (1977) Newer concepts of atopic dermatitis. Arch Dermatol, **113**(5): 663–670.

Hanifin JM & Rajka G (1980) Diagnostic features of atopic dermatitis. Acta Dermatol Venereol, **92**(suppl): 44–47.

Hanson DG, Roy MJ, Green GM, & Miller SD (1993) Inhibition of orally-induced immune tolerance in mice by prefeeding an endopeptidase inhibitor. Region Immunol, **5**: 76–84.

Hariya T, Ikezawa Z, Aihara M, Kitamura K, Osawa J, & Nakajima H (1994) Allergenicity and tolerogenicity of amlexanox in the guinea pig. Contact Dermatitis, **31**: 31–36.

Hasselmark L, Malmgren R, Zetterstrom O, & Unge G (1993) Selenium supplementation in intrinsic asthma. Allergy, **48**(1): 30–36.

Hatch M & Thomas D (1993) Measurement issues in environmental epidemiology. Environ Health Perspect, **101**(Suppl 4): 49–57.

Hattevig G, Kjellman B, Sigurs N, Bjorksten B, & Kjellman NI (1989) Effect of maternal avoidance of eggs, cow's milk and fish during lactation upon allergic manifestations in infants. Clin Exp Allergy, **19**(1): 27–32.

Heine J, Ignotz RA, Hemler ME, Crouse C, & Massague J (1989) Regulation of cell adhesion receptors by transforming growth factor-ß. Concomitant regulation of integrins that share a common ß1 subunit. J Biol Chem, **264**: 380–388.

Heiner DC, Sears JW, & Kniker WT (1962) Multiple precipitins to cow's milk chronic respiratory disease: A syndrome including poor growth, gastrointestinal symptoms, evidence of allergy, iron deficiency anemia and pulmonary hemosiderosis. Am J Dis Child, **103**: 634–651.

Henderson CR & Riley EC (1945) Certain statistical considerations in patch testing. J Invest Dermatol, **6**: 227–232.

Hennekens LIM & Buring JE (1987) Epidemiology in medicine. Boston, Toronto, Little, Brown & Co., 400 pp.

Hennekens LIM, Cook NR, Hebert PR, Karlson EW, LaMotte F, Manson JE, & Buring JE (1996) Self-reported breast implants and connective tissue diseases in female health professionals. J Am Med Assoc, **275**: 616–621.

Henschler D, Assman W, & Meyer K (1962) [The toxicology of toluene diisocyanate.] Arch Toxicol, **19**: 364–387 (in German).

Heppel LMJ & Kilshaw PJ (1982) Immune responses of guinea pigs to dietary protein: I. Induction of tolerance by feeding with ovalbumin. Int Arch Allergy Appl Immunol, **68**: 54–59.

Herbert CA, King CM, Ring PC, Holgate ST, Stewart GA, Thompson PJ, & Robinson C (1995) Augmentation of permeability in the bronchial epithelium by the house dust mite allergen Der p 1. Am J Respir Cell Mol Biol, **12**: 369–378.

Hertzman PA, Blevins WL, Mayer J, Greenfield B, Ting M, & Gleich GJ (1990) Association of the eosinophilia-myalgia syndrome with the ingestion of tryptophan. N Eng J Med, **322**(130): 869–873.

Hess AD & Fischer AC (1989) Immune mechanisms in cyclosporine-induced syngeneic graft-versus- host disease. Transplantation, **48**: 895–900.

Heufler C, Koch F, & Schuler G (1988) Granulocyte/macrophage colony-stimulating factor and interleukin-1 mediate the maturation of murine epidermal Langerhans cells into potent immunostimulatory dendritic cells. J Exp Med, **167**: 700–705.

Hewitt CRA, Brown AP, Hart BJ, & Pritchard DI (1995) A major house dust mite allergen disrupts the immunoglobulin E network by selectively cleaving CD23: innate protection by antiproteases. J Exp Med, **182**: 1537–1544.

Hide DW, Matthews S, Matthews L, Stevens M, Ridout S, Twiselton R, Gant C, & Arshad SH (1994) Effect of allergen avoidance in infancy on allergic manifestations at age two years. J Allergy Clin Immunol, **93**(5): 842–846.

Hill DJ & Hosking CS (1997) Emerging disease profiles in infants and young children with food allergy. Pediatr Allergy Immunol, 8(suppl 10): 21–26.

Hill LW & Sulzberger MB (1935) Evolution of atopic dermatitis. Arch Dermatol Syph, **32**: 451.

Hjorth N & Menné T (1990) Prevention of allergic contact sensitization: a historical perspective. In: Menné T & Maibach HI ed. Exogenous dermatoses. Boca Raton, Florida, CRC Press, chapter 30.

Hjorth N & Roed-Petersen J (1976) Occupational protein contact dermatitis in food handlers. Contact Dermatitis, **2**(1): 28–42.

Hochberg MC, Perlmutter DL, Medsger TA Jr, Nguyen K, Steen V, Weisman MH, White B, & Wigley FM (1996) Lack of association between augmentation mammoplasty and systemic sclerosis (scleroderma). Arthritis Rheum, **39**: 1125–1131.

Hodge L, Salome CM, Peat JK, Haby MM, Xuan W, & Woolcock AJ (1996) Consumption of oily fish and childhood asthma risk. Med J Aust, **164**(3): 137–140.

Holgate ST, Beasley R, & Twentyman OP (1987) The pathogenesis and significance of bronchial hyperresponsiveness in airways disease. Clin Sci, **73**(6): 561–572.

Hollady SD & Luster MI (1996) Alterations in fetal thymic and liver hematopoietic cells as indicators of exposure to developmental immunotoxicants. Environ Health Perspect, **104**(suppl 4): 809–813.

Holt PG (1994) A potential vaccine strategy for asthma and allied atopic diseases during early childhood. Lancet, **344**: 456–458.

Holt PG (1996) Infections and the development of allergy. Toxicol Lett, **86**: 205–210.

Holt PG & Sedgwick JD (1987) Suppression of IgE responses following antigen inhalation: A natural homeostatic mechanism which limits sensitization to aeroallergens. Immunol Today, **60**(1): 97–102.

Holt PG, Schon-Hegrad MA, & McMenamin PG (1990) Dendritic cells in the respiratory tract. Int. Rev Immunol, **6**(2–3): 139–149.

Hopkins JM (1997) Genetics of atopy. In: Kay AB ed. Allergy and allergic diseases. Oxford, London, Boston, Blackwell Scientific Publications, pp 1187–1195.

Høst A & Halken S (1990) A prospective study of cow milk allergy in Danish infants during the first 3 years of life. Allergy, **45**: 587.

Høst A, Husby S, & Østerballe O (1988) A prospective study of cow's milk allergy in exclusively breast-fed infants: Incidence, pathogenetic role of inadvertent exposure to cow's milk formula, and characterisation of bovine milk protein in human milk. Acta Pædiatr Scand, **77**(5): 663–670.

Hostynek JJ, Magee PS, & Maibach HI (1996) QSAR predictive of contact allergy: scope and limitations. Curr Probl Dermatol, **14**: 59–106.

Howe W, Venables KM, Topping MD, Dally MB, Hawkins R, Law SJ, & Newman Taylor AJ (1983) Tetrachlorophthalic anhydride asthma: evidence for specific IgE antibody. J Allergy Clin Immunol, **71**: 5–11.

HSE (1996) Good health is good business: Employers guide — Work-related dermatitis. London, United Kingdom, Health and Safety Executive, 3 pp.

Hsieh KH & Shen JJ (1988) Prevalence of childhood asthma in Taipei, Taiwan, and other Asian Pacific countries. J Asthma, **25**(2): 73–82.

Hunter D, Milton R, & Perry KMA (1945) Asthma caused by the complex salts of platinum. Br J Ind Med, **2**: 92–98.

Hurtenbach U, Gleichmann H, Nagata N, & Gleichmann E (1987) Immunity to D-penicillamine: genetic, cellular, and chemical requirements for induction of popliteal lymph node enlargement in the mouse. J Immunol, **139**: 411–416.

IARC (1993) Beryllium, cadmium, mercury, and exposures in the glass industry. Lyon, International Agency for Research on Cancer, pp 88–89 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 58).

Ignotz RA & Massague J (1986) Transforming growth factor-ß stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. J Biol Chem, **261**(9): 4337–4345.

Imbus HR (1985) Clinical evaluation of patients with complaints related to formaldehyde exposure. J Allergy Clin Immunol, **76**(6): 831–840.

International Rhinitis Management Working Group (1994) International consensus report on the diagnosis and management of rhinitis. Allergy, **49**(suppl 19): 1–34.

ISAAC (International Study of Asthma and Allergies in Childhood) (1998) ISAAC Steering Committee — Worldwide variation in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema. Lancet, **351**: 1225–1232.

IPCS (1996) Environmental health criteria 180: Principles and methods for assessing direct immunotoxicity associated with exposure to chemicals. Geneva, World Health Organization, International Programme on Chemical Safety, 390 pp.

Irani AA, Schechter NM, Craig SS, DeBlois G, & Schwartz LB (1986) Two types of human mast cells that have distinct neutral protease compositions. Proc Natl Acad Sci (USA), **83**: 4464–4468.

Irvine C, Pugh CE, Hansen EJ, & Rycroft RJG (1994) Cement dermatitis in underground workers during construction of the Channel Tunnel. Occup Med, **44**: 17–23.

Ishizaka K & Ishizaka T (1966) Physicochemical properties of reginic antibody: 1. Association of reaginic activity with an immunoglobulin other than gammaA- or gammaG-globulin. J Allergy, **37**(3): 169–185.

Ishizaki T, Koizumi K, Ikemori R, Ishiyama Y, & Kushibiki E (1987) Studies of prevalence of Japanese cedar pollinosis among the residents in a densely cultivated area. Ann Allergy, **58**: 265–270.

Iverson GM (1970) Ability of CBA mice to produce anti-idiotypic sera to 5563 myeloma protein. Nature (Lond), **227**: 273–274.

Iwami T, Nagai H, Tsuruoka N, & Koda A (1993) Effect of murine recombinant interleukin-5 on bronchial reactivity in guinea-pigs. Clin Exp Allergy, **23**(1): 32–38.

Izquierdo MA, Scheffer GL, Flens MJ, Giaccone G, Broxterman HJ, Van der Valk P, Meijer CJLM, & Scheper RJ (1996) Broad distribution of the multidrug resistance — related Vault Lung Resistance Protein in normal human tissues and tumors. Am J Pathol, **148**: 877–887.

Jacobs MC, White IR, Rycroft RJG, & Taub N (1995) Patch testing with preservatives at St. John's from 1982 to 1993. Contact Dermatitis, **33**: 247–255.

Jakob T, Hermann K, & Ring J (1991) Eosinophil cationic protein in atopic eczema. Arch Dermatol Res, **283**: 5–6.

Jakobsson I & Lindberg T (1979) A prospective study of cow's milk protein intolerance in Swedish infants. Acta Pædiatr Scand, **68**: 853–859.

Janeway CA, Travers P, Hunt S, & Walport M (1997) Immunobiology: The immune system in health and disease. Edinburgh, Churchill Livingstone, 400 pp.

Jarrett EEE (1984) Immunoregulation of IgE responses: the role of the gut in perspective. Ann Allergy, **53**: 550–556.

Jarrett EEE & Hall E (1984) The development of IgE suppressive immunocompetence in young animals: influence of exposure to antigen in the presence or absence of maternal immunity. Immunology, **53**: 365–373.

Jarrett EEE, Haig DM, McDougall W, & McNulty E (1976) Rat IgE production: II. Primary and booster reaginic antibody responses following intradermal or oral immunization. Immunology, **30**: 671–677.

Jarvis J, Agius R, & Sawyer L (1996) Odds on for asthma. Chem Br, **32**: 51–53.

Jenkins MK, Schwartz RH, & Pardoll DM (1988) Effects of cyclosporine A on T-cell development and clonal deletion. Science, **24**: 1655–1658.

Jiang X, Khursigara G, & Rubin RL (1994) Transformation of lupus-inducing drugs to cytotoxic products by activated neutrophils. Science, **266**: 810–813.

Johansen J & Menné T (1995) The fragrance mix and its constituents: a 14-year material. Contact Dermatitis, **32**: 18–23.

Johansen J, Rastogi SC, & Menné T (1996a) Exposure to selected fragrance materials. A case study of fragrance-mix-positive eczema patients. Contact Dermatitis, **34**: 106–110.

Johansen JD, Andersen KE, & Menné T (1996b) Quantitative aspects of isoeugenol contact allergy assessed by use and patch tests. Contact Dermatitis, **34**: 414–418.

Johansen JD, Rastogi SC, Andersen KE, & Menné T (1997) Content and reactivity to product perfumes in fragrance mix positive and negative eczema patients: A study of perfumes used in toiletries and skin-care products. Contact Dermatitis, **36**(6): 291–296.

Johansson SGO & Juhlin L (1970) Immunoglobulin-E in "healed" atopic dermatitis and after treatment with corticosteroids and azathioprine. Br J Dermatol, **82**: 10–13.

Johansson SGO, Dannaeus A, & Lilja G (1984) The relevance of anti-food antibodies for the diagnosis of food allergy. Ann Allergy, **53**: 665.

Johnston SL, Pattemore PK, Sanderson G, Smith S, Lampe F, Josephs L, Symington P, O'Toole S, Myint SH, & Tyrrell DAJ (1995) Community study of role of viral infections in exacerbations of asthma in 9–11 year old children. Br Med J, **310**: 1225–1229.

Jones RJ, Vogelsang GB, Hess AD, Farmer ER, Mann RB, Geller RB, Piantadosi S, & Santos GW (1989) Induction of graft-versus-host disease after autologous bone marrow transplantation. Lancet, **1**: 754–757.

Jones LA, Chin LT, Longo DL, & Kruisbeek AM (1990) Peripheral clonal elimination of functional T cells. Science, **250**: 1726–1729.

Jones RN, Rando RJ, Glindmeyer HW, Foster TA, Hughes JM, O'Neil CE, & Weill H (1992) Abnormal lung function in polyurethane foam producers: Weak relationship to toluene diisocyanate exposures. Am Rev Respir Dis, **146**(4): 871–877.

Jones DB, Coulson AFW, & Duff GW (1993) Sequence homologies between hsp60 and autoantigens. Immunol Today, **14**: 115–118.

Jones Williams W (1988) Beryllium disease. Postgrad Med J, **64**: 511–516.

Jones Williams W & Wallach ER (1989) Laser probe mass spectrometry (LAMMS) analysis of beryllium, sarcoidosis and other granulomatous diseases. Sarcoidosis, **6**: 111–117.

Jordan JM & Whitlock FA (1972) Emotions and the skin: the conditioning of scratch responses in cases of atopic dermatitis. Br J Dermatol, **86**(6): 574–585.

Jordan JM & Whitlock FA (1974) Atopic dermatitis, anxiety and conditioned scratch responses. J Psychosom Res, **18**(5): 297–299.

Jungers P, Dougados M, Pelissier C, Kutten F, Tron F, Lesavre P, & Bach JF (1982) Influence of oral contraceptive therapy on the activity of systemic lupus erythematosus. Arthritis Rheum, **25**: 618–623.

Juniper CP, How MJ, Goodwin BFJ, & Kinshott AK (1977) *Bacillus subtilis* enzymes: a seven year clinical, epidemiological and immunological study of an industrial allergen. J Soc Occup Med, **27**: 3–12.

Kagnoff MF (1982) Oral tolerance. Ann NY Acad Sci, **392**: 248–265.

Kagnoff MF (1992) Celiac disease: A gastrointestinal disease with environmental, genetic, and immunologic components. Gastr Clin North Am, **21**(2): 405–425.

Kajosaari M & Saarinen UM (1983) Prophylaxis of atopic disease by six month's total solid food elimination. Acta Paediatr Scand, **72**: 411–414.

Kammüller ME (1996) Drug-induced autoimmune disorders. Drug Inf J, **30**: 293–299.

Kammüller ME & Seinen W (1988) Structural requirements for hydantoins and 2-thiohydantoins to induce lymphoproliferative popliteal lymph node reactions in the mouse. Int J Immunopharmacol, **10**: 997–1010.

Kammüller ME, Penninks AH, & Seinen W (1984) Spanish toxic oil syndrome is a chemically induced GVHD-like epidemic. Lancet, 1: 1174–1175.

Kammüller ME, Bloksma N, & Seinen W ed. (1989a) Autoimmunity and toxicology: Immune disregulation induced by drugs and chemicals. Amsterdam, Oxford, New York, Elsevier Science Publishers, pp 3–34.

Kammüller ME, Thomas C, De Bakker JM, Bloksma N, & Seinen W (1989b) The popliteal lymph node assay in mice to screen for the immune disregulating potential of chemicals — a preliminary study. Int J Immunopharmacol, 11: 293–300.

Kaposi M (1895) Pathology and treatment of diseases of the skin, 4th ed. New York, William Wood.

Kapp A (1995) [Atopic dermatitis: The skin manifestations.] Allergo J, **4**: 229–238 (in German).

Kapsenberg ML, Wierenga, EA, Bos JD, & Jansen HM (1991) Functional subsets of allergen-reactive human CD4$^+$ T cells. Immunol Today, **12**: 392–395.

Karol MH (1983) Concentration-dependent immunologic response to toluene diisocyanate (TDI) following inhalation exposure. Toxicol Appl Pharmacol, **68**: 229–241.

Karol MH (1986) Respiratory effects of inhaled isocyanates. CRC Crit Rev Toxicol, **16**: 349–380.

Karol MH (1991) Allergic reactions to indoor air pollutants. Environ Health Perspect, **95**: 45–51.

Karol MH (1992) Occupational asthma and allergic reactions to inhaled compounds. In: Miller K, Turk J, & Nicklin S ed. Principles and practice of immunotoxicology. Oxford, London, Boston, Blackwell Scientific Publications, pp 228–241.

Karol MH (1993) Concentration-dependent immunologic response to toluene diisocyanate (TDI) following inhalation exposure. Toxicol Appl Pharmacol, **68**: 229.

Karol MH (1994a) Occupational asthma pig predictive tests for respiratory allergy. In: Dean JH, Luster MI, Munson AE, & Kimber I ed. Immunotoxicology and immunopharmacology, 2nd ed. New York, Raven Press, pp 703–720.

Karol MH (1994b) Animal models of occupational asthma. Eur Respir J, **7**(3): 555–568.

Karol MH & Thorne PS (1988) Pulmonary hypersensitivity and hyperreactivity: implications for assessing allergic responses. In: Gardner DE, Crapo JD, & Massaro EJ ed. Toxicology of the lung. New York, Raven Press, p 427.

Karol MH, Graham C, Gealy R, Macina OT, Sussman N, & Rosenkranz HS (1996) Structure-activity relationships and computer-assisted analysis of respiratory sensitization potential. Toxicol Lett, **86**: 187–191.

Karpatkin S (1988) Immunological platelet disorders. In: Immunological diseases, 4th ed. Boston, Little Brown, vol 2, pp 1631–1662.

Karpus WJ & Swanborg RH (1991) CD4+ suppressor cells inhibit the function of effector cells of experimental autoimmune encephalomyelitis through a mechanism involving transforming growth factor-ß. J Immunol, **146**: 1163–1168.

Katsutani N & Shionoya H (1992) Popliteal lymph node enlargement induced by procainamide. Int J Immunopharmacol, **14**: 681–686.

Kaufman LD, Gruber BL, & Gregersen PK (1991) Clinical follow-up and immunogenetic studies of 32 patients with eosinophilia-myalgia syndrome. Lancet, **337**: 1071–1074.

Kaufman DL, Clare-Salzler M, Tian J, Forsthuber T, Ting GSP, Robinson P, Atkinson MA, Sercarz EE, Tobin AJ, & Lehmann PV (1993) Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. Nature (Lond), **366**: 69–72.

Kayser D & Schlede E (1995) [Chemicals and contact allergy — a summary evaluation.] Berlin, Federal Institute for Consumer's Health Protection and Veterinary Medicine (BGVV) (in German).

Keeley DJ, Neill P, & Gallivan S (1991) Comparison of the prevalence of reversible airways obstruction in rural and urban Zimbabwean children. Thorax, **46**: 549–553.

Keil U, Weiland SK, Duhme H, & Chambless L (1996) The international study of asthma and allergies in childhood (ISAAC): Objectives and methods — Results from German ISAAC centres concerning traffic density and wheezing and allergic rhinitis. Toxicol Lett, **86**: 99–103.

Kemp T, Pearce N, Fitzharris P, Crane J, Fergusson D, St. George I, Wickens K, & Beasley R (1997) Is infant immunization a risk factor for childhood asthma or allergy? Epidemiology, **8**: 678–680.

Keskinen H, Alanko K, & Saarinen L (1978) Occupational asthma in Finland. Clin Allergy, **8**(6): 569–579.

Keskinen H, Tupasela O, Tiikkainen U, & Nordman H (1988) Experience of specific in IgE asthma due to diisocyanates. Clin Allergy, **18**(6): 597–604.

Khoury SJ, Hancock WW, & Weiner HL (1992) Oral tolerance to myelin basic protein and natural recovery from experimental autoimmune encephalomyelitis are associated with downregulation of inflammatory cytokines and differential upregulation of transforming growth factor ß, interleukin 4 and prostaglandin E expression in the brain. J Exp Med, **176**: 1355–1364.

Kimber I & Basketter DA (1997) Contact sensitisation: a new approach to risk assessment. Hum Ecol Risk Assess, **3**: 385–395.

Kimber I & Cumberbatch M (1992a) Dendritic cells and cutaneous immune responses to chemical allergens. Toxicol Appl Pharmacol, **117**: 137–146.

Kimber I & Cumberbatch M (1992b) Stimulation of Langerhans cell migration by tumour necrosis factor alpha. J Invest Dermatol, **99**: 48S–50S.

Kimber I & Maurer T ed. (1996) Toxicology of contact hypersensitivity. London, Taylor & Francis, 170 pp.

Kimber I & Wilks MF (1995) Chemical respiratory allergy: Toxicological and occupational health issues. Hum Exp Toxicol, **14**(9): 735–736.

Kimber I, Hilton J, & Weisenberger C (1989) The murine local lymph node assay for identification of contact allergens: a preliminary evaluation of in situ measurement of lymphocyte proliferation. Contact Dermatitis, **21**: 215.

Kimber I, Kinnaird A, Peters SW, & Mitchell JA (1990) Correlation between lymphocyte proliferative responses and dendritic cell migration in regional lymph nodes following skin painting with contact sensitizing agents. Int Arch Allerg Appl Immunol, **93**: 47–53.

Kimber I, Dearman RJ, Scholes EW, & Basketter DA (1994) The local lymph node assay developments and applications. Toxicology, **93**: 13–31.

King TP, Hoffman D, Lφwenstein H, Marsh DG, Platts-Mills TAE, & Thomas W (1995) Allergen nomenclature. J Allergy Clin Immunol, **96**: 5–14.

Kirton V (1978) Contact urticaria and cinnamic aldehyde. Contact Dermatitis, **4**(6): 374–375.

Kishimoto T & Hirano T (1984) β-Lymphocyte activation, proliferation, and immunoglobulin secretion. In: Paul WE ed. Fundamental immunology, 2nd ed. New York, Raven Press, pp 385–411.

Kitagaki H, Fujisawa S, Watanabe K, Hayakawa K, & Shiohara T (1995) Immediate type hypersensitivity response followed by a late reaction is induced by repeated epicutaneous application of contact sensitizing agents in mice. J Invest Dermatol, **105**: 749–755.

Kjellman NI & Croner S (1984) Cord blood IgE determination for allergy prediction — a follow-up to seven years of age in 1,651 children. Ann Allergy, **53**(2): 167–171.

Kligman AM (1958a) Poison ivy (Rhus) dermatitis. Arch Dermatol, **77**: 159–180.

Kligman AM (1958b) Hyposensitization against Rhus dermatitis. Arch Dermatol, **78**: 47–72.

Kligman AM (1966a) The identification of contact allergies by human assay: II. Factors influencing the induction and measurement of allergic contact dermatitis. J Invest Dermatol, **47**: 375–393.

Kligman AM (1966b) The identification of contact allergens by human assay: III. The maximization test — A procedure for screening and rating contact sensitizers. J Invest Dermatol, **47**: 393–409.

Kligman AM (1966c) The SLS provocative patch test in allergic sensitization. J Invest Dermatol, **46**: 573–595.

Knippels LM, Penninks AH, Spanhaak S, & Houben GF (1998) Oral sensitization to food proteins: a Brown Norway rat model. Clin Exp Allergy, **28**(3): 368–375.

Koenig JQ (1988) Indoor and outdoor pollutants and the upper respiratory tract. J Allergy Clin Immunol, **81**: 1055–1059.

Koenig JQ (1995) Effect of ozone on respiratory responses in subjects with asthma. Environ Health Perspect, **103**(suppl 2): 103–105.

Koenig JQ, Pierson WE, & Horike M (1983) The effects of inhaled sulfuric acid on pulmonary function in adolescent asthmatics. Am Rev Respir Dis, **128**(2): 221–225.

Koenig JQ, Pierson WE, Covert DS, Marshall SG, Morgan MS, & van Belle G (1988) The effects of ozone and nitrogen dioxide on lung function in healthy and asthmatic adolescents. Res Rep Health Eff Inst, **14**: 5–24.

Koenig JQ, Covert DS, Hanley QS, van Belle G, & Pierson WE (1990) Prior exposure to ozone potentiates subsequent response to sulfur dioxide in adolescent asthmatic subjects. Am Rev Respir Dis, **141**: 377–380.

Koëter HBWM (1995) International harmonisation of immunotoxicity testing. Hum Exp Toxicol, **14**: 151–154.

Kogevinas M, Anto J, Soriano JB, Tobias A, & Burney P (1996) The risk of asthma attributable to occupational exposures. Ann Resp Crit Care Med, **154**: 137–143.

Kohler C, Jeanvoine G, Pierrez J, Olive D, & Gerard H (1987) Modifications of the thymus and splenic thymic dependent zones after in utero exposure to phenytoin: Qualitative and quantitative analysis in C3H mice. Dev Pharmacol Therap, **10**: 405–412.

Koren HS (1995) Associations between criteria air pollutants and asthma. Environ Health Perspect, **103**(suppl 6): 235–242.

Koren HS & Bromberg PA (1995) Respiratory responses of asthmatics to ozone. Int Arch Allergy Immunol, **107**: 236–238.

Koren HS, Devlin RB, Graham DE, Mann R, McGee MP, Horstman DH, Kozumbo WJ, Becker S, House DE, McDonnel WF, & Bromberg PA (1989) Ozone-induced inflammation in the lower airways of human subjects. Am Rev Respir Dis, **139**: 407–415.

Koren HS, Hatch GE, & Graham DE (1990) Nasal lavage as a tool in assessing acute inflammation in response to inhaled pollutants. Toxicology, **60**: 15–25.

Korman NJ (1995) In situ bound antibodies eluted from the skin of patients with bullous pemphigoid are preferentially directed against the 230-kD bullous pemphigoid antigen. J Invest Dermatol, **105**: 824–830.

Korman NJ, Eyre RW, Zone J, & Stanley JR (1991) Drug-induced pemphigus: autoantibodies directed against the pemphigus antigen complexes are present in penicillamine and captopril-induced pemphigus. J Invest Dermatol, **96**: 273–276.

Kortekangas-Savolainen O, Savolainen J, & Einarsson R (1993) Gastrointestinal stability of baker's yeast allergens: an *in vitro* study. Clin Exp Allergy, **23**(7): 578–590.

Krämer U, Behrendt H, & Ring J (1996) Air pollution as a risk factor for allergy: The East-West German experience. In: Ring J, Behrendt H, & Vieluf D ed. New trends in allergy. Berlin, Heidelberg, New York, Springer Verlag, vol IV, pp 25–35.

Kremer AM, Pal TM, Schouten JP, & Rijcken B (1995) Airway hyperresponsiveness in workers exposed to low levels of irritants. Eur Respir J, **8**(1): 53–61.

Kremser M (1989) [Food allergies — oropharyngeal reactions.] Wien Med Wochenschr, **139**(6/7): 135–139.

Kröger S, Neuber K, Gruseck E, Ring J, & Abeck D (1995) *Pityrosporum ovale* extracts increase interleukin-4, interleukin-10 and IgE synthesis in patients with atopic eczema. Acta Dermatol Venereol, **75**: 357–360.

Krzystyniak K, Brouland J-P, Panayi G, Patriarca C, Verdier F, Descotes J, & Revillard J-P (1992) Activation of $CD4^+$ and $CD8^+$ lymphocyte subsets by streptozotocin in murine popliteal lymph node (PLN) test. J Autoimmun, **5**: 183–197.

Krzyzanowski M, Quackenboss JJ, & Lebowitz MD (1992) Relation of peak expiratory flow rates and symptoms to ambient ozone. Arch Environ Health, **47**(2): 107–115.

Kubicka-Muranyi M, Goebels R, Goebel C, Uetrecht J, & Gleichmann E (1993) T lymphocytes ignore procainamide, but respond to its reactive metabolites in peritoneal cells: demonstration by the adoptive transfer popliteal lymph node assay. Toxicol Appl Pharmacol, **122**: 88–94.

Kubicka-Muranyi M, Griem P, Lübben B, Rottmann N, Lührmann R, & Gleichmann E (1996) Mercuric chloride-induced autoimmunity in mice involves an upregulated presentation of altered and unaltered nucleolar self antigen. Int Arch Allergy Immunol, **108**: 1–10.

Kuchroo VK, Byrne MC, Greenfield E, Whitters MJ, Nalefsky EA, Rao A, Collins M, & Dorf ME (1995) Transfection of TCR alpha-chains into suppressor and T helper cell hybridomas. J Immunol, **154**: 5030–5038.

Kuhn R, Rajewsky K, & Muller W (1991) Generation and analysis of interleukin-4 deficient mice. Science, **254**(5032): 707–710.

Kurisaki J, Konishi Y, Kaminogawa S, & Yamauchi K (1981) Studies on the allergenic structure of hen ovomucoid by chemical and enzymatic fragmentation. Agric Biol Chem, **45**: 879.

Küster W, Petersen M, Christophers E, Goos M, & Sterry W (1990) A family study of atopic dermatitis. Arch Dermatol Res, **282**: 98–102.

Lake AM, Kagey-Sobotka A, Jakubowicz T, & Lichtenstein LM (1984) Histamine release in acute anaphylactic enteropathy of the rat. J Immunol, **133**(3): 1529–1534.

Lammintausta K, Kalimo K, & Havu VK (1982) Contact allergy in atopics who perform wet work in hospital. Derm Beruf Umwelt, **30**(6): 184–188.

Lammintausta K, Kalimo K, & Fagerlund VL (1992) Patch test reactions in atopic patients. Contact Dermatitis, **26**(4): 234–240.

Lamont AG, Mowat A McI, Browning MJ, & Parrott DMV (1988) Genetic control of oral tolerance to ovalbumin in mice. Immunology, **63**: 737–739.

Landsteiner K & Rostenberg A Jr (1939) Individual differences in susceptibility to eczematous sensitization with simple chemical substances. J Invest Dermatol, **2**: 25–29.

Lane HC & Fauci AS (1985) Immunologic abnormalities in the acquired immunodeficiency syndrome. Annu Rev Immunol, **3**: 477–500.

Lange CE, Jühe S, Stein G, & Veltman G (1974) [The so-called vinyl chloride disease — an occupational scleroderma?] Int Arch Arbeitsmed, **32**: 1–32 (in German).

Laor A, Cohen L, & Danon YL (1993) Effects of time, sex, ethnic origin, and area of residence on prevalence of asthma in Israeli adolescents. Br Med J, **307**: 841–844.

Larsen CG, Thomsen MK, Gesser B, Deleuran BW, & Thestrup-Pedersen K (1995) The delayed type hypersensitivity reaction is dependent on IL-8. Inhibition of a tuberculin skin reaction by an anti-IL-8 monoclonal antibody. J Immunol, **155**(4S): 2151–2157.

Lau S, Falkenhorst G, Weber A, Werthmann I, Lind P, Buettner Goetz P, & Wahn U (1989) High mite-allergen exposure increases the risk of sensitization in atopic children and young adults. J Allergy Clin Immunol, **84**: 718–725.

Lau S, Ehnert B, Cremer B, Nasert S, Buettner P, Czarnetzki BM, & Wahn U (1995) [Reduction of house dust mite exposure in sensitized patients with atopic eczema.] Allergo J, **4**: 432–437 (in German).

Lauer K (1990) Environmental nitrophenols and autoimmunity (Letter to the Editor). Mol Immunol, **27**: 697–698.

Lauerma AI (1992) Contact hypersensitivity to glucocorticosteroids. Am J Contact Dermatitis, **3**: 112–132.

Lauwerys R & Lison D (1994) Health risks associated with cobalt exposure — an overview. Sci Total Environ, **150**(1–3): 1–6.

Law LW, Goldstein AL, & White A (1968) Influence of thymosin on immunological competence of lymphoid cells from thymectosized mice. Nature (Lond), **219**(161): 1391–1392.

Leach CL, Hatoum NS, Ratajcak HV, Zeiss CR, Roger JC, & Garvin PJ (1987) Pathologic and immunologic response to inhaled trimellitic anhydride in rats. Toxicol Appl Pharmacol, **87**: 67–80.

Ledford DK (1994) Indoor allergens. J Allergy Clin Immunol, **94**: 327–334.

Lee HK, Alarie Y, & Karol MH (1984) Induction of formaldehyde sensitivity in guinea pigs. Toxicol Appl Pharmacol, **75**: 147–155.

Lehmann PV, Secarz EE, Fortshuber T, Dayan CM, & Gammon G (1993) Determinant spreading and the dynamics of the autoimmune T cell repertoire. Immunol Today, **14**: 203–208.

Lejman E, Stoudemayer T, Grove G, & Kligman AM (1984) Age differences in poison ivy dermatitis. Contact Dermatitis, **11**: 163–167.

Lenshow DJ, Walunas TL, & Bluestone JA (1996) CD28/B7 system of T cell co-stimulation. Annu Rev Immunol, **14**: 233–258.

Leung R & Ho P (1994) Asthma, allergy, and atopy in three south-east Asian populations. Thorax, **49**: 1205–1210.

Lewis S, Richards D, Bynner J, Butler N, & Britton J (1995) Prospective study of risk factors for early and persistent wheezing in childhood. Eur Respir J, **8**(3): 349–356.

Leyden JJ & Kligman AM (1977) Allergic contact dermatitis: Sex differences. Contact Dermatitis, **3**: 333–336.

Leyden JE, Marples RR, & Kligman AM (1974) *Staphylococcus aureus* in the lesions of atopic dermatitis. Br J Dermatol, **90**(5): 525–530.

Li LF (1995) A clinical and patch test study of contact dermatitis from traditional Chinese medical materials. Contact Dermatitis, **33**: 392–395.

Lidén C, Menné T, & Burrows D (1996) Nickel-containing alloys and platings and their ability to cause dermatitis. Br J Dermatol, **134**: 193–198.

Lindskov R & Hølmer G (1992) Polyunsaturated fatty acid in plasma, red blood cells and mononuclear phospholipids in patients with atopic dermatitis. Allergy, **47**: 517–521.

Linn WS, Shamoo DA, Spier CE, Valencia LM, Anzar UT, Venet TG, & Hackney JD (1983a) Respiratory effects of 0.75 ppm sulfur dioxide in exercising asthmatics: influence of upper-respiratory defenses. Environ Res, **30**: 340–348.

Linn WS, Venet TG, Shamoo DA, Valencia LM, Anzar UT, Spier CE, & Hackney JD (1983b) Respiratory effects of sulfur dioxide in heavily exercising asthmatics: A dose–response study. Am Rev Respir Dis, **127**: 278–283.

Lippmann M (1989) Health effects of ozone: a critical review. J Air Pollut Control Assoc, **39**: 672–695.

Liu MC, Bleecker ER, Lichtenstein LM, Kagey-Sobotka A, Niv Y, Mclemore TL, Permutt S, Proud D, & Hubbard WC (1990) Evidence for elevated levels of histamine, prostaglandin D_2, and other bronchoconstricting prostaglandins in the airways of subjects with mild asthma. Am Rev Respir Dis, **142**: 126–132.

Lo D (1996) Animal models of human disease — Transgenic and knockout models of autoimmunity: building a better disease? Clin Immunol Immunopathol, **79**(2): 96–104.

Logan RFA (1992) In: Auricchio S & Visakorpi JK ed. Problems and pitfalls in epidemiological studies of coeliac disease, common food intolerances: 1. Epidemiology of coeliac disease. Basel, Karger, p 14.

Lonkar A, Mitchell JC, & Calnan CD (1974) Contact dermatitis from parthenium hysterophorus. Trans St. John's Hosp Derm Soc, **60**: 43–53.

Løvik M, Høgseth A-K, Gaarder PI, Hagemann R, & Eide I (1997) Diesel exhaust particles and carbon black have adjuvant activity on the local lymph node response and systemic IgE production to ovalbumin. Toxicology, **121**: 165–178.

Lucas A, Brooke OG, Morley R, Cole TJ, & Bamford MF (1990) Early diet of preterm infants and development of allergic or atopic disease: randomised prospective study. Br Med J, **300**(6728): 837–840.

Lucente FE (1989) Rhinitis and nasal obstruction. Otolaryngol Clin North Am, **22**: 307–318.

Luczynska CM & Topping MD (1986) Specific IgE antibodies to reactive dye-albumin conjugates. J Immunol Methods, **95**: 177–186.

Luo J-C, Nelsen KG, & Fischbein A (1990) Persistent reactive airways dysfunction syndrome after exposure to toluene di-isocyanate. Br J Ind Med, **47**: 239–241.

Lutsky I, Baum GL, Teichtahl H, Mazar A, Aizer F, & Bar Sela S (1985) Occupational respiratory disease in veterinarians. Ann Allergy, **55**(2): 153–156.

Lynch NR, Hagel IA, Palenque ME, Di Prisco MC, Escudero JE, Corao LA, Sandia JA, Ferreira LJ, Botto C, Perez M, & Le Souef PN (1998) Relationship between helminthic infection and IgE response in atopic and nonatopic children in a tropical environment. J Allergy Clin Immunol, **101**(2): 217–221.

McAlindon T, Gianotta L, Taub N, D'Cruz D, & Hughes GRV (1993) Environmental factors predicting nephritis in systemic lupus erythematosus. Ann Rheum Dis, **52**: 720–724.

Maccia CA, Berstein IL, Emmett EA, & Brookes SSM (1976) *In vitro* demonstration of specific IgE in phthalic anhydride sensitivity. Am Rev Respir Dis, **113**: 701–704.

MacDonald TT (1983) Immunosuppression caused by antigen feeding: II. Suppressor T cells mask Peyer's patch B cell priming to orally administered antigen. Eur J Immunol, **13**: 138–142.

McGrath H Jr, Bell JM, & Haycock JW (1994) Fluorescent light activates the immunomodulator cis-urocanic acid *in vitro*: Implications for patients with systemic lupus erythematosus. Ann Rheum Dis, **53**(6): 396–399.

Machado DC, Horton D, Harrop R, Peachell PT, & Helm BA (1996) Potential allergens stimulate the release of mediators of the allergic response from cells of mast cell lineage in the absence of sensitization with antigen-specific IgE. Eur J Immunol, **26**: 2972–2980.

McMillan C & Burrows D (1995) *In vitro* testing in contact hypersensitivity. In: Rycroft RJG, Menné T, Frosch PJ, & Benezra C ed. Textbook of contact dermatitis, 2nd ed. Berlin, Heidelberg, New York, Springer Verlag, pp 306–318.

Madara JL & Stafford J (1989) Interferon-gamma directly affects barrier function of cultured intestinal epithelial monolayers. J Clin Invest, **83**(2): 724–727.

Maestrelli P, Di Stefano A, Occari P, Turato G, Milani G, Pivirotto F, Mapp CE, Fabbri LM, & Saetta M (1995) Cytokines in the airway mucosa of subjects with asthma induced by toluene diisocyanate. Am J Resp Crit Care Med, **151**: 607–612.

Magnus P & Jaakkola JJ (1997) Secular trend in the occurrence of asthma among children and young adults: critical appraisal of repeated cross sectional surveys. Br Med J, **314**: 1795–1799

Magnusson CG (1986) Maternal smoking influences cord serum IgE and IgD levels and increases the risk for subsequent infant allergy. J Allergy Clin Immunol, **78**: 898–904.

Magnusson B & Gilje O (1973) Allergic contact dermatitis from a dish-washing liquid containing laurylethersulphate. Acta Dermatol Venereol, **53**: 136–140.

Magnusson B & Hersle K (1965) Patch test methods. Acta Dermatol Venereol, **45**: 123–128.

Magnusson B & Kligman AM (1970a) Allergic contact dermatitis in the guinea pig. Springfield, Charles C Thomas, pp 3–120.

Magnusson B & Kligman AM (1970b) The identification of contact allergens by animal assay: The guinea pig maximisation test. J Invest Dermatol, **52**: 268–276.

Magnussen H, Holz O, & Jörres RA (1998) Asthma and the environment: Is ozone really important? Eur Respir Rev, **8**: 141–144.

Mahzoon S, Yamamoto S, & Greaves MW (1977) Response of skin to ammonium persulphate. Acta Dermatol Venereol, **57**(2): 125–126.

Maibach H (1976) Immediate hypersensitivity in hand dermatitis. Arch Dermatol, **112**(9): 1289–1291.

Malo J-L, Ouimet G, Cartier A, Lebitz D, & Ziess CR (1983) Combined alveolitis and asthma due to hexamethylene di-isocyanate (HDI) with evidence of crossed respiratory and immunologic reactivities to diphenylmethane di-isocyanate MDI. J Allergy Clin Immunol, **72**: 413–419.

Manetti R, Parronchi P, Giudizi MG, Piccinni MP, Maggi E, Trinchieri G, & Romagnani S (1993) Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4 producing Th cells. J Exp Med, **177**(4): 1199–1204.

Mantle J & Pepys J (1974) Asthma amongst Tristan da Cunha islanders. Clin Allergy, **4**: 161–170.

Marchal G, Seman M, Milon G, Truffa-Bacchi P, & Zilberfarb V (1982) Local adoptive transfer of skin delayed type hypersensitivity initiated by a single T lymphocyte. J Immunol, **129**(3): 954–958.

Marchionini A (1960) [New studies of constitutional neurodermatitis.] In: Marchionini A & Röckl M ed. [Dermatology and venereology.] Berlin, Heidelberg, New York, Springer Verlag, vol 3, pp 42–46 (in German).

Marcon-Genty D, Tomé D, Dumontier AM, Kheroua O, & Desjeux JF (1989) Permeability of milk protein antigens across the intestinal epithelium *in vitro*. Reprod Nutr Dev, **29**(6): 717–723.

Marfaing-Koka A, Devergne O, Gorgone G, Portier A, Schall TJ, Galanaud P, & Emilie D (1995) Regulation of the production of RANTES chemokine by endothelial cells — Synergistic induction by IFN-gamma plus TNF-alpha and inhibition by IL-4 and IL-13. J Immunol, **154**(suppl 4): 1870–1878.

Marsh DG (1990) Immunogenetic and immunochemical factors determining immune responsiveness to allergens: studies in unrelated subjects. In: Marsh DG & Blumenthal M ed. Genetic and environmental factors in clinical allergy. Minneapolis, University of Minneapolis Press, pp 97–120.

Marsh DG & Norman PS (1988) Antigens that cause atopic disease. In: Immunological diseases, 4th ed. Boston, Little Brown, vol 2, pp 981–1008.

Martin S & Weltzien HU (1994) T cell recognition of haptens, a molecular view. Int Arch Allergy Immunol, **104**: 10–16.

Martinez FD (1994) Role of viral infections in the inception of asthma and allergies during childhood: could they be protective? Thorax, **49**: 1189–1191.

Martinez FD (1995) Viral infections and the development of asthma. Am J Respir Crit Care Med, **151**: 1644–1648.

Martinez FD, Antognoni G, Macri F, Bonci E, Midulla F, De Castro G, & Ronchetti R (1988) Parental smoking enhances bronchial responsiveness in nine-year-old children. Am Rev Respir Dis, **138**(3): 518–523.

Martinez FD, Cline M, & Burrows B (1992) Increased incidence of asthma in children of smoking mothers. Pediatrics, **89**: 21–26.

Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, & Morgan WJ (1995) Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med, **332**: 133–138.

Marzulli FN & Maibach HI (1973) Antimicrobials: Experimental contact sensitization in man. J Soc Cosmet Chem, **24**: 399–421.

Marzulli FN & Maibach HI (1974) The use of graded concentrations in studying skin sensitizers: experimental contact sensitization in man. Food Cosmet Toxicol, **12**: 219–227.

Marzulli FN & Maibach HI (1996) Test methods for allergic contact dermatitis in humans. In: Marzulli FN & Maibach HI ed. Dermatotoxicology, 5th ed. Washington, New York, London, Hemisphere Publishing Corporation, pp 477–482.

Matricardi PM (1997) Infections preventing atopy: Facts and new questions. Allergy, **52**: 879–882.

Matricardi PM, Rosmini F, Ferrigno L, Nasion R, Rapicetta M, Chionne P, Stroffolini T, Pasquini P, & D'Amelio R (1997) Cross sectional retrospective study of the prevalence of atopy among Italian military students with antibodies against hepatitis A virus. Br Med J, **314**: 999–1003.

Mattingly JA (1984) Immunologic suppression after oral administration of antigen: III. Activation of suppressor-inducer cells in the Peyer's patches. Cell Immunol, **86**: 46–52.

Matzner Y, Erlich HA, Brautbar C, Sanilevitch A, Landau M, Brenner S, & Friedmann A (1995) Identical HLA class II alleles predispose to drug-triggered and idiopathic pemphigus vulgaris. Acta Dermatol Venereol, **75**: 12–14.

Maulitz RM, Pratt DS, & Schocket AL (1979) Exercise-induced anaphylactic reaction to shellfish. J Allergy Clin Immunol, **63**(6): 433–434.

Maurer T, Thomann P, Weirich EG, & Hess R (1975) The optimization test in the guinea pig: A method for the predictive evaluation of the contact allergenicity of chemicals. Agents Actions, **5**: 174–179.

Maynard AD, Northage C, Hemingway M, & Bradley SD (1997) Measurement of short-term exposure to airborne soluble platinum in the platinum industry. Ann Occup Hyg, **41**(1): 77–94.

Meade R, Askenase PW, Geba GP, Neddermann K, Jacoby RO, & Pasternak RD (1992) Transforming growth factor-ß1 inhibits murine immediate and delayed type hypersensitivity. J Immunol, **149**(2): 521–528.

Medici TC & Vetter W (1991) [Bronchial asthma and kitchen salt.] Schweiz Med Wochenschr, **121**(**14**): 501–508 (in German).

Meding B (1990) Epidemiology of hand eczema in an industrial city. Acta Dermatol Venereol, **153**(suppl): 2–43.

Melamed D & Friedman A (1993) Direct evidence for anergy in T lymphocytes tolerized by oral administration of ovalbumin. Eur J Immunol, **23**: 935–942.

Mellström G, Lindahl G, & Wahlberg J (1989) DAISY: reference database on protective gloves. Semin Dermatol, **8**: 75–79.

Melnik B & Plewig G (1991) Atopic dermatitis and disturbances of essential fatty acid in prostaglandin E metabolism. J Am Acad Dermatol, **25**: 859–860.

Menné T & Bachmann E (1979) Permanent disability from skin diseases. Dermatosen, **27**: 37–42.

Menné T & Holm NV (1983) Nickel allergy in a female twin population. Int J Dermatol, **22**: 22–28.

Menné T & Holm NV (1986) Gene susceptibility in human allergic contact sensitization. Semin Dermatol, **5**: 301–306.

Menné T & Maibach HI (1991) Systemic contact-type dermatitis. In: Marzulli FN & Maibach HI ed. Dermatotoxicology. Washington, New York, London, Hemisphere Publishing Corporation, p 453.

Menné T & Maibach HI ed. (1994) Hand eczema. Boca Raton, Florida, CRC Press, 334 pp.

Menné T & Wilkinson JD (1995) Individual predisposition to contact dermatitis. In: Rycroft RJG, Menné T, Frosch PJ, & Benezra C ed. Textbook of contact dermatitis. Berlin, Heidelberg, New York, Springer Verlag, pp 123–130.

Menné T, Christophersen J, & Maibach HI (1987) Epidemiology of allergic contact sensitization. Monogr Allergy, **21**: 132–161.

Menné T, Dooms-Goossens A, Wahlberg JE, White I, & Wilkinson J (1992) How large a proportion of contact sensitivities are diagnosed with the European standard series. Contact Dermatitis, **26**: 201–202.

Menné T, Veien N, Sjølin KE, & Maibach HI (1994) Systemic contact dermatitis. Am J Contact Dermatitis, **5**: 1–12.

Meredith S (1993) Reported incidence of occupational asthma in the United Kingdom, 1989–90. J Epidemiol Community Health, **47**(6): 459–463.

Meredith SK & McDonald JC (1994) Work-related respiratory disease in the United Kingdom, 1989–1992: Report on the SWORD project. Occup Med, **44**(4): 183–189.

Meredith S & Nordman H (1996) Occupational asthma: measures of frequency from four countries. Thorax, **51**(4): 435–440.

Merget R, Reineke M, Rueckmann A, Bergmann EM, & Schultze Werninghaus G (1994) Nonspecific and specific bronchial responsiveness in occupational asthma caused by platinum salts after allergen avoidance. Am J Respir Crit Care Med, **150**(4): 1146–1149.

Merget R, Dierkes A, Rueckmann A, Bergmann EM, & Schultze Werninghaus G (1996) Absence of relationship between degree of nonspecific and specific bronchial responsiveness in occupational asthma due to platinum salts. Eur Respir J, **9**(2): 211–216.

Metcalfe DD (1985) Food allergens. Clin Rev Allergy, **3**(3): 331–349.

Metcalfe DD & Sampson HA (1990) Workshop on experimental methodology for clinical studies of adverse reactions to foods and food additives. J Allergy Clin Immunol, **86**: 421–442.

Metzger WJ, Zavala D, Richerson HB, Mosely P, Iwamota P, Monick M, Sjoerdsma K, & Hunninghake GW (1987) Local allergen challenge and bronchoalveolar lavage of allergic asthmatic lungs. Am Rev Respir Dis, **135**: 433–440.

Miedema I, Feskens EJ, Heederik D, & Kromhout D (1993) Dietary determinants of long-term incidence of chronic nonspecific lung diseases: The zutphen study. Am J Epidemiol, **138**(1): 37–45.

Miller K & Nicklin S (1988) Mechanisms of food intolerances: development and use of experimental animal models. In: Walker R & Quattrucci E ed. Nutritional and toxicological aspects of food processing. London, New York, Philadelphia, Taylor & Francis, pp 351–364.

Minowada G & Welch WJ (1995) Clinical implications of the stress response. J Clin Invest, **95**: 3–12.

Mitchell EA, Stewart AW, Pattemore PK, Asher MI, Harrison AC, & Rea HH (1989) Socioeconomic status in childhood asthma. Int J Epidemiol, **18**(4): 888–890.

Molfino NA, Wright SC, Katz I, Tarlo S, Silverman F, McClean PA, Szalai JP, Raizenne M, Slutsky AS, & Zamel N (1991) Effect of low concentrations of ozone on inhaled allergen responses in asthmatic subjects. Lancet, **338**: 199–203.

Moller DR, Gallagher JS, Benstein DI, Wilcox TG, Burroughs HE, & Bernstein IL (1985) Detection of IgE mediated respiratory sensitisation in workers exposed to hexahydrophthalic anhydride. J Allergy Clin Immunol, **75**: 663–672.

Monick M, Glazier J, & Hunninghake GW (1987) Human alveolar macrophages suppress interleukin 1 activity via the secretion of prostaglandin E2. Am Rev Respir Dis, **135**: 72–77.

Montgomery-Smith J (1983) Epidemiology and natural history of asthma, allergic rhinitis and atopic dermatitis (eczema). In: Allergy: Principles and practice. St Louis, Missouri, C.V. Mosby & Co., pp 771–803.

Montgomery-Smith J, Middleton E, Reed CE, Ellis EF, Adkinson NF, & Yunginger JW ed. (1983) Allergy: Principles and practice — Epidemiology and natural history of asthma, allergic rhinitis and atopic dermatitis (eczema). St Louis, Missouri, C.V. Mosby & Co., pp 771–803.

Moore SA, Strieter RM, Rolfe MW, Standiford TJ, Burdick MD, & Kunkel SL (1992) Expression and regulation of human alveolar macrophage-derived interleukin-1 receptor antagonist. Am J Respir Cell Mol Biol, **6**(6): 569–575.

Morel E, Feuillet-Fieux MN, Vernet-der Garabedian B, Raimond F, D'Anglejan J, Bataille R, Sany J, & Bach JF (1991) Autoantibodies in D-penicillamine-induced myasthenia gravis: a comparison with idiopathic myasthenia and rheumatoid arthritis. Clin Immunol Immunopathol, **58**: 318–330.

Morgenstern H & Thomas D (1993) Principles of study design in environmental epidemiology. Environ Health Perspect, **101**(suppl 4): 23–28.

Morren MA, Przybilla B, Bamelis M, Heykants B, Reynaers A, & Degreef H (1994) Atopic dermatitis: triggering factors. J Am Acad Dermatol, **31**: 467–473.

Mosimann B, Peitrequin R, Blanc C, & Pecoud A (1992) Allergie aux blattes (cafards) dans une population suisse souffrant d'asthme et de rhinite chroniques. Schweiz Med Wochenschr, **122**(34): 1245–1248.

Mosmann TR & Sad S (1996) The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today, **17**: 138–146.

Moulon C, Vollmer J, & Weltzien HU (1995) Characterization of processing requirements and metal cross-reactivities in T cell clones from patients with allergic contact dermatitis to nickel. Eur J Immunol, **25**: 3308–3315.

Mowatt AMcI (1984) The immunopathogenesis of food-sensitive enteropathies. In: Newly TJ & Stokes CR ed. Local immune responses of the gut. Boca Raton, Florida, CRC Press, pp 199–225.

Mowatt AMcI (1987) The regulation of immune responses to dietary protein antigens. Immunol Today, **8**: 93–98.

Mowatt AMcI, Lamont AG, Strobel S, & Mackenzie S (1986) The role of antigen processing and suppressor T cells in immune responses to dietary proteins in mice. Adv Exp Med Biol, **216A**: 709–720.

M'Raihi L, Charpin D, Pons A, Bongrande P, & Vervloet D (1991) Cross-reactivity between latex and banana. J Allergy Clin Immunol, **87**: 129–130.

Mullan RJ & Murthy LI (1991) Occupational sentinel health events: An up-dated list for physician recognition and public health surveillance. Am J Ind Med, **19**(6): 775–799.

Mullick FG, McAllister HA, Wagner BM, & Fenoglio JJ (1979) Drug related vasculitis: Clinicopathologic correlations in 30 patients. Hum Pathol, **10**: 313–325.

Munir AK, Einarsson R, Schou C, & Dreborg SK (1993) Allergens in school dust: I. The amount of the major cat (Fel d I) and dog (Can f I) allergens in dust from Swedish schools is high enough to probably cause perennial symptoms in most children with asthma who are sensitized to cat and dog. J Allergy Clin Immunol, **91**(5): 1067–1074.

Munir AK, Einarsson R ,& Dreborg SK (1994a) Indirect contact with pets can confound the effect of cleaning procedures for reduction of animal allergen levels in house dust. Pediatr Allergy Immunol, **5**(1): 32–39.

Munir AK, Bjorksten B, Einarsson R, Schou C, Ekstrand Tobin A, Warner A, & Kjellman NI (1994b) Cat (Fel d I), dog (Can f I), and cockroach allergens in homes of asthmatic children from three climatic zones in Sweden. Allergy, **49**(7): 508–516.

Muraille E & Leo O (1998) Revisiting the Th1/Th2 paradigm. Scand J Immunol, **47**: 1–9.

Muranaka M, Suzuki S, Koizumi K, Takafuji S, Miyamoto T, Ikemori R, & Tokiwa H (1986) Adjuvant activity of diesel-exhaust particulates for the production of IgE antibody in mice. J Allergy Clin Immunol, **77**: 616–623.

Mygind N (1986) Essential allergy, 1st ed. Oxford, London, Boston, Blackwell Scientific Publications, 480 pp.

Mygind N (1989) Nasal allergy, 2nd ed. Oxford, London, Boston, Blackwell Scientific Publications, pp 219–223.

Nagata S, Yamashiro Y, Ohtuska Y, Shioya T, Oguchi S, Shimizu T, & Maeta M (1995) Quantitative analysis and immunohistochemical studies on small intestinal mucosa of food-sensitive enteropathy. J Pediatr Gastroenterol Nutr, **20**: 44–48.

Naparstek Y & Plotz PH (1993) The role of autoantibodies in autoimmune disease. Annu Rev Immunol, **11**: 79–104.

NAS (National Academy of Science) (1983) Committee on the Institutional Means for Assessment of Risks to Public Health — Risk assessment in the Federal Government: Managing the process. Washington, DC, National Academy Press, pp 1–50.

NAS (National Academy of Science) (1993) Committee on Risk Assessment Methodology, Board on Environmental Studies and Toxicology — Issues in risk assessment. Washington, DC, National Academy Press, 356 pp.

Neas LM, Dockery DW, Ware JH, Spengler JD, Speizer FE, & Ferris BG Jr (1991) Association of indoor nitrogen dioxide with respiratory symptoms and pulmonary function in children. Am J Epidemiol, **134**(2): 204–219.

Nelson HS (1985) The atopic diseases. Ann Allergy, **55**: 441–447.

Nelson HS, Rosloniec DM, McCall LI, & Ikle D (1993) Comparative performance of five commercial prick skin test devices. J Allergy Clin Immunol, **92**(5): 750–756.

Nemery B, Casier P, Roosels D, Lahaye D, & Demedts M (1992) Survey of cobalt exposure and respiratory health in diamond polishers. Am Rev Respir Dis, **145**(3): 610–616.

Newly TJ, Stokes CR, & Bourne FJ (1980) Effects of feeding bacterial lipopolysaccharide and dectran sulphate on the development of oral tolerance to contact sensitizing agents. Immunology, **41**: 617–621.

Newman LS & Kreiss K (1992) Nonoccupational beryllium disease masquerading as sarcoidosis: identification by blood lymphocyte proliferative response to beryllium. Am Rev Resp Dis, **145**: 1212–1214.

Newman LS, Bobka C, Schumacher B, Daniloff E, Zhen B, Mroz MM, & King TE Jr (1994) Compartmentalized immune response reflects clinical severity of beryllium disease. Am J Resp Crit Care Med, **150**: 135–142.

Newman Taylor AJ (1988) Occupational asthma. Postgrad Med J, **64**: 505–510.

Newman Taylor AJ (1995) Environmental determinants of asthma. Lancet, **345**(8945): 296–299.

Newman Taylor AJ, Venables KM, Durham S, Graneek BJ, & Topping MD (1987) Acid anhydrides and asthma. Int Arch Allergy Appl Immunol, **82**: 435–439.

Ng TP & Tan WC (1994a) Epidemiology of chronic (perennial) rhinitis in Singapore: prevalence estimates, demographic variation and clinical allergic presentation. Ann Acad Med Singap, **23**: 83–88.

Ng TP & Tan WC (1994b) Epidemiology of allergic rhinitis and its associated risk factors in Singapore. Int J Epidemiol, **23**(3): 553–558.

Niebel G (1995) [Behavioural medicine of chronic skin disease.] Bern, Göttingen, Toronto, Seattle, Hogrefe & Huber Publishers.

Nielsen NH & Menné T (1992) Allergic contact sensitization in an unselected Danish population — the Glostrup allergy study, Denmark. Acta Dermatol Venereol, **72**: 456–460.

Nielsen NH & Menné T (1993) Nickel sensitization and ear piercing in an unselected Danish population — The Glostrup allergy study, Denmark. Contact Dermatitis, **29**: 16–21.

Niermann H (1964) [Dermatology in twins.] Berlin, Heidelberg, New York, Springer Verlag, 108 pp.

Niestijl Jansen JJ, Kardinaal AFM, Huijbers G, Vlieg-Boerstra BJ, Martens BPM, & Ockhuizen T (1994) Prevalence of food allergy and intolerance in the adult Dutch population. J Allergy Clin Immunol, **93**: 446–456.

NIH (National Institutes of Health) (1991) National Asthma Education Program Expert Panel — Guidelines for the diagnosis and management of asthma. Bethesda, Maryland, National Heart, Lung and Blood Institute, 72 pp (NIH Publication No. 91-3042A).

Nilsson B & Kristofferson A (1989) Zimelidine: Febrile reaction and peripheral neuropathy. In: Kammüller ME, Bloksma N, & Seinen W ed. Autoimmunity and toxicology. Amsterdam, Oxford, New York, Elsevier Science Publishers, pp 183–214.

Ninan TK & Russell G (1992) Respiratory symptoms and atopy in Aberdeen schoolchildren: Evidence from two surveys 25 years apart. Br Med J, **304**: 873–875.

NIOSH (1997) NIOSH alert: Preventing allergic reactions to natural rubber latex in the workplace. Cincinnati, Ohio, National Institute for Occupational Safety and Health, 11 pp.

Nitta H, Sato T, Nakai S, Maeda K, Aoki S, & Ono M (1993) Respiratory health associated with exposure to automobile exhaust. I. Results of cross-sectional studies in 1979, 1982, and 1983. Arch Environ Health, **48**: 53–58.

Noelle RJ, Daum J, Bartlett WC, McCann J, & Shepherd DM (1990) Cognate interactions between helper T-cells and B-cells. V. Reconstitution of helper T-cell function using purified plasma membranes from activated Th1 and Th2 helper T-cells and lymphokines. J Immunol, **146**(4): 1118–1124.

Norback D, Bjornsson E, Janson C, Widstrom J, & Boman G (1995) Asthmatic symptoms and volatile organic compounds, formaldehyde, and carbon dioxide in dwellings. Occup Environ Med, **52**(6): 388–395.

Nordman H, Keskinen H, & Tuppurainen M (1985) Formaldehyde asthma — rare or overlooked? J Allergy Clin Immunol, **75**: 91–99.

Nussler AK & Billiar TR (1993) Inflammation, immunoregulation, and inducible nitric oxide synthase. J Leuk Biol, **54**(2): 171–178.

Nyren O, Yin L, Josefsson S, McLaughlin JK, Blot WJ, Engqvist M, Hakelius L, Boice JD Jr, & Adami HO (1998) Risk of connective tissue disease and related disorders among women with breast implants: a nation-wide retrospective cohort study in Sweden. Br Med J, **316**(7129): 417–422.

O'Brien IM, Harries MG, Burge PS, & Pepys J (1979) Toluene di-isocyanate induced asthma. Reactions to TDI, MDI, HDI and histamine. Clin Allergy, **19**: 1–6.

O'Byrne PM, Dolovich J, & Hargreave FE (1987) Late asthmatic responses. Am Rev Respir Dis, **134**: 740–751.

Odom RB & Maibach HI (1976) Contact urticaria: a different contact dermatitis. Cutis, **18**(5): 672–676.

O'Donnell TV (1995) Asthma and respiratory problems — a review. Sci Total Environ, **163**(1–3): 137–145.

OECD (1992) Guideline 406 for testing chemicals. Paris, Organisation for Economic Cooperation and Development.

Oettigen HC, Martin TR, Wynshaw-Boris A, Deng C, Drazen JM, & Leder P (1994) Active anaphylaxis in IgE-deficient mice. Nature (Lond), **370**: 367–370.

Ogawa M, Berger PA, McIntyre OR, & Clendenning WE (1971) IgE in atopic dermatitis. Arch Dermatol, **103**: 575.

Ohashi PS, Oehnen S, Buerki K, Pircher H, Ohashi CT, Odermatt B, Malissen B, Zinkernagel RM, & Hengartner H (1991) Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. Cell, **65**: 305–317.

Ollerenshaw S & Woolcock AJ (1992) Characteristic of inflammation in biopsies from large airways of subjects with asthma and subjects with chronic airflow limitation. Am Rev Respir Dis, **145**: 922–927.

Ollier S & Davies RJ (1994) A report of the relationship between allergen load, primary immunologic sensitization and the expression of allergic disease. Respir Med, **88**: 407–415.

Oostendorp RAJ, Meijer CJLM, & Scheper RJ (1993) Immunosuppression by retroviral-envelope-related peptides, and their role in non-retroviral human disease. Crit Rev Oncol Hematol, **14**: 189–206.

Oosterlee A, Drijver M, Lebret E, & Brunekreef B (1996) Chronic respiratory symptoms in children and adults living along streets with high traffic density. Occup Environ Med, **53**: 241–247.

Ortolani C, Ispano M, Pastorello EA, Ansaloni R, & Magri GC (1989) Comparison of results of skin prick tests (with fresh foods and commercial food extracts) and RAST in 100 patients with oral allergy syndrome. J Allergy Clin Immunol, **83**: 683–690.

Osebold JW, Gershwin LJ, & Zee YC (1980) Studies on the enhancement of allergic lung sensitization by inhalation of ozone and sulfuric acid aerosol. J Environ Pathol Toxicol, **3**: 221–234.

Osterland CK (1994) Laboratory diagnosis and monitoring in chronic systemic autoimmune diseases. Clin Chem, **40**(11B): 2146–2153.

Ostro BD, Lipsett MJ, Wiener MB, & Selner JC (1991) Asthmatic responses to airborne acid aerosols. Am J Public Health, **81**(6): 694–702.

Oswald IP, Gazzinelli RT, Sher A, & James SL (1992) IL-10 synergizes with IL-4 and transforming growth factor-ß to inhibit macrophage cytotoxic activity. J Immunol, **148**(11): 3578–3582.

Paggiaro P, Bacci E, Paoletti P, Bernard P, Dente FL, Marchetti G, Talini D, Menconi GF, & Giuntini C (1990) Bronchoalveolar lavage and morphology of the airways after cessation of exposure in asthmatic subjects sensitised to toluene di-isocyanate. Chest, **98**(3): 536–542.

Park HS, Yu HJ, & Jung KS (1994) Occupational asthma caused by chromium. Clin Exp Allergy, **24**: 676–681.

Parker D, Sommer G, & Turk JL (1975) Variation in guinea pig responsiveness. Cell Immunol, **18**: 233–238.

Parronchi P, Macchia D, Piccini M-P, Biswas P, Simonelli C, Maggi E, Ricci M, Ansari A, & Romagnani S (1991) Allergen-and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. Proc Natl Acad Sci (USA), **88**: 4538–4542.

Pastorello E, Stocchi L, Bigi A, Pravettoni V, Schilke ML, Valente D, & Zanussi C (1989) Value and limits of diagnostic tests in food hypersensitivity. Allergy, **44**(suppl): 151–158.

Pastorello EA, Incorvaia C, & Ortolani C (1995) The mouth and pharynx. In: Ortolani C ed. Atlas of mechanisms in adverse reactions to food. Allergy, **50**(20): 41–44.

Pauli G, de Blay F, Bessort JC, & Dietermann A (1992) The association between respiratory allergies and food hypersensitivities. ACI News, **4**: 43–47.

Pauluhn J (1996) Predictive testing for respiratory sensitisation. Toxicol Lett, **86**(2–3): 177–185.

Pauluhn J & Eben A (1991) Validation of a non-invasive technique to assess immediate or delayed onset of airway hypersensitivity in guinea-pigs. J Appl Toxicol, **11**: 423–431.

Pauluhn J & Mohr U (1994) Assessment of respiratory hypersensitivity in guinea pigs sensitized to diphenylmethane-4,4-diisocyanate (MDI) and challenged with MDI acetylcholine or MDI albumin conjugate. Toxicology, **92**: 53–74.

Payne MP & Walsh PT (1994) Structure-activity relationships for skin sensitization potential: development of structural alerts for use in knowledge-based toxicity prediction systems. J Chem Inf Comput Sci, **34**(1): 154–161.

PEACE(1998) Study of pollution effects on asthmatic children in Europe (PEACE). Eur Respir Rev, **8**: 1–130.

Pearce N, Weiland S, Keil U, Langridge P, Anderson HR, Strachan D, Bauman A, Young L, Gluyas P, Ruffin D, Crane J, & Beasley R (1993) Self-reported prevalence of asthma symptoms in children in Australia, England, Germany and New Zealand: an international comparison using the ISAAC protocol. Eur Respir J, **6**: 1455–1461.

Peat JK (1996) Prevention of asthma. Eur Respir J, **9**: 1545–1555.

Peat J., Woolcock A, Leeder SR, & Blackburn CR (1980) Asthma and bronchitis in Sydney schoolchildren: II. The effect of social factors and smoking on prevalence. Am J Epidemiol, **111**(6): 728–735.

Peat J., van den Berg RH, Green WF, Mellis CM, Leeder SR & Woolcock A (1994) Changing prevalence of asthma in Australian children. Br Med J, **308**: 1591–1596.

Peden DB, Carter J, Dailey LA, & Devlin R (1994) The effect of ozone on house dust mite allergen-induced nasal inflammation in asthmatics. Am J Respir Crit Care Med, **149**: A154 (abstract).

Pelletier L, Castedo M, Bellon B, & Druet P (1994) Mercury and autoimmunity. In: Dean JH, Luster MI, Munson AE, & Kimber I ed. Immunotoxicology and immunopharmacology, 2nd ed. New York, Raven Press, pp 539–552.

Pepys J (1994) "Atopy": a study in definition. Allergy, **49**(6): 397–399.

Pepys J, Pickering CAC, & Hughes EG (1972) Asthma due to inhaled chemical agents — complex salts of platinum. Clin Allergy, **2**: 391–396.

Peters A, Dockery DW, Heinrich J, & Watchman HE (1997) Short-term effects of particulate air pollution on respiratory morbidity in asthmatic children. Eur Resp J, **10(4)**: 872–879.

Pfeiffer C, Murray J, Madri J, & Bottomly K (1991) Selective activation of Th1- and Th2-like cells *in vivo*: response to human collagen IV. Immunol Rev, **123**: 65–84.

Philen RM & Posada M (1993) Toxic oil syndrome and eosinophilia-myalgia syndrome: May 8–10, 1991, World Health Organization Meeting report. Semin Arthritis Rheum, **23**: 104–124.

Picker LJ, Treer JR, Ferguson-Darnell B, Collins PA, Bergstresser PR, & Terstappen LWMM (1993) Control of lymphocyte recirculation in man: II. Differential regulation of the cutaneous lymphocyte associated antigen, a tissue-selective homing receptor for skin-homing T cells. J Immunol, **150**: 1122–1136.

Pinkston P, Bitterman PB, & Crystal RG (1984) Interleukin-2 in the alveolitis of beryllium-induced lung disease. Am Rev Respir Dis, **129(411)**: A161 (abstract).

Pistoor FAM., Kapsenberg ML, Bos JD, Meinardi MMHM, von Blomberg BME, & Scheper RJ (1995) Cross-reactivity of human nickel-reactive T lymphocyte clones with copper and palladium. J Invest Dermatol, **105**: 92–95.

Planchon SM, Martins CAP, Guerrant RL, & Roche J. (1994) Regulation of intestinal epithelial barrier function by TGF-ß1. J Immunol, **153**: 5730–5739.

Platts-Mills TA (1994) How environment affects patients with allergic disease: indoor allergens and asthma. Ann Allergy, **72**: 381–384.

Platts-Mills TA & Chapman MD (1987) Dust mites: immunology, allergic disease, and environmental control. J Allergy Clin Immunol, **80**: 755–775.

Platts-Mills TAE, Chapman MD, Mitchell B, Heymann PW, & Deuell B (1991) Role of inhalant allergens in atopic eczema. In: Ruzicka T, Ring J, & Przybilla B ed. Handbook of atopic eczema. Berlin, Heidelberg, New York, Springer Verlag, pp 192–203.

Platt-Mills TA, Sporik RB, Chapman MD, & Heymann PW (1995) The role of indoor allergens in asthma. Allergy, **50**(suppl 22): 5–12.

Podmore P (1995) Shoes. In: Rycroft RJG, Menné T, Frosch PJ, & Benezra C ed. Textbook of contact dermatitis, 2nd ed. Berlin, Heidelberg, New York, Springer Verlag, pp 516–527.

Polak L (1980) Immunological aspects of contact sensitivity: An experimental study. Monogr Allergy, **15**: 1–170.

Pollart SM, Chapman MD, Fiocco GP, Rose G, & Platts-Mills TA (1989) Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. J Allergy Clin Immunol, **83**: 875–882.

Pope CA III, Schwarz J, & Ransom MR (1992) Daily mortality and PM10 pollution in Utah Valley. Arch Environ Health, **47**: 211–217.

Pope CA III, Bates DV, & Raizenne ME (1995) Health effects of particulate air pollution: Time for reassessment. Environ Health Perspect, **103**(5): 472–480.

Posselt AM, Barker CF, Friedman AL, & Naji A (1992) Prevention of autoimmune diabetes in the BB rat by intrathymic islet transplantation at birth. Science, **256**: 1321–1324.

Potter DW & Wederbrand KS (1995) Total IgE antibody in BALB/c mice after dermal exposure to chemicals. Fundam Appl Toxicol, **26**: 127–135.

Prausnitz C & Kustner H (1921) [Studies concerning sensitivity.] Zent. bl Bakteriol I Orig, **86**: 160–169

Prentice RL & Thomas D (1993) Methodologic research needs in environmental epidemiology: Data analysis. Environ Health Perspect, **101**(suppl 4): 39–48.

Prud'Homme GJ, Parfrey NA, & Vanier LE (1991) Cyclosporine-induced autoimmunity and immune hyperreactivity. Autoimmunity, **9**: 345–356.

Przybilla B & Ring J (1990) Food allergy and atopic eczema. Semin Dermatol, **9**: 220–225.

Przybilla B, Eberlein-König B, & Rueff F (1994) Practical management of atopic eczema. Lancet, **343**: 1342–1346.

Punnonen J & de Vries JE (1994) IL-13 induces proliferation, Ig isotype switching, and Ig synthesis by immature human fetal B cells. J Immunol, **152**: 1094–1102.

Punnonen J, Aversa G, Cocks BG, McKenzie AN, Menon S, Zurawski G, de Waal Malefyt R, & de Vries JE (1993) Interleukin-13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. Proc Natl Acad Sci (USA), **90**(8): 3730–3734.

Raaschou Nielsen O, Nielsen ML, & Gehl J (1995) Traffic-related air pollution: exposure and health effects in Copenhagen street cleaners and cemetery workers. Arch Environ Health, **50**(3): 207–213.

Radl J, Valentijn RM, Haaijman JJ, & Paul LC (1985) Monoclonal gamma-pathies in patients undergoing immunosuppressive treatment after renal transplantation. Clin Immunol Immunopathol, **37**: 98–102.

Rajka G (1990) Essential aspects of atopic eczema. Berlin, Heidelberg, New York, Springer Verlag, 261 pp.

Rastogi SK, Gupta BN, Husain T, Mathur N, Pangtey BS, & Garg N (1992) Respiratory symptoms and ventilatory capacity in metal polishers. Hum Exp Toxicol, **11**(6): 466–472.

Rattray NJ, Botham PA, Hext PM, Woodcock DR, Fielding I, Dearman RJ, & Kimber I (1994) Induction of respiratory hypersensitivity to diphenylmethane-4,4-diisocyanate in guinea pigs. Influence of route of exposure. Toxicology, **88**: 15–30.

Raulf M & Konig W (1991) Modulation of leukotriene generation from human polymorphonuclear granulocytes by polychlorinated biphenyls (PCB). Immunology, **73(4)**: 485–490.

Ravetch JV (1994) Fc receptors: rubor redux. Cell, **78**: 553–560

Ravetch JV (1997) Fc receptors. Curr Opin Immunol, **9**: 121–125.

Rea TH (1979) Quantitative enhancement of dinitrochlorobenzene responsivity in women receiving oral contraceptives. Arch Dermatol, **115**(3): 361–362.

Reijula K & Patterson R (1994) Occupational allergies in Finland in 1981–91. Allergy Proc, **15**(3): 163–168.

Reitamo S, Visa K, Kahonen K, Kayhko K, Stubb S, & Salo OP (1986) Eczematous reactions in atopic patients caused by epicutaneous testing with inhalant allergens. Br J Dermatol, **114**: 303–309.

Renstrom A, Malmberg P, Larsson K, Sundblad BM, & Larsson PH (1994) Prospective study of laboratory-animal allergy: factors predisposing to sensitization and development of allergic symptoms. Allergy, **49**(7): 548–552.

Richardson BC, Liebling MR, & Hudson JL (1990) CD4+ cells treated with DNA methylation inhibitors induce autologous B cell differentiation. Clin Immunol Immunopathol, **55**: 368–381.

Richardson B, Powers D, Hooper F, Yung RL, & O'Rourke K (1994) Lymphocyte function-associated antigen 1 overexpression and T cell autoreactivity. Arthritis Rheum, **37**: 1363–1372.

Ridings JE, Barrat MD, Cary R, Earnshaw CG, Eggington CE, Ellis MK, Judson PN, Langowski JJ, Marchant CA, Payne MP, Watson WP, & Yih TD (1996) Computer prediction of possible toxic action from chemical structure: an update on the DEREK system. Toxicology, **106**: 267–279.

Riedel F, Kramer M, Scheibenbogen C, & Rieger CH (1988) Effects of SO_2 exposure on allergic sensitization in the guinea pig. J Allergy Clin Immunol, **82**: 527–534.

Riedler J, Reade T, Dalton M, Holst D, & Robertson C (1994) Hypertonic saline challenge in an epidemiologic survey of asthma in children. Am J Respir Crit Care Med, **150**: 1632–1639.

Ring J (1988) [Applied allergology], 2nd ed. Munich, MMW Medizin Verlag GmbH, pp 85–86 (in German).

Ring J (1991) Atopy: Conditions, disease or syndrome? In: Ruzicka T, Ring J, & Przybilla B ed. Handbook of atopic eczema. Berlin, Heidelberg, New York, Springer Verlag, pp 3–8.

Ring J & Thomas P (1989) Histamine and atopic eczema. Acta Dermatol Venereol, **144**: 70–77.

Ring J, Sedlmeier F, von der Helm D, Mayr T, Walz U, Ibel H, Przybilla B, Reimann HJ, & Dorsch W (1988) Histamine and allergic disease. In: Ring J & Burg G ed. New trends in allergy. Berlin, Heidelberg, New York, Springer Verlag, pp 44–77.

Ring J, Bieber T, Vieluf D, Kunz B, & Przybilla B (1991a) Atopic eczema, Langerhans cells and allergy. Int Arch Allergy Immunol, **94**: 194–201.

Ring J, Ruzicka T, & Przybilla B (1991b) The pathophysiology of atopic eczema. In: Ruzicka T, Ring J, & Przybilla B ed. Handbook of atopic eczema. Berlin, Heidelberg, New York, Springer Verlag, pp 330–335.

Ring J, Abeck D, & Neuber K (1992) Atopic eczema: role of microorganisms on the skin surface. Allergy, **47**: 265–269.

Ring J, Behrendt H, Schäfer T, Vieluf D, & Krämer U (1995) Impact of air pollution on allergic diseases: Clinical and epidemiologic studies. In: Johansson SGO ed. Progress in allergy and clinical immunology. Bern, Göttingen, Toronto, Seattle, Hogrefe & Huber Publishers, vol 3, pp 174–179.

Ring J, Brockow K, & Abeck D (1996) The therapeutic concept of patient management in atopic eczema. Allergy, **51**: 206–215.

Ritz HL, Conner DS, & Sauter ED (1975) Contact sensitization of guinea-pigs with unsaturated and halogenated sultones. Contact Dermatitis, **1**: 349–359.

Ritz HL, Evans BL, Bruce RD, Fletcher ER, Fisher GL, & Sarlo K (1993) Respiratory and immunological responses of guinea pigs to enzyme-containing detergents: A comparison of intratracheal and inhalation modes of exposure. Fundam Appl Toxicol, **21**: 31–37.

Roberts DW & Basketter DA (1990a) A quantitative structure activity/dose relationship for contact allergenic potential of alkyl group transfer agents. Toxicology *in vitro*, **4**(4/5): 686–687.

Roberts DW & Basketter DA (1990b) A quantitative structure activity/dose response relationship for contact allergic potential of alkyl group transfer agents. Contact Dermatitis, **23**: 331–335.

Roberts SA, Reinhardt MC, Paganelli R, & Levinsky RJ (1981) Specific antigen exclusion and non-specific facilitation of antigen entry across the gut in rats allergic to food proteins. Clin Exp Immunol, **45**: 131–136.

Robertson CF, Heycock E, Bishop J, Nolan T, Olinsky A, & Phelan PD (1991) Prevalence of asthma in Melbourne schoolchildren: Changes over 26 years. Br Med J, **302**: 1116–1118.

Robinson DS (1998) T cell co-stimulation: a potential therapeutic target in asthma? Clin Exp Allergy, **28**: 788–790.

Roche WR, Beasley R, Williams JH, & Holgate ST (1989) Subepithelial fibrosis in the bronchi of asthmatics. Lancet, **1**: 520–523.

Röcken M, Racke M, & Shevach EM (1996) IL-4-induced immune deviation as antigen-specific therapy for inflammatory autoimmune disease. Immunol Today, **17**: 225–231.

Roed-Petersen J (1989) A new glove material protective against epoxy and acrylate monomer. In: Frosch PJ, Dooms-Goossens A, Lachapelle J-M, Rycroft RJG, & Scheper RJ ed. Current topics in contact dermatitis. Berlin, Heidelberg, New York, Springer Verlag, pp 603–606.

Roitt IM, Brostoff J, & Male DK ed. (1998) Immunology, 5 th ed. St. Louis, Missouri, C.V. Mosby & Co., 424 pp.

Romagnani S (1991) Human Th_1 and Th_2 subsets: doubt no more. Immmunol Today, **12**(8): 256–257.

Romagnani S (1992a) Induction of Th1 and Th2 responses: A key role for the "natural" immune response? Immunol Today, **13**(10): 379–381.

Romagnani S (1992b) Human TH1 and TH2 subsets: Regulation of differentiation and role in protection and immunopathology. Int Arch Allergy Immunol, **98**: 279–285.

Romanski B (1987) The pathology of food allergy studied by gastric allergen challenge. In: Brostoff J & Challacombe SJ ed. Food allergy and intolerance. London, Baillière Tindall, pp 917–931.

Romanski B (1989) The pathology of food allergy studied by gastric allergen challenge. In: Paul WE ed. Fundamental immunology. New York, Raven Press, p 41.

Rose G (1985) Sick individuals and sick populations. Int J Epidemiol, **14**: 32–38.

Rose NR & Potter M (1995) The silicone controversy: towards a resolution. Immunol Today, **16**: 459–460.

Rosen FS (1987) Autoimmunity and immunodeficiency disease. In: Evered D & Whelan J ed. Autoimmunity and autoimmune disease. New York, Chichester, Brisbane, Toronto, John Wiley & Sons, pp 135–148 (Ciba Foundation Symposium No. 129).

Rosenstreich DL, Eggleston P, Kattan M, Baker D, Slavin RG, Gergen P, Mitchell H, McNiff-Mortimer K, Lynn H, Ownby D, & Malveaux F (1997) The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. N Engl J Med, **336**(19): 1356–1363.

Ross B, McCullough J, & Ownby DR (1991) Evidence of allergenic cross-reactivity between banana and watermelon. J Allergy Clin Immunol, **87**: 274 (abstract).

Rossman MD, Kern JA, Elias JA, Cullen MR, Epsein PE, Preuss OP, Markham TN, & Daniele RP (1988) Proliferative response of bronchoalveolar lymphocytes to beryllium. Ann Intern Med, **108**: 687–693.

Roth MD & Golub SH (1993) Human pulmonary macrophages utilize prostaglandins and transforming growth factor beta 1 to suppress lymphocyte activation. J Leuk Biol, **53**(4): 366–371.

Rothman KJ (1993) Methodologic frontiers in environmental epidemiology. Environ Health Perspect, **101**(suppl 4): 19–21.

Rubin RL (1989) Autoimmune reactions induced by procainamide and hydralazine. In: Kammüller M, Bloksma N, & Seinen W ed. Autoimmunity and toxicology: Immune disregulation induced by drugs and chemicals. Amsterdam, Oxford, New York, Elsevier Science Publishers, pp 119–150.

Rubin RL, Burlingame RW, Arnott JE, Totoritis MC, McNally EM, & Johnson AD (1995) IgG but not other classes of anti-[(H2A-H2B)-DNA] is an early sign of procainamide-induced lupus. J Immunol, **154**: 2483–2493.

Ruffin J, Liu MYG, Sessions R, Banerjee S, & Banerjee UC (1986) Effects of certain atmospheric pollutants (SO_2, NO_2, CO) on the soluble amino acids, molecular weight and antigenicity of some airborne pollen grains. Cytobios, **46**: 119–129.

Ruzicka T, Ring J, & Przybilla B ed. (1991) Handbook of atopic eczema. Berlin, Heidelberg, New York, Springer Verlag, 481 pp.

Rycroft RJG (1995) Occupational contact dermatitis. In: Rycroft RJG, Menné T, Frosch PJ, & Benezra C ed. Textbook of contact dermatitis, 2nd ed. Berlin, Heidelberg, New York, Springer Verlag, pp 343–400.

Rycroft RJG, Menné T, Frosch PJ, & Benezra C ed. (1995) Textbook of contact dermatitis, 2nd ed. Berlin, Heidelberg, New York, Springer Verlag, 400 pp.

Rystedt I (1985) Atopic background in patients with occupational hand eczema. Contact Dermatitis, **12**(5): 247–254.

Saarinen UM & Kajosaari M (1995) Breastfeeding as prophylaxis against atopic disease: Prospective follow-up study until 17 years old. Lancet, **346**(8982): 1065–1069.

Saihan EM, Burton JL, & Heaton KW (1978) A new syndrome with pigmentation, scleroderma, gynaecomastia, Raynaud's phenomenon and peripheral neuropathy. Br J Dermatol, **99**: 437–440.

Saint-Remy J-MR (1997) Epitope mapping, a new method for biological evaluation and immunotoxicology. Toxicology, **119**: 77–81.

Sakaguchi S & Sakaguchi N (1989) Organ-specific autoimmune disease induced in mice by elimination of T cell subsets: V. Neonatal administration of cyclosporine A causes autoimmune disease. J Immunol, **142**: 471–480.

Sakaguchi S & Sakaguchi N (1990) Thymus and autoimmunity: capacity of the normal thymus to produce pathogenic self-reactive T cells and conditions required for their induction of autoimmune disease. J Exp Med, **172**: 537–545.

Salome CM, Peat J., Britton WJ, & Woolcock A (1987) Bronchial hyperresponsiveness in two populations of Australian schoolchildren: I. Relation to respiratory symptoms and diagnosed asthma. Clin Allergy, **17**: 271–281.

Saltini C, Winestock K, Kirby M, Pinkston P, & Crysal RG (1989) Maintenance of the alveolitis in patients with chronic beryllium disease by beryllium-specific helper T-cells. N Engl J Med, **320**: 1103–1109.

Samet JM, Lambert WE, Skipper BJ, Cushing AH, Hunt WC, Young SA, McLaren LC, Schwab M, & Spengler JD (1993) Nitrogen dioxide and respiratory illnesses in infants. Am Rev Respir Dis, **148**: 1258–1265.

Sampson HA (1982) The immunopathogenic role of food hypersensitivity in atopic dermatitis. Acta Dermatol Venereol, **176**(suppl): 34–37.

Sampson HA (1983) Role of immediate food hypersensitivity in the pathogenesis of atopic dermatitis. J Allergy Clin Immunol, **71**: 473–480.

Sampson HA & Albergo R (1984) Comparison of results of skin tests, RAST, and double-blind, placebo-controlled food challenges in children with atopic dermatitis. J Allergy Clin Immunol, **74**: 26–33.

Sampson HA & Metcalfe DD (1992) Food allergies. J Am Med Assoc, **268**(20): 2840–2844.

Sanchez-Guerrero J, Schur PH, Sergent JS, & Liang MH (1994) Silicone breast implants and rheumatic diseases. Arthritis Rheum, **37**: 158–168.

Sanchez-Guerrero J, Colditz GA, Karlson EW, Hunter DJ, Speizer FE, & Liang MH (1995) Silicone breast implants and the risk of connective-tissue diseases and symptoms. N Engl J Med, **332**(25): 1666–1670.

Sanderson CJ (1990) Eosinophil differentiation factor (interleukin-5). Immunol Ser, **49**: 231–256.

Sanderson DM & Earnshaw CG (1991) Computer prediction of possible toxic action from chemical structure; The DEREK system. Hum Exp Toxicol, **10**: 261–273.

Sandford A, Shirakawa T, Moffatt MF, Daniels SE, Ra C, Faux JA, Young RP, Nakamura Y, Lathrop GM, Cookson WOCM, & Hopkin JM (1993) Localization of atopy and ß subunit of high-affinity IgE receptor (FcεR1) on chromosome 11q. Lancet, **341**: 332–334.

Sarlo K & Clark ED (1992) A tier approach for evaluating the respiratory allergenicity of low molecular weight chemicals. Fundam Appl Toxicol, **18**: 107–114.

Sarlo K & Karol MH (1994) Guinea pig predictive tests for respiratory allergy. In: Dean JH, Luster MI, Munson AE, & Kimber I ed. Immunotoxicology and immunopharmacology, 2nd ed. New York, Raven Press, pp 703–720.

Sarlo K, Ritz HL, Fletcher ER, Schrotel KR, & Clark ED (1997) Proteolytic detergent enzymes enhance the allergic antibody responses of guinea pigs to nonproteolytic detergent enzymes in a mixture: implications for occupational exposure. J Allergy Clin Immunol, **100**: 480–487.

Satoh T, Kramarik JA, Tollerud DJ, & Karol MH (1995) A murine model for assessing the respiratory hypersensitivity potential of chemical allergens. Toxicol Lett, **78**: 57–66.

Saval P, Fuglsang G, Madsen C, & Østerballe O (1993) Prevalence of atopic disease among Danish school children. J Pediatr Allergy Immunol, **4**: 117–122.

Savilahti E & Kuitunen M (1992) Allergenicity of cow milk proteins. J Pediatr, **121**: S12–S20.

Savonius B, Keskinen H, Tuppurainen M, & Kanerva L (1993) Occupational respiratory disease caused by acrylates. Clin Exp Allergy, **23**(5): 416–424.

Schachter J & Higgins MW (1976) Median age at onset of asthma and allergic rhinitis in Tecumseh, Michigan. J Allergy Clin Immunol, **57**(4): 342–351.

Schäfer T & Ring J (1997) Epidemiology of allergic diseases. Allergy, **52**(suppl 38): 14–22.

Schäfer T, Przybilla B, Überla K, Pöschl C, & Ring J (1994) Frequency of atopic diseases in children and parental predisposition — results of an epidemiological survey. ACI News, **2**(suppl): 170.

Scheinbart LS, Johnson MA, Gross LA, Edelstein SR, & Richardson BC (1991) Procainamide inhibits DNA methyltransferase in a human T cell line. J Rheumatol, **18**: 530–534.

Scheinman PL (1996) Allergic contact dermatitis to fragrances: A review. Am J Contact Dermatitis, **7**: 65–76.

Scheper RJ & von Blomberg BME (1992) Cellular mechanisms in allergic contact dermatitis. In: Rycroft RJG, Menné T, Frosch PJ, & Benezra C ed. Textbook of contact dermatitis. Berlin, Heidelberg, New York, Springer Verlag, pp 11–27.

Scheper RJ, von Blomberg M, Boerrigter GH, Bruynzeel DP, von Dinther-Janssen ACHM, & Vos A (1983) Induction of immunological memory in the skin. Role of local T cell retention. Clin Exp Immunol, **51**: 141–148.

Schleimer RP & Bochner BS (1998) The role of adhesion molecules in allergic inflammation and their suitability as targets of antiallergic therapy. Clin Exp Allergy, **28**(suppl 39): 15–23.

Schlipköter HW, Krämer U, Behrendt H, Dolgner R, Stiller-Winkler R, Ring J, & Willer HJ (1992) Impact of air pollution on children's health: Results from Saxony-Anhalt and Saxony as compared to Northrhine-Westphalia. In: Health and ecological effects — Critical issues in the global environment. Pittsburgh, Pennsylvania, Air and Waste Management Association, vol 5 (Publication IU-A 2103).

Schnyder UW (1960) [Neurodermitis, asthma, rhinitis: A genetic allergological study.] Basel, Karger, 106 pp.

Schon-Hegrad MA, Oliver J, McMenamin PG, & Holt PG (1991) Studies on the density, distribution and surface phenotype of intraepithelial class II MHC antigen (Ia)-bearing dendritic cells (DC) in the conducting airways. J Exp Med, **173**(6): 1345–1356.

Schönrich G, Kalinke U, Momburg F, Malissen M, Schmitt-Verhulst AM, Malissen B, Hämmerling GJ, & Arnold B (1991) Down-regulation of T cell receptors on self-reactive T cells as a novel mechanism for extrathymic tolerance induction. Cell, **65**: 293–304.

Schöpf E, Mueller JM, & Ostermann T (1995) [Significance of adjuvant basis therapy in chronic relapsing skin disease.] Hautarzt, **44:** 451–454 (in German).

Schuhmann D, Kubicka-Muranyl M, Mirtschewa J, Gunther J, Kind P, & Gleichmann E (1990) Adverse reactions to gold: I. Chronic treatment with a Au(I) drug sensitizes mouse T cells not to Au(I) but to Au(III) and induces autoantibody formation. J Immunol, **145**: 2132–2139.

Schulz O, Sutton BJ, Beavil RL, Shi J, Sewell HF, Gould HJ, Laing P, & Shakib F (1997) Cleavage of the low-affinity receptor for human IgE (CD23) by a mite cysteine protease: nature of the cleaved fragment in relation to the structure and function of CD23. Eur J Immunol, **27:** 584–588.

Schulz O, Sewell HF, & Shakib F (1998) Proteolytic cleavage of CD25, the a subunit of the human T cell interleukin 2 receptor, by Der p 1, a major mite allergen with cysteine protease activity. J Exp Med, **18**: 271–275.

Schultz Larsen F (1991) Genetic aspects of atopic eczema. In: Ruzicka T, Ring J, & Przybilla B ed. Handbook of atopic eczema. Berlin, Heidelberg, New York, Springer Verlag, pp 15–26.

Schultz-Larsen F (1993) The epidemiology of atopic dermatitis. Monogr Allergy, **31**: 9–28.

Schultz Larsen F & Hanifin JM (1992) Secular change in the occurrence of atopic dermatitis. Acta Dermatol Venereol, **176**(suppl): 7–12.

Schultz-Larsen F, Holm NV, & Hennigsen K (1986) Atopic dermatitis: A genetic-epidemiology study in a population-based twin sample. J Am Assoc Dermatol, **15**: 487–494.

Schultze-Werninghaus G, Roesch A, Wilhelms OH, Gonsior E, & Meir Sydow J (1978) [Bronchial asthma due to occupational allergy of immediate type (I) to platinum salts.] Dtsch Med Wochenschr, **103**: 972–975 (in German).

Schutz-Kiss D, Popp W, Wagner C, Reiser K, Havelec L, & Zwick H (1995) [Sensitization to inhaled allergens in the Vienna population.] Wien Klin Wochenschr, **107**(11): 331–335 (in German).

Schuurman H-J, Van Loveren H, Rozing J, & Vos JG (1992) Chemicals trophic for the thymus: risk for immunodeficiency and autoimmunity. Int J Immunopharmac, **14**: 369–375.

Schwartz J & Morris R (1995) Air pollution and hospital admissions for cardiovascular disease in Detroit, Michigan. Am J Epidemiol, **142**: 23–35.

Schwartz J & Weiss ST (1990) Dietary factors and their relation to respiratory symptoms — The second national health and nutrition examination survey. Am J Epidemiol, **132**(1): 67–76.

Schwartz HR, Nerurkar LS, Spies JR, Scanlon RT, & Bellanti JA (1980) Milk hypersensitivity: RAST studies using new antigens generated by pepsin hydrolysis of beta-lactoglobulin. Ann Allergy, **45**: 242–245.

Scott P (1993) IL-12: Initiation cytokine for cell-mediated immunity. Science, **260**(5107): 496–497.

Seager J, Jamison DL, Wilson J, Hayward AR, & Soothill JF (1975) IgA deficiency, epilepsy, and phenytoin treatment. Lancet, **II**: 632–635.

Sears MR, Herbison GP, Holdaway MD, Hewitt CJ, Flannery EM, & Silva PA (1989) The relative risks of sensitivity to grass pollen, house dust mite and cat dander in the development of childhood asthma. Clin Exp Allergy, **19**: 419–424.

Seaton A, Godden DJ, & Brown K (1994) Increase in asthma: a more toxic environment or a more susceptible population? Thorax, **49**: 171–174.

Seifert J, Ring J, & Brendel W (1974) Prolongation of skin allografts after oral application of ALS in rats. Nature (Lond), **249**(459): 776.

Seifert J, Ring J, Steininger J, & Brendel W (1977) Influence of the immune response on the absorption of protein from the gut. Nutr Metab, **21**(Suppl 1): 256–258.

Sell S (1987) Immune deficiency states. In: Immunology, immunopathology, and immunity. Amsterdam, Oxford, New York, Elsevier Science Publishers, 617 pp.

Senthilselvan A & Habbick BF (1995) Increased asthma hospitalizations among registered Indian children and adults in Saskatchewan, 1970–1989. J Clin Epidemiol, **48**(10): 1277–1283.

Sequeira J, Cesic D, Keser G, Bukelica M, Karanagnostis S, Khamashta MA, & Hughes GRV (1993) Allergic disorders in systemic lupus erythematosus. Lupus, **2**: 187–192.

Serafini U (1997) Do infections protect against asthma and atopy? Allergy, **52**(9): 955–957.

Shaheen SO, Aaby P, Hall A, Barker DJP, Heyes CB, Shiell AW, & Goudiaby A (1996) Measles and atopy in Guinea-Bissau. Lancet, **347**: 1792–1796.

Shaw RA, Crane J, O'Donnell TV, Porteous LE, & Coleman ED (1990) Increasing asthma prevalence in a rural New Zealand adolescent population: 1975–89. Arch Dis Child, **65**: 1319–1323.

Shaw RA, Crane J, & O'Donnell TV (1991) Asthma symptoms, bronchial hyperresponsiveness and atopy in a Maori and European adolescent population. NZ Med J, **104**: 175–179.

Shaw RA, Crane J, O'Donnell TV, Lewis ME, Stewart B, & Beasley R (1992a) The use of a videotaped questionnaire for studying asthma prevalence: A pilot study among New Zealand adolescents. Med J Aust, **157**(5): 311–314.

Shaw RA, Crane J, Pearce N, Burgess CD, Bremner P, Woodman K, & Beasley R (1992b) Comparison of a video questionnaire with the IUATLD written questionnaire for measuring asthma prevalence. Clin Exp Allergy, **22**: 561–568.

Shaw R, Woodman K, Crane J, Moyes C, Kennedy J, & Pearce N (1994) Risk factors for asthma symptoms in Kawerau children. NZ Med J, **107**: 387–391.

Shaw R, Woodman K, Ayson M, Dibdin S, Winkelmann R, Crane J, Beasley R, & Pearce N (1995) Measuring the prevalence of bronchial hyper-responsiveness in children. Int J Epidemiol, **24**(3): 597–602.

Sherrill DL, Halonen M, & Burrows B (1994) Relationships between total serum IgE, atopy, and smoking: a twenty-year follow-up analysis. J Allergy Clin Immunol, **94**: 954–962.

Shimizu H, Masunaga T, Ishiko A, Kikuchi A, Hashimoto T, & Nishikawa T (1995) *Pemphigus vulgaris* and *Pemphigus foilaceus* sera show an inversely graded pattern to extracellular regions of desmosomes in different layers of human epidermis. J Invest Dermatol, **105**: 153–159.

Shirakawa T, Enomoto T, Shimazu S, & Hopkin JM (1997) The inverse association between tuberculin responses and atopic disorder. Science, **275**: 77–79.

Sibbald B (1993) Epidemiology of allergic rhinitis. In: Burr ML ed. Epidemiology of clinical allergy. Basel, Karger, pp 61–79 (Monograph on Allergy Series).

Sibbald B (1997) Familial inheritance of asthma and allergy. In: Kay AB ed. Allergy and allergic diseases. Oxford, London, Boston, Blackwell Scientific Publications, pp 1177–1186.

Sibbald B & Rink E (1991a) Labelling of rhinitis and hayfever by doctors. Thorax, **46**: 378–381.

Sibbald B & Rink E (1991b) Epidemiology of seasonal and perennial rhinitis: Clinical presentation and medical history. Thorax, **46**: 895–901.

Sigurs N, Bjarnson R, Sigurbergsson F, Kjellman B, & Björkstén (1995) Asthma and immunoglobulin E antibodies after respiratory syncitial virus bronchiolitis: a prospective cohort study with matched controls. Pediatrics, **95**: 500–505.

Silman A & Hochberg MC (1996) Occupational and environmental influences on scleroderma. Rheum Dis Clin North Am, **22**(4): 737–749.

Silman A, Black CM, & Welsh KL (1996) Epidemiology, demographics, genetics. In: Clements PJ & Furst DE ed. Systemic sclerosis. Baltimore, Maryland, Williams and Wilkins, pp 23–49.

Silvennoinen-Kassinen S (1981) The specificity of nickel sulphate reaction *in vitro*: A family study and a study of chromium-allergic subjects. Scand J Immunol, **13**: 231–235.

Silverman GA, Peri BA, & Rothberg RM (1982) Systemic antibody responses of different species following ingestion of soluble protein antigens. Dev Comp Immunol, **6**: 747–752.

Singer PA & Theofilopoulos AN (1990) T-cell receptor Vb repertoire expression in murine models of SLE. Immunol Rev, **118**: 103–127.

Sinigaglia F (1994) The molecular basis of metal recognition by T cells. J Invest Dermatol, **102**: 398–401.

Smalley DL, Shanklin DR, Hall MF, Stevens MV, & Hanissian A (1995) Immunologic stimulation of T lymphocytes by silica after use of silicone mammary implants. FASEB J, **9**: 424–427.

Smalley DL, Levine JJ, Shanklin DR, Hall MF, & Stevens MV (1997) Lymphocyte response to silica among offspring of silicone breast implant recipients. Immunobiology, **196**: 567–574.

Smith IM (1983) Epidemiology and natural history of asthma, allergic rhinitis and atopic dermatitis (eczema). In: Middleton E, Reed Cellis EF, Adkinson NF, Junginger JW, & Busse WW ed. Allergy: Principles and practice. St. Louis, Missouri, C.V. Mosby & Co., pp 771–804.

Song YH, Li Y, & Maclaren NK (1996) The nature of autoantigens targeted in autoimmune endocrine diseases. Immunol Today, **17**: 232–237.

Song Z, Casolaro V, Chen R, Georas SN, Monos D, & Ono SJ (1996) Polymorphic nucleotides within the human IL-4 promoter that mediate over expression of the gene. J Immunol, **156**: 424–429.

Sonnhag C, Karlsson E, & Hed J (1979) Procainamide induced lupus erythematosus like syndrome in relation to acetylator phenotype and plasma levels of procainamide. Acta Med Scand, **206**: 245–251.

Sparrow GP (1977) A connective tissue disorder similar to vinyl chloride disease in a patient exposed to perchlorethylene. Clin Exp Dermatol, **2**: 17–22.

Speizer FE & Ferris BGJ (1973) Exposure to automobile exhaust: I. Prevalence of respiratory symptoms and disease. Arch Environ Health, **26**: 313–318.

Spier HW & Sixt J (1955) [Investigation of the dependence of eczema on the thickness of the stratum corneum.] Hautarzt, **6**: 152–159 (in German).

Sporik R, Holgate ST, Platts Mills TA, & Cogswell JJ (1990) Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood: A prospective study. N Engl J Med, **323**: 502–507.

Stadler JC & Karol MH (1985) Use of dose–response data to compare the skin sensitizing abilities of dicyclohexylmethane-4,4'-diisocyanante and picryl chloride in two animal species. Toxicol Appl Pharmacol, **78**: 445–450.

Stadler JC & Loveless SE (1992) Guinea pigs exhibit extended latency period for the development of sensitivity to an amine. Toxicologist, **12**: 44 (abstract).

Stahl RE, Friedman-Kien A, Dubin R, Marmor M, & Zolla-Pazner S (1982) Immunologic abnormalities in homosexual men. Am J Med, **73**(2): 171–178.

Starzl TE, Nalesnik MA, Porter KA, Ho M, Iwatsuki S, Griffith BP, Rosenthal JT, Hakala TR, Shaw BW Jr, Hardesty RL, Atchinson RW, Jaffe R, & Bahnson HT (1984) Reversibility of lymphomas and lymphoproliferative lesions developing under cyclosporin-steroid therapy. Lancet, **8377**: 583–587.

Steinman R, Hoffman L, & Pope M (1995) Maturation and migration of cutaneous dendritic cells. J Invest Dermatol, **105**: 2S–7S.

Sterk PJ, Fabbri LM, Quanjer PH, Cockcroft DW, O'Byrne PM, Anderson SD, Juniper EF, & Malo JL (1993) Airway responsiveness: Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Report of Working Party on Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J, **16**(suppl): 53–83.

Stewart GA (1994) Molecular biology of allergens. In: Busse WW & Holgate ST ed. Asthma and rhinitis. Oxford, London, Boston, Blackwell Scientific Publications, pp 898–932.

Stiller-Winkler R, Radaszkiewicz T, & Gleichmann E (1988) Immunopathological signs in mice treated with mercury compounds-I. Identification by the popliteal lymph node assay of responder and non-responder strains. Int J Immunopharmacol, **10**: 475–484.

Stites DP & Terr AI (1991) Basic and clinical immunology. London, Appleton & Lange, p 797.

Stoddard JJ & Miller T (1995) Impact of parental smoking on the prevalence of wheezing respiratory illness in children. Am J Epidemiol, **141**: 96–102.

Stokes CR, Newly JR, Huntley JH, Patel D, & Bourne FJ (1979) The immune response of mice to bacterial antigens given by mouth. Immunology, **38**: 497–502.

Stokes CR, Newly TJ, & Bourne FJ (1983a) The influence of oral immunization on local and systemic immune response to heterologous antigens. Clin Exp Immunol, **52**: 399–406.

Stokes CR, Swarbrick ET, & Soothill JF (1983b) Genetic differences in immune exclusion and partial tolerance to ingested antigens. Clin Exp Immunol, **52**(3): 678–684.

Strachan DP (1988) Damp housing and childhood asthma: validation of reporting of symptoms. Br Med J, **297**: 1223–1226.

Strachan DP (1989) Hay fever, hygiene, and household size. Br Med J, **299**: 1259–1260.

Strachan DP (1995) Epidemiology of hay fever: towards a community diagnosis. Clin Exp Allergy, **25**(4): 296–303.

Strachan DP (1996) Socioeconomic factors and the development of allergy. Toxicol Lett, **86**: 199–203.

Strachan DP & Anderson HR (1992) Trends in hospital admission rates for asthma in children. Br Med J, **304**: 819–820.

Strachan DP & Carey IM (1995) Home environment and severe asthma in adolescents: a population based case-control study. Br Med J, **311**: 1053–1056.

Strachan DP, Golding J, & Anderson HR (1990) Regional variations in wheezing illness in British children: effect of migration during early childhood. J Epidemiol Community Health, **44**: 231–236.

Strachan DP, Cox BD, Erzinclioglu SW, Walters DE, & Whichelow MJ (1991)Ventilatory function and winter fresh fruit consumption in a random sample of British adults. Thorax, **46**: 624–629.

Strachan DP, Anderson HR, Limb ES, O'Neill A, & Wells N (1994) A national survey of asthma prevalence, severity, and treatment in Great Britain. Arch Dis Child, **70**: 174–178.

Strachan DP, Sibbald B, Weiland SK, Ait-Khaled N, Anabwani G, Anderson HR, Asher MI, Beasley R, Bjorksten B, Burr M, Clayton T, Crane J, Ellwood P, Keil U, Lai C, Mallol J, Martinez

F, Mitchell E, Montefort S, Pearce N, Robertson C, Shah J, Stewart A, von Mutius E, & Williams H (1997) Worldwide variations in prevalence of symptoms of allergic rhinoconjunctivitis in children: the International study of asthma and allergies in childhood (ISAAC). Pediatr Allergy Immunol, **8**: 161–176.

Strandberg I, Boman G, Hassler L, & Sjoqvist F (1976) Acetylator phenotype in patients with hydralazine induced lupoid syndrome. Acta Med Scand, **200**: 367–371.

Strobel S & Ferguson A (1984) Immune responses to fed protein antigens in mice: III. Systemic tolerance or priming is related to age at which antigen is first encountered. Pediatr Res, **18**: 588–594.

Suzuki T, Kanoh T, Kanbayashi M, Todome Y, & Ohkuni H (1993) The adjuvant activity of pyrene in diesel exhaust on IgE antibody production in mice. Jpn J Allergol, **42**: 963–968.

Swennen B, Buchet JP, Stanescu D, Lison D, & Lauwerys R (1993) Epidemiological survey of workers exposed to cobalt oxides, cobalt salts, and cobalt metal. Br J Ind Med, **50**(9): 835–842.

Szentivanyi A (1968) The beta adrenergic theory of the atopic abnormality in asthma. J Allergy, **42**: 203.

Takafuji S, Suzuki S, Koizumi K, Tadokoro K, Miyamoto T, Ikemori R, & Muranaka M (1987) Diesel-exhaust particulates inoculated by the intranasal route have an adjuvant activity for IgE production in mice. J Allergy Clin Immunol, **79**: 639–645.

Takafuji S, Suzuki S, Koizumi K, Tadokoro K, Ohashi H, Muranaka M, & Miyamoto T (1989) Enhancing effect of suspended particulate matter on the IgE antibody production in mice. Int Arch Allergy Appl Immunol, **90**: 1–7.

Tanser AR, Bourke MP, & Blandford AG (1973) Isocyanate asthma: respiratory symptoms caused by diphenylmethane di-isocyanate. Thorax, **28**(5): 596–600.

Tariq SM, Stevens M, Matthews S, Ridout S, Twiselton R, & Hide DW (1996) Cohort study of peanut and tree nut sensitisation by the age of 4 years. Br Med J, **313**: 514–517.

Tarvainen K, Jolanki R, Estlander T, Tupasela O, Pfaffli P, & Kanerva L (1995) Immunologic contact urticaria due to airborne methylhexahydrophthalic and methyltetrahydrophthalic anhydrides. Contact Dermatitis, **32**(4): 204–209.

Taylor SL (1986) Immunologic and allergic properties of cow's milk proteins in humans. J Food Protect, **49**: 239–250.

Taylor SL (1992) Chemistry and detection of food allergens. Food Technol, **46**: 146–152.

Taylor JS & Praditsuwan P (1996) Latex allergy. Arch Dermatol, **132**: 265–271.

Taylor B, Wadsworth J, Wadsworth M, & Peckham C (1984) Changes in the reported prevalence of childhood eczema since the 1939–45 war. Lancet, **2**: 1255–1257

Tee RD, Gordon DJ, Gordon S, Crook B, Nunn A, Musk AW, Venables KM, & Taylor A (1992a) Immune response to flour and dust mites in a United Kingdom bakery. Br J Ind Med, **49**(8): 581–587.

Tee RD, Gordon DJ, van Hage Hamsten M, Gordon S, Nunn A, Johansson SG, & Taylor A (1992b) Comparison of allergic responses to dust mites in UK bakery workers and Swedish farmers. Clin Exp Allergy, **22**(2): 233–239.

Teichberg S, Isolauri E, Wapnir RA, Roberts B, & Lifshitz F (1990) Development of the neonatal rat small intestinal barrier to nonspecific macromolecular absorption: Effect of early weaning to artificial diets. Pediatr Res, **28**(1): 31–37.

Tenenbaum SA, Rice JC, Espinoza LR, Cuéllar ML, Plymale DR, Sander DM, Williamson LL, Haislip AM, Gluck OS, Tesser JRP, Nogy L, Stribrny Km, Bevan JA, & Garry RF (1997) Use of antipolymer assay in recipients of silicone breast implants. Lancet, **349**: 449–454.

Terr AI (1994a) The atopic diseases. In: Stites DP, Terr AI, & Parslow TG ed. Basic and clinical immunology, 8th ed. East Norwalk, Connecticut, Appleton & Lange, pp 27–346.

Terr AI (1994b) Immune-complex allergic disease. In: Stites DP, Terr AI, & Parslow TG ed. Basic and clinical immunology, 8th ed. East Norwalk, Connecticut, Appleton & Lange, pp 357–362.

Tharp MD (1990) Immunodermatology. IgE and immediate hypersensitivity. Dermatol Clin North Am, **8**(4): 619–631.

Theofilopoulos AN (1995a) The basis of autoimmunity: Part I. Mechanisms of aberrant self-recognition. Immunol Today, **16**: 90–98.

Theofilopoulos AN (1995b) The basis of autoimmunity: Part II. Genetic predisposition. Immunol Today, **16**: 150–158.

Theofilopoulos AN & Dixon FJ (1985) Murine models of systemic lupus erythematosus. Adv Immunol, **37**: 269–390.

Thomas C, Groten J, Kammüller ME, De Bakker JM, Seinen W, & Bloksma N (1989) Popliteal lymph node reactions in mice induced by the drug zimeldine. Int J Immunopharmacol, **11**: 693–702.

Thomas C, Lippe W, Seinen W, & Bloksma N (1991) Popliteal lymph node enlargement and antibody production in the mouse induced by drugs affecting monoamine levels in the brain. Int J Immunopharmacol, **13**: 621–629.

Thompson HSG & Staines NA (1990) Could specific oral tolerance be a therapy for autoimmune disease? Immunol Today, **11**: 398–399.

Thorne PS & Karol MH (1989) Association of fever with late-onset pulmonary hypersensitivity responses in the guinea pig. Toxicol Appl Pharmacol, **100**: 247–258.

Thorne PS, Hillebrand J, Magreni C, Riley EJ, & Karol MH (1986) Experimental sensitization to subtilisin: I. Production of immediate- and late-onset pulmonary reactions. Toxicol Appl Pharmacol, **86**: 112–123.

Thorne PS, Hawk C, Kaliszewski SD, & Gurney PD (1991) The noninvasive mouse ear swelling assay. I. Refinements for detecting weak contact sensitizers. Fundam Appl Toxicol, **17**: 790–806.

Thurston GD (1996) A critical review of PM10-mortality time-series studies. J Expo Anal Environ Epidemiol, **6**(1): 3–21.

Thurston GD, Ito K, Kinney PL, & Lippmann M (1992a) A multi-year study of air pollution and respiratory hospital admissions in three New York State metropolitan areas: Results for 1988 and 1989 summers. J Expo Anal Environ Epidemiol, **2**: 429–450.

Thurston G, D'Souza N, Lippmann M, Bartoszek M, & Fine I (1992b) Associations between summer haze air pollution and asthma exacerbations: A pilot camp study. Am Rev Respir Dis, **145**: A429 (abstract).

Tisch R, Yang XD, Singer SM, Liblau LS, Fugger L, & McDevitt HO (1993) Immune response to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mice. Nature (Lond), **366**: 72–75.

Tollerud DJ, Weiss ST, Elting E, Speizer FE, & Ferris B (1983) The health effects of automobile exhaust. VI. Relationship of respiratory symptoms and pulmonary function in tunnel and turnpike workers. Arch Environ Health, **38**: 334–340.

Tomasi TB, Barr WG, Challacombe SJ, & Curran G (1983) Oral tolerance and accessory-cell function of Peyer's patches. Ann NY Acad Sci, **409**: 145–163.

Tomura T & van Lancker JL (1988) Procainamide — DNA interaction. J Rheumatol, 15: 59–64.

Topping MD, Venables KM, Luczynska CM, Howe W, & Newman Taylor A (1986) Specificity of the human IgE response to inhaled acid anhydride. J Allergy Clin Immunol, **77**: 834–842.

Toren K, Brisman J, & Jarvholm B (1993) Asthma and asthma-like symptoms in adults assessed by questionnaires: A literature review. Chest, **104**(2): 600–608.

Tournade H, Pelletier L, Pasquier R, Vial MC, Mandet C, & Druet P (1990) D-penicillamine-induced autoimmunity in Brown Norway rats: Similarities with $HgCl_2$-induced autoimmunity. J Immunol, **144**: 2985–2991.

Tribe RM, Barton JR, Poston L, & Burney PG (1994) Dietary sodium intake, airway responsiveness, and cellular sodium transport. Am J Respir Crit Care Med, **149**(6): 1426–1433.

Troisi RJ, Willett WC, Weiss ST, Trichopoulos D, Rosner B, & Speizer FE (1995) A prospective study of diet and adult-onset asthma. Am J Respir Crit Care Med, **151**(5): 1401–1408.

Tsicopoulos A, Hamid Q, Varney V, Ying S, Moqbel R, Durham SR, & Kay AB (1992) Preferential messenger RNA expression of Th1-type cells (IFN-gamma+, IL2+) in classical delayed-type (tuberculin) hypersensitivity reactions in human skin. J Immunol, **148**(7): 2058–2061.

Tsunoda K, Ohta Y, Shinogami M, & Soda Y (1995) Does passive smoking affect the incidence of nasal allergies? Am J Public Health, **85**(7): 1019–1020.

Turjanmaa K (1987) Incidence of immediate allergy to latex gloves in hospital personnel. Contact Dermatitis, **17**(5): 270–275.

Turjanmaa K (1994) Allergy to natural rubber latex: a growing problem. Ann Med, **26**: 297–300.

Turjanmaa K, Alenius H, Mäkinen-Kiljunen S, Reunala T, & Palosuo T (1996) Natural rubber latex allergy. Allergy, **51**: 593–602.

Turner MW, Boulton P, Shields JG, Strobel S, Gibson S, Miller HRP, & Levinsky RJ (1988) Intestinal hypersensitivity reactions in the rat: I. Uptake of intact protein, permeability to sugars and their correlation with mucosal mast-cell activation. Immunology, **63**(1): 119–124.

Turner MW, Barnett GE, & Strobel S (1990) Mucosal mast cell activation patterns in the rat following repeated feeding of antigen. Clin Exp Allergy, **20**(4): 421–427.

Uehara M (1991) Dry skin and inflammation. In: Ruzicka T, Ring J, & Przybilla B ed. Handbook of atopic eczema. Berlin, Heidelberg, New York, Springer Verlag, pp 84–89.

Uetrecht JP (1992) The role of leukocyte-generated reactive metabolites in the pathogenesis of idiosyncratic drug reactions. Drug Metab Rev, **24**: 299–366.

Ulfvarson U & Alexandersson R (1990) Reduction in adverse effect on pulmonary function after exposure to filtered diesel exhaust. Am J Ind Med, **17**: 341–347.

Utell MJ, Warren J, & Sawyer RF (1994) Public health risks from motor vehicle emissions. Annu Rev Public Health, **15**: 157–178.

Vandenplas O (1995) Occupational asthma caused by natural rubber latex. Eur Respir J, **8**: 1957–1965.

Vandenplas O, Delwiche JP, Evrard G, Aimont P, van der Brempt X, Jamart J, & Delaunois L (1995) Prevalence of occupational asthma due to latex among hospital personnel. Am J Respir Crit Care Med, **151**(1): 54–60.

Van der Heijden FL, Van Neerven RJJ, Van Katwijk M, Bos JD, & Kapsenberg ML (1993) Serum IgE-facilitated allergen presentation in atopic disease. J Immunol, **150**: 3643–3650.

Van der Valk P, van Kalken CK, Ketelaars H, Broxterman HJ, Kuiper CM, Tsuruo T, Lankelma J, Meijer CJLM, Pinedo HM, & Scheper RJ (1990) Distribution of multi-drug resistance-associated P-glycoprotein in normal and neoplastic human tissues — Analysis with 3 monoclonal antibodies recognizing different epitopes of the P-Glycoprotein molecule. Ann Oncol, **1**: 56–64.

Van Hoogstraten IMW, Boden D, von Blomberg BME, Kraal G, & Scheper RJ (1992) Persistent immune tolerance to nickel and chromium by oral administration prior to cutaneous sensitization. J Invest Dermatol, **99**: 608–616.

Van Hoogstraten IMW, Boos C, Boden D, von Blomberg BME, Scheper RJ, & Kraal G (1993) Oral induction of tolerance to nickel sensitization in mice. J Invest Dermatol, **101**: 26–31.

Van Hoogstraten IMW, von Blomberg BME, Boden D, Kraal G, & Scheper RJ (1994) Non-sensitizing percutaneous skin contacts prevent subsequent induction of immune tolerance. J Invest Dermatol, **102**: 80–83.

Van Lee Uwen BH, Martinsom ME, Webb GC, & Young IG (1989) Molecular organization of the cytokine gene cluster, involving the human IL-3, IL-4, IL-5, and GM-CSF genes on human chromosome 5. Blood, **73**: 1142–1148.

Van Loveren H, Redegeld F, Matsuda H, Buckley T, Teppema JS, & Garssen J (1997) Mast cells. In: Bos JD ed. Skin immune system (SIS), 2nd ed. Boca Raton, Florida, CRC Press, pp 160–184.

Van Niekerk CH, Weinberg EG, Shore SC, Heese HV, & Van Schalkwyk J (1979) Prevalence of asthma: A comparative study of urban and rural Xhosa children. Clin Allergy, **9**: 319–324.

Varjonen E, Kalimo K, Lammintausta K, & Terho P (1992) Prevalence of atopic disorders among adolescents in Turku, Finland. Allergy, **47**: 243–248.

Veien NK, Hattel T, Justese O, & Norholm A (1987) Dietary restrictions in the treatment of adult patients with eczema. Contact Dermatitis, **17**: 223–228.

Veien NK, Hattel T, & Laurberg G (1993) Low nickel diet: An open prospective trial. J Am Acad Dermatol, **29**: 1002–1007.

Veltman C, Lange CE, Juhe S, Stein G, & Bachner V (1975) Clinical manifestations and course of vinyl chloride disease. Proc NY Acad Sci, **246**: 6–17.

Venables KM & Chan Yeung M (1997) Occupational asthma. Lancet, **349**(9063): 1465–1469.

Venables KM & Newman Taylor A (1990) Exposure-response relationships in tetrachlorophthalic anhydride asthma. J Allergy Clin Immunol, **85**: 55–58.

Venables KM, Dally MB, Burge PS, Pickering CAC, & Newman Taylor A (1985a) Occupational asthma in a steel coating plant. Br J Ind Med, **42**: 517–524.

Venables KM, Topping MD, Howe W, Luczynska CM, Hawkins R, & Newman Taylor A (1985b) Interaction of smoking and atopy in producing specific IgE antibody against a hapten protein conjugate. Br Med J, **290**: 201–204.

Venables KM, Topping MD, Nunn A, Howe W, & Newman Taylor A (1987) Immunologic and functional consequences of chemical (tetrachlorophthalic anhydride) induced asthma after 4 years of avoidance of exposure. J Allergy Clin Immunol, **80**: 212–218.

Venables KM, Dally MB, Nunn A, Stevens JF, Stephens R, Farrer N, Hunter JV, Stewart M, Hughes EG, & Newman Taylor A (1989) Smoking and occupational allergy in a platinum refinery. Br Med J, **299**: 939–942.

Verdier F, Virat M, & Descotes J (1990) Applicability of the popliteal lymph node assay in the Brown-Norway rat. Immunopharmacol Immunotoxicol, **12**: 669–677.

Verdier F, Patriarca C, & Descotes J (1997) Autoantibodies in conventional toxicity testing. Toxicology, **119**: 51–58.

Verhoeff AP, van Strien RT, van Wijnen JH, & Brunekreef B (1995) Damp housing and childhood respiratory symptoms: the role of sensitization to dust mites and molds. Am J Epidemiol, **141**: 103–110.

Vial T & Descotes J (1994) Contact sensitization assays in guinea pigs: are they predictive of the potential for systemic allergic reactions? Toxicology, **93**: 63–75.

Vickers CFH (1991) Natural history of atopic eczema. In: Ruzicka T, Ring J, & Przybilla B ed. Handbook of atopic eczema. Berlin, Heidelberg, New York, Springer Verlag, pp 80–83.

Vieluf D, Przybilla B, Traenkner I, & Ring J (1990) Oral provocation with food additives in atopic eczema. J Allergy Clin Immunol, **85**: 206 (abstract).

Vieluf D, Kunz B, Bieber T, Przybilla B, & Ring J (1993) "Atopy patch test" with aeroallergens in patients with atopic eczema. Allergo J, **2**: 9–12

Vincent A, Roberts M, Willison H, Lang B, & Newsom-Davis J (1995) Autoantibodies, neurotoxins and the nervous system. J Physiol, **89**(3): 129–136.

Viner AS & Jackman N (1976) Retrospective survey of 1271 patients diagnosed as perennial rhinitis. Clin Allergy, **6**(3): 251–259.

Vivier E & Daeron M (1997) Immunoreceptor tyrosine-based inhibition motifs. Immunol Today, **18**: 286–291.

Von Blomberg BME, Bruynzeel DP, & Scheper RJ (1990) Advances in mechanisms of allergic contact dermatitis: *in vitro* and *in vivo* research. In: Marzulli FN & Maibach HI ed. Dermatotoxicology, 4th ed. Washington, New York, London, Hemisphere Publishing Corporation, pp 255–362.

Von Mutius E, Fritzsch C, Weiland SK, Roll G, & Magnussen H (1992) Prevalence of asthma and allergic disorders among children in united Germany: A descriptive comparison. Br Med J, **305**: 1395–1399.

Von Mutius E, Martinez FD, Fritzsch C, Nicolai T, Roell G, & Thiemann HH (1994a) Prevalence of asthma and atopy in two areas of West and East Germany. Am J Respir Crit Care Med, **149**: 358–364.

Von Mutius E, Martinez FD, Fritzsch C, Nicolai T, Reitmeir P, & Thiemann HH (1994b) Skin test reactivity and number of siblings. Br Med J, **308**: 692–695.

Von Mutius E, Weiland SK, Fritzsch C, Duhme H, & Keil U (1998) Increasing prevalence of hay fever and atopy among children in Leipzig, East Germany. Lancet, **351**: 862–866.

Vos JG, Younes M, & Smith E ed. (1996) Allergic hypersensitivities induced by chemicals: Recommendations for prevention. Boca Raton, Florida, CRC Press, 348 pp.

Vreeburg KJJ, Wilkinson JD, & Scheper RJ (1991) Reduced frequency of nickel allergy upon oral nickel contact at an early age. Clin Exp Immunol, **85**: 441–445.

Wade JF 3rd & Newman LS (1993) Diesel asthma: Reactive airways disease following overexposure to locomotive exhaust. J Occup Med, **35**(2): 149–154.

Wahlberg JE (1995) Patch testing. In: Rycroft RJG, Menné T, Frosch PJ, & Benezra C ed. Textbook of contact dermatitis, 2nd ed. Berlin, Heidelberg, New York, Springer Verlag, pp 241–265.

Wahlberg JE & Boman A (1985) Guinea pig maximisation test. Curr Probl Dermatol, **14**: 59–106.

Waite DA, Eyles EF, Tonkin SL, & O'Donnell TV (1980) Asthma prevalence in Tokelauan children in two environments. Clin Allergy, **10**: 71–75.

Walker RB & Warin RP (1956) The incidence of eczema in early childhood. Br J Dermatol, **68**: 182–183.

Walker FB, Smith PD, & Maibach HI (1967) Genetic factors in human allergic contact dermatitis. Int Arch Allergy, **32**: 453–462.

Walker C, Kaegi MK, Braun MD, & Blaser K (1991) Activated T cells and eosinophilia in bronchoalveolar lavages from subjects with asthma correlated with disease severity. J Allergy Clin Immunol, **88**: 935–942.

Walker-Smith JA (1992) Immunology of gastrointestinal food allergy in infancy and early childhood. In: MacDonald TT ed. Immunology of gastrointestinal disease. Dodrecht, Kluwer Academic Publishers, pp 61–73.

Ward AM, Udnoon S, Watkins J, Walker AE, & Darke CS (1976) Immunological mechanisms in the pathogenesis of vinyl chloride disease. Br Med J, **1**: 936–938.

Wardlaw A (1993) The role of air pollution in asthma. Clin Exp Allergy, **23**: 81–96.

Ware JH, Dockery DW, Spiro A 3rd, Speizer FE, & Ferris BG Jr (1984) Passive smoking, gas cooking, and respiratory health of children living in six cities. Am Rev Respir Dis, **129**(3): 366–374.

Ware JH, Spengler JD, Neas LM, Samet JM, Wagner GR, Coultas D, Ozkaynak H, & Schwab M (1993) Respiratory and irritant health effects of ambient volatile organic compounds: The Kanawha County health study. Am J Epidemiol, **137**(12): 1287–1301.

Warren DL, Shiotsuka RN, Sangha GK, & Lyon JP (1993) Comparison of respiratory sensitization responses to 2,4 and 2,6 toluene diisocyanate. Toxicologist, **13**: 41 (abstract).

Wass U & Belin L (1990) An *in vitro* method for predicting sensitising properties of inhaled chemicals. Scand J Work Environ Health, **16**(3): 208–214.

Weeke ER (1987) Epidemiology of hay fever and perennial allergic rhinitis. Monogr Allergy, **21**: 1–20.

Weigle WO (1965) The production of thyroiditis and antibody following injection of unaltered thyroglobulin into rabbits previously stimulated with altered thyroglobulin. J Exp Med, **122**: 1049–1062.

Weiland SK, Mundt KA, Rückmann A, & Keil U (1994) Self-reported wheezing and allergic rhinitis in children and traffic density on street of residence. Ann Epidemiol, **4**: 243–247.

Weill H, Butcher B, & Charmarajan V (1981) Respiratory and immunologic evaluation of isocyanate exposure in a new manufacturing plant. Cincinnati, Ohio, National Institute of Occupational Safety and Health (NIOSH Technical Report; DHHS (NIOSH) Publication No. 81-125).

Weinberg DA, Lesser RL, & Vollmer TL (1994) Ocular myasthenia: A protean disorder. Surv Ophthalmol, **39**: 169–210.

Weiner HL, Friedman A, Miller A, Khoury SJ, Al-Sabbagh A, Santos L, Sayegh M, Nussenblatt RB, Trentham DE, & Hafler DA (1994) Oral tolerance: Immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. Annu Rev Immunol, **12**: 809–837.

Weinstein RS, Grogan TM, Kuszak JR, Jakate SM, Kluskens LF, & Coon JS (1991) Multidrug resistance gene product (P-glycoprotein) in normal tissue and tumors. St. Louis, Missouri, Mosby Year Book, Inc., pp 207–234 (Advances in Pathology and Laboratory Medicine Series).

Weiss KB, Wagener DK, Aberg N, Engstrom I, & Lindberg U (1989) Geographic variations in US asthma mortality: Small-area analyses of excess mortality, 1981–1985 — Allergic diseases in Swedish school children. Am J Epidemiol, **78**(2): 246–252.

Weiss KB, Gergen PJ, & Wagener DK (1993) Breathing better or wheezing worse? The changing epidemiology of asthma morbidity and mortality. Annu Rev Public Health, **14**: 491–513.

Weitzman M, Gortmaker SL, Sobol AM, & Perrin JM (1992) Recent trends in the prevalence and severity of childhood asthma. J Am Med Assoc, **268**: 2673–2677.

Weltzien HU, Moulon C, Martin S, Padovan E, Hartmann U, & Kohler J (1996) T cell immune responses to haptens: Structural models for allergic and autoimmune reactions. Toxicology, **107**: 141–151.

White IR (1995) Phototoxic and photoallergic reactions. In: Rycroft RJG, Menné T, Frosch PJ, & Benezra C ed. Textbook of contact dermatitis. Berlin, Heidelberg, New York, Springer Verlag, pp 75–91.

WHO (in press) Air quality guidelines. Copenhagen, World Health Organization, Regional Office for Europe.

Wick G, Sundick RS, & Albini B (1974) The obese strain (OS) of chickens: An animal model with spontaneous autoimmune thyroiditis. Clin Immunol Immunopathol, **3**: 272–300.

Widmann FK (1989) An introduction to clinical immunology. Philadelphia, Pennsylvania, F.A. Davis, 424 pp.

Wierenga EA, Smoek M, de Groot C, Chrétien I, Bos JD, Jansen HM, & Kapsemberg ML (1990) Evidence for compartmentalization of functional subsets of CD4+ T lymphocytes in atopic patients. J Immunol, **144**: 4651–4656.

Wilkinson JD (1995) The management of contact dermatitis. In: Rycroft RJG, Menné T, Frosch PJ, & Benezra C ed. Textbook of contact dermatitis, 2nd ed. Berlin, Heidelberg, New York, Springer Verlag, pp 660–683.

Williams WJ (1989) Beryllium workers — sarcoidosis or chronic beryllium disease. Sarcoidosis, **6**(suppl): 34–35.

Williams HC (1992) Is the prevalence of atopic dermatitis increasing? Clin Exp Dermatol, **17**: 385–391.

Williams IR & Unanue ER (1990) Costimulatory requirements of murine Th1 clones: The role of accessory cell-derived signals in response to anti-CD3 antibody. J Immunol, **145**(1): 85–93.

Williams WR & Williams WJ (1982) Development of beryllium lymphocyte transformation tests in chronic beryllium disease. Int Arch Allergy Appl Immunol, **67**: 175–180.

Williams ME, Lichtman AH, & Abbas AK (1990) Anti-CD3 antibody induces unresponsiveness to IL-2 in Th1 clones but not in Th2 clones. J Immunol, **144**(4): 1208–1214.

Williams HC, Burney PG, Hay RJ, Archer CB, Shipley MJ, Hunter JJ, Bingham EA, Finlay AY, Pembroke AC, Graham Brown RA, Atherton DA, Lewis-Jones MS, Holden CA, Harper JI, Champion RH, Poyner TF, Launer J, & David TJ (1994a) The UK Working Party's diagnostic criteria for atopic dermatitis: I. Derivation of a minimum set of discriminators for atopic dermatitis. Br J Dermatol, **131**(3): 383–396.

Williams HC, Strachan DP, & Hay RJ (1994b) Childhood eczema: disease of the advantaged? Br Med J, **308**: 1132–1135.

Williams HC, Pembroke AC, Forsdyke H, Boodoo G, Hay RJ, & Burney PG (1995a) London-born black Caribbean children are at increased risk of atopic dermatitis. J Am Acad Dermatol, **32**(2/1): 212–217.

Williams PB, Buhr MP, Weber RW, Volz MA, Koepke JW, & Selner JC (1995b) Latex allergen in respirable particulate air pollution. J Allergy Clin Immunol, **95**(1/1): 88–95.

Witt C, Stuckey MS, Woolcock A, & Dawkins RL (1986) Positive allergy prick tests associated with bronchial histamine responsiveness in an unselected population. J Allergy Clin Immunol, **77**: 698–702.

Wjst M, Reitmeir P, Dold S, Wulff A, Nicolai T, von Loeffelholz Colberg EF, & von Mutius E (1993) Road traffic and adverse effects on respiratory health in children. Br Med J, **307**: 596–600.

Wjst M, Heinrich J, Liu P, Dold S, Wassmer G, Merkel G, Huelsse C, & Watchman HE (1994) Indoor factors and IgE levels in children. Allergy, **49**: 766–771.

Wolf R & Brenner S (1994) An active amide group in the molecule of drugs that induce pemphigus: a casual or causal relationship? Dermatology, **189**: 1–4.

Wood RA, Mudd KE, & Eggleston PA (1992) The distribution of cat and dust mite allergens on wall surfaces. J Allergy Clin Immunol, **89**(1/1): 126–130.

Wucherpfennig KW, Yu B, Monos DS, Argyris E, Karr RW, Ahmed AR, & Strominger JL (1995) Structural basis for major histocompatibility complex (MHC)-linked susceptibility to autoimmunity: charged residues of a single MHC binding pocket confer selective presentation of self-peptides in pemphigus vulgaris. Proc Natl Acad Sci (USA), **92**(25): 11935–11939.

Wüthrich B (1975) [Immunopathology of constitutional neurodermatitis.] Bern, Göttingen, Toronto, Seattle, Hogrefe & Huber Publishers, 178 pp (in German).

Wüthrich B (1990) [Do food allergies of Type III exist?] Allergologie, **13**: 371 (in German).

Wüthrich B (1991) Minimal forms of atopic eczema. In: Ruzicka T, Ring J, & Przybilla B ed. Handbook of atopic eczema. Berlin, Heidelberg, New York, Springer Verlag, pp 46–53.

Wüthrich B (1993) [Food allergies: Frequency of symptoms and the allergy-eliciting foods in 402 patients.] Allergologie, **16**: 280–287 (in German).

Wüthrich B & Hofer T (1984) [Food allergy: The "celery-mugwort-spices syndrome" association with mango allergy?] Dtsch Med Wochenschr, **109**(250): 981–986 (in German).

Wüthrich B, Stäger J, & Johansson SGO (1990) Celery allergy associated with birch and mugwort pollinosis. Allergy, **45**(80): 566–571.

Wüthrich B, Schindler C, Leuenberger P, Ackermann-Liebrich U, & SAPALDIA Team (1995) Prevalence of atopy and pollinosis in the adult population of Switzerland (SAPALDIA-study). Int Arch Allergy Immunol, **106**: 149.

Xu X & Wang L (1993) Association of indoor and outdoor particulate level with chronic respiratory illness. Am Rev Respir Dis, **148**(6/1): 1516–1522.

Yamakage A, Ishikawa H, Saito Y, & Hattori A (1980) Occupational scleroderma-like disorder occurring in men engaged in the polymerization of epoxy resins. Dermatologica, **161**: 33–44.

Yamashita U, Takami T, Hamasaka T, & Kitigawa M (1976) The role of hapten-reactive T-lymphocytes in the induction of autoimmunity in mice: II. Termination of self tolerance to erythrocytes by immunization with hapten-isologous erythrocytes. Cell Immunol, **25**: 32–40.

Yamashita N, Natsuaki M, & Sagami S (1989) Flare-up reaction on murine contact hypersensitivity: I. Description of an experimental model: rechallenge system. Immunology, **67**(3): 365–369.

Yokoyama Y, Nitta H, Maeda K, & Aoki S (1985) What interaction does indoor nitrogen dioxide have on the effect of the automobile exhaust. Tokai J Exp Clin Med, **10**: 379–384.

Young RP, Barker R, Cookson WOCM, & Newman Taylor A (1993) HLA-DR and DP antigen frequencies and acid anhydride sensitization. Am Rev Respir Dis, **147**(suppl): A113 (abstract).

Young E, Stoneham MD, Petruckevitch A, Barton J, & Rana R (1994) A population study of food intolerance. Lancet, **343**: 1127–1130.

Young RP, Barker RD, Pile KD, Cookson WOCM, & Newman Taylor A (1995) The association of HLA-DR3 with specific IgE to inhaled acid anhydrides. Am J Resp Crit Care Med, **151**: 219–221.

Yssel H, Johnson KE, Schneider PV, Wideman J, Terr A, Kastelein R, & de Vries JE (1992) T cell activation inducing epitopes of the house dust mite allergen Der p I — Induction of a restricted cytokine production profile of Der p I-specific T cell clones upon antigen-specific activation. J Immunol, **148**: 738–745.

Yssel H, Fasler S, Lamb J, & de Vries JE (1994) Induction of non-responsiveness in human allergen-specific type 2 T helper cells. Curr Opin Immunol, **6**: 847–852.

Yung RL, Quddus J, Crisp CE, Johnson KJ, & Richardson BC (1995) Mechanisms of drug induced lupus: I. Cloned Th2 cells modified with DNA methylation inhibitors *in vitro* cause autoimmunity *in vivo*. J Immunol, **154**: 3025–3035.

Zeiss CR, Patterson R, Pruzansky JJ, Miller MM, Resonberg M, & Levitz D (1977) Trimellitic anhydride induced airways syndrome: Clinical and immunologic studies. J Allergy Clin Immunol, **60**: 96–103.

Zeiss CR, Kanellaks TM, Bellone TD, Levitz D, Pruzansky JJ, & Patterson R (1980) Immunoglobulin E mediated asthma and hyper-sensitivity pneumonitis with precipitating antihapten antibodies due to diphenylmethane di-isocyanate (MDI) exposure. J Allergy Clin Immunol, **65**: 347–352.

Zinkernagel RM & Doherty PC (1975) H-2 compatibility requirement for T-cell mediated lysis of target cells infected with lymphocytic choriomeningitis virus — Different cytotoxic T-cell specifities are associated with structures coded for H-2K or H-2D. J Exp Med, **141**(6): 1427–1436.

Zoia MC, Fanfulla F, Bruschi C, Basso O, De Marco R, Casali L, & Cerveri I (1995) Chronic respiratory symptoms, bronchial responsiveness and dietary sodium and potassium: a population-based study. Monaldi Arch Chest Dis, **50**(2): 104–108.

Zummo SM & Karol MH (1996) Indoor air pollution: Acute adverse health effects and host susceptibility. Environ Health Sci, **Jan/Feb**: 25–29.

Zurawski G & de Vries JE (1994) Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. Immunol Today, **15**(1): 19–26.

1. CONCLUSIONS

L'allergie constitue un problème de santé à l'échelle mondiale. Elle touche une proportion importante de la population et toutes les tranches d'âge. Pour des raisons mal connues, sa fréquence globale est en augmentation.

La présente monographie fait le point des interactions diverses et complexes entre produits chimiques, médicaments, système immunitaire et organes cibles, interactions qui aboutissent à des manifestations d'hypersensibilité allergique et à des troubles d'origine autoimmune. D'importantes recherches sont menées dans tous ces domaines et l'on peut donc s'attendre à de nouvelles découvertes. Les études portent sur les facteurs endogènes et exogènes multiples qui interviennent dans l'allergie. Les facteurs exogènes ont tous leur importance, qu'il s'agisse des allergènes eux-mêmes ou encore des maladies infectieuses, de la pollution de l'air et du mode de vie (par exemple, le tabagisme). Par ailleurs, les prédispositions génétiques à un trouble allergique donné déterminent en grande partie la réactivité du sujet.

L'épidémiologie de l'allergie montre que cet ensemble d'affections est très répandu et que, par conséquent, il importe d'accorder l'attention voulue à l'identification du risque allergique et à son évaluation pour être à même de mettre en oeuvre des stratégies de prise en charge adaptées au risque.

Les méthodes d'identification des substances produisant une sensibilisation cutanée sont bien connues. Elles restent à normaliser dans le cas des sensibilisants respiratoires et font encore défaut pour les autres types d'allergènes. On utilise les techniques de mesure de l'activité des sensibilisants cutanés pour l'évaluation du risque.

Une fois le risque allergique bien caractérisé, l'évaluation de ce risque et sa prise en charge sont d'une importance primordiale pour réduire l'incidence des troubles allergiques. Pour évaluer le risque, il faut déterminer l'activité allergisante de l'agent en cause en fonction de la nature et de l'intensité de l'exposition. Si l'évaluation en fait ressortir la nécessité, il faudra alors prendre en charge ce risque en

mettant en oeuvre un certain nombre de mesures consistant par exemple à limiter l'exposition et à étiqueter le produit.

Les progrès accomplis dans la connaissance des caractéristiques physicochimiques et immunologiques des allergènes alimentaires pourraient déboucher sur la mise au point d'indicateurs prédictifs.

En raison de la complexité des mécanismes qui sont à la base des troubles allergiques, il est encore difficile de proposer des tests prédictifs *in vitro* qui soient d'une portée générale, mais l'application des relation structure–activité mérite d'être étudiée de manière plus approfondie.

2. RECOMMANDATIONS POUR LA PROTECTION DE LA SANTÉ HUMAINE

a) Il convient de mettre en oeuvre des stratégies efficaces pour prévenir les allergies, fondées sur des informations fiables relatives aux agents en cause et à l'environnement. L'action en vue de prévenir ou de réduire au minimum la fréquence des allergies doit reposer sur la limitation de l'exposition.

b) Elucider la cause de la fréquence accrue des allergie est devenu d'une urgente nécessité.

c) Il faut mettre en oeuvre des méthodes de surveillance afin de déterminer la fréquence des divers types d'allergie.

d) Il peut s'avérer difficile de mesurer l'exposition des individus et des populations, mais une évaluation correcte est néanmoins nécessaire à toute analyse de la relation effet–exposition. Du fait de sa spécificité, la réponse immunitaire constitue un biomarqueur unique en son genre pour étudier les antécédents d'exposition, par exemple par apposition d'un timbre cutané, par intradermoréaction ou par titrage des anticorps IgE pour mettre en évidence une sensibilisation par des allergènes déterminés.

e) Il faut améliorer les systèmes de surveillance sanitaire des travailleurs, la qualité des visites médicales effectuées dans le cadre de la médecine du travail ainsi que l'information de ceux qui sont exposés à des produits chimiques, afin de mettre en évidence le plus tôt possible toute maladie allergique d'origine professionnelle. La surveillance de ces affections sur le lieu de travail est particulièrement importante, du fait que l'exposition y est vraisemblablement plus intense qu'ailleurs.

f) Il faut évaluer périodiquement l'efficacité et l'intérêt de la prévention primaire et secondaire, de même que celle des stratégies d'intervention en utilisant des techniques d'évaluation épidémiologique dûment validées.

g) Dans le cas de la dermatite de contact d'origine allergique, les modèles prédictifs *in vivo* dont on dispose à l'heure actuelle ont fait la preuve de leur valeur pour les antigènes de faible masse moléculaire relative. Il faut maintenant trouver le moyen de les utiliser au mieux pour mesurer l'activité des allergènes.

h) Dans le cas des troubles allergiques intéressant les voies respiratoires, les tests *in vivo* relatifs aux substances de faible masse moléculaire relative sont prometteurs, mais il faut encore s'assurer de leur valeur prédictive et de leur spécificité. Dans le cas des allergènes de nature protéique, on est en train de mettre au point des méthodes d'épreuve, mais il faudra encore les évaluer de manière plus approfondie sur des substances dont on connaît le pouvoir allergénique chez l'Homme.

i) Il importe que les services de contrôle et les consommateurs puissent s'informer facilement de la présence éventuelle d'allergènes dans certains produits (par ex. les denrées alimentaires) en consultant par exemple des bases de données ou grâce à un étiquetage approprié, et soient de la sorte en mesure de prendre les précautions voulues.

j) Le principal moyen dont on dispose pour éviter les affections autoimmunes provoquées par des produits chimiques sur le lieu de travail consiste à limiter l'exposition. Il convient d'envisager un examen préalable des personnes qui vont être exposées à des produits

chimiques à action immunomodulatrice pour rechercher tout signe de maladie affectant le tissu conjonctif. Il est souhaitable que les consignes visant à éviter ou à réduire l'exposition soient scrupuleusement suivies, notamment en ce qui concerne les règles d'hygiène et sécurité. Il faut aussi réduire au maximum les autres facteurs de risque, comme le tabagisme, et envisager des contrôles médicaux réguliers dans le cadre de la médecine du travail.

k) Il est nécessaire d'étudier les relations quantitatives entre la réponse immunitaire suscitée par une exposition à des produits chimiques et la gravité des réactions allergiques.

l) Il est également important de déterminer, pour lutter contre ce type de maladies, quelles sont l'intensité et la durée minimales d'exposition nécessaires pour provoquer une sensibilisation ou déclencher une réaction allergique.

m) Il importe de mettre au point des stratégies normalisées pour l'examen clinique et le diagnostic des allergies et de les mettre en oeuvre à l'échelle internationale afin de déterminer les causes et l'incidence des maladies d'origine allergique. A cet effet, il faudra produire et mettre à disposition des extraits normalisés d'allergènes biologiques d'activité contrôlée et procéder périodiquement à l'examen des produits qui entreront dans la composition des batteries normalisées d'allergènes destinées aux divers tests.

n) Les autorités en charge de la santé publique, les membres des professions de santé et les organismes publics devront réfléchir aux moyens d'évaluer le coût humain et économique des maladies allergiques pour l'individu et la société.

o) Les autorités en charge de la santé publique, les membres des professions de santé, le public en général et les travailleurs en particulier tireront avantage d'une meilleure information sur la fréquence, les causes, les manifestations cliniques et les conséquences des différents types d'allergie.

1. CONCLUSIONES

La alergia es un importante problema de salud de alcance mundial. Afecta a una proporción sustancial de la población y a todos los grupos de edad. Por razones poco conocidas, la frecuencia general de la alergia está en aumento.

En esta monografía se examinan las interacciones diversas y complejas entre productos químicos, medicamentos y proteínas, el sistema inmunitario y el(los) órgano(s) diana que dan lugar a la manifestación de la hipersensibilidad alérgica y la autoinmunidad. Estos temas son objeto de una investigación extensa continua, de manera que se prevén nuevos descubrimientos. Se examina la multiplicidad de factores endógenos y exógenos que tienen repercusiones sobre la alergia. Los factores exógenos, como los alergenos mismos, las infecciones, la contaminación del aire y el modo de vida (por ejemplo, el tabaquismo) son todos ellos importantes. Además, la predisposición genética a un trastorno alérgico específico es un determinante importante de la reactividad.

La epidemiología de la alergia demuestra el carácter extendido de este grupo de trastornos y, como consecuencia, pone de relieve la necesidad de prestar suficiente atención a la identificación de los riesgos de alergia y la evaluación de éstos en la aplicación de estrategias apropiadas de gestión de riesgos.

Hay métodos de reconocida eficacia para la determinación de riesgos en relación con los sensibilizadores de la piel. Hace falta normalizar los aplicables a los sensibilizadores a nivel respiratorio y aún no se dispone de métodos aplicables a otros tipos de alergenos. En la evaluación de riesgos se están aplicando técnicas de determinación de la potencia de los sensibilizadores de la piel.

Una vez que los riesgos de alergia se han caracterizado bien, la evaluación de riesgos, la gestión de riesgos y la comunicación sobre los riesgos son elementos decisivos para reducir la incidencia de los trastornos alérgicos. La evaluación de riesgos requiere que el riesgo, de una potencia conocida, se evalúe atendiendo a la naturaleza y la magnitud de la exposición. Cuando la evaluación de riesgos indica que

es necesario, se deben aplicar medidas de gestión de los riesgos, tales como el control de la exposición y el etiquetado de productos.

Se están adqduiriendo más conocimientos sobre las características fisicoquímicas e inmunológicas de alergenos alimentarios, que con el tiempo podrán llegar a tener valor predictivo.

Dada la complejidad de los mecanismos de los trastornos alérgicos, por el momento resulta difícil proponer métodos preditivos *in vitro* de aplicabilidad general; no obstante, la aplicación de las relaciones entre actividad y estructura merece mayor consideración.

2. RECOMENDACIONES PARA LA PROTECCIÓN DE LA SALUD HUMANA

a) Para prevenir la alergia se deben aplicar estrategias eficaces basadas en buena información acerca de los alergenos presentes en los productos y el medio ambiente. El control de la exposición debe ser la base de la prevención o la reducción al mínimo de la aparición de enfermedades alérgicas.

b) Hay una necesidad urgente de determinar las causas del aumento de la frecuencia de la alergia.

c) Se deben adoptar métodos de vigilancia para definir la frecuencia de alergias de diferentes tipos.

d) Puede ser difícil medir la exposición de los individuos y las poblaciones, pero una evaluación adecuada es esencial para cualquier análisis de la asociación entre la exposición y el efecto. La especificidad de las respuestas inmunitarias representa un tipo único de marcador biológico a la hora de estudiar la exposición pasada, por ejemplo mediante la utilización de pruebas epicutáneas o de puntura, o la valoración de los anticuerpos de IgE para detectar la sensibilización por alergenos específicos.

e) Se deben mejorar los sistemas de vigilancia de los trabajadores, la calidad del examen médico de éstos y la educación de los expuestos

a productos químicos para detectar enfermedades alérgicas ocupacionales en un estadio inicial. Los sistemas relativamente sencillos de notificación de los trastornos ocupacionales y la vigilancia posterior a la comercialización de los medicamentos constituyen formas económicas de reconocimiento y alerta en relación con las enfermedades alérgicas. La vigilancia de estos trastornos en el lugar de trabajo es particularmente valiosa porque es allí donde la exposición probablemente sea mayor que en cualquier otro lugar.

f) La eficacia y el valor de las estrategias de prevención primaria y secundaria e intervención deben evaluarse a intervalos que utilizan técnicas epidemiológicas validadas.

g) Con respecto a la dermatitis de contacto alérgica, los modelos preditivos *in vivo* disponibles tienen un valor comprobado para los antígenos de masa molecular relativa baja. Es necesario descubrir la mejor manera de utilizarlos para determinar la potencia de los alergenos.

h) Con respecto a los trastornos alérgicos de las vías respiratorias, los métodos de prueba *in vivo* disponibles para las sustancias de masa molecular relativa baja son prometedores, pero es necesario comprobar su valor predictivo y su especificidad. Para los alergenos proteicos, están desarrollándose algunos métodos de prueba en animales, pero es preciso someterlos a evaluaciones ulteriores utilizando sustancias de potencial alergénico conocido en los seres humanos.

i) Es importante que la información acerca de la presencia de alergenos en los productos (por ejemplo alimentos) esté fácilmente disponible, por ejemplo en bases de datos y en las etiquetas de los productos, para que las instancias reguladoras y los individuos puedan tomar precauciones apropiadas.

j) El método principal para prevenir enfermedades autoinmunitarias ocupacionales inducidas químicamente es el control de la exposición. Se debe considerar la evaluación previa a la exposición de los expuestos a los productos químicos con potencial inmunomodelador para documentar cualquier característica preexistente de morbilidad del tejido conectivo. Se recomienda una adhesión estricta a las normas

para evitar o reducir al mínimo la exposición, inclusive la utilización de buenas prácticas de higiene ocupacional. Se deben reducir al mínimo otros factores de riesgo, como el consumo de tabaco y se debe considerar la posibilidad de efectuar exámenes médicos ocupacionales regulares.

k) Es necesario investigar las relaciones cuantitativas existentes entre las respuestas inmunitarias inducido por los productos químicos y la gravedad de las reacciones alérgicas.

l) Para controlar las enfermedades alérgicas es importante determinar la exposición mínima y su duración necesaria para causar sensibilización o producir una respuesta alérgica.

m) Es importante idear estrategias normalizadas de investigación y diagnóstico clínicos de la alergia y aplicarlas internacionalmente para examinar las causas y la incidencia de los trastornos alérgicos. Ello requerirá la producción y la disponibilidad de extractos normalizados de alergenos biológicos de potencia controlada, así como el examen regular de los componentes de series normalizadas de alergenos utilizados en las pruebas.

n) Las autoridades de salud pública, los profesionales de la salud y las dependencias gubernamentales deben examinar la manera de calcular los costos humanos y económicos de las enfermedades alérgicas para los individuos y la sociedad.

o) Las autoridades de salud pública, los profesionales de la salud, el público y, especialmente, los trabajadores necesitan mejor información acerca de la frecuencia, las causas, las manifestaciones clínicas y las consecuencias de diferentes tipos de alergia.

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