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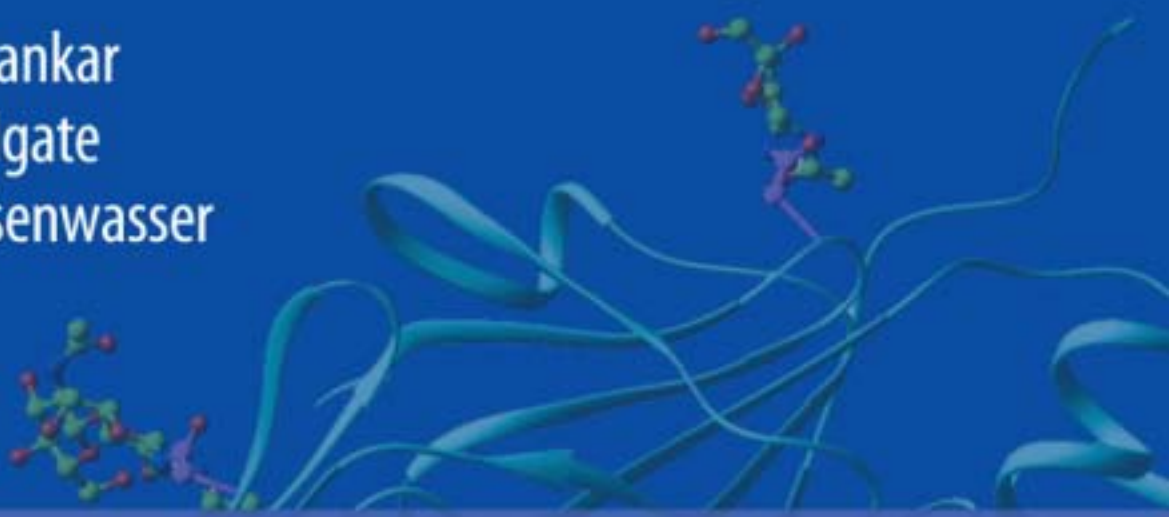

Allergy Frontiers:

Epigenetics, Allergens and Risk Factors

Volume 1

Springer

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ISBN: 978-4-431-72801-6 Springer Tokyo Berlin Heidelberg New York
e-ISBN: 978-4-431-72802-3
DOI: 10.1007/978-4-431-72802-3

Library of Congress Control Number: 2008941262

Springer is a part of Springer Science+Business Media
springer.com

Foreword

When I entered the field of allergy in the early 1970s, the standard textbook was a few hundred pages, and the specialty was so compact that texts were often authored entirely by a single individual and were never larger than one volume. Compare this with *Allergy Frontiers: Epigenetics, Allergens, and Risk Factors*, the present six-volume text with well over 150 contributors from throughout the world. This book captures the explosive growth of our specialty since the single-author textbooks referred to above.

The unprecedented format of this work lies in its meticulous attention to detail yet comprehensive scope. For example, great detail is seen in manuscripts dealing with topics such as "Exosomes, naturally occurring minimal antigen presenting units" and "Neuropeptide S receptor 1 (NPSR1), an asthma susceptibility gene." The scope is exemplified by the unique approach to disease entities normally dealt with in a single chapter in most texts. For example, anaphylaxis, a topic usually confined to one chapter in most textbooks, is given five chapters in *Allergy Frontiers*. This approach allows the text to employ multiple contributors for a single topic, giving the reader the advantage of being introduced to more than one viewpoint regarding a single disease.

This broad scope is further illustrated in the way this text deals with the more frequently encountered disorder, asthma. There are no fewer than 26 chapters dealing with various aspects of this disease. Previously, to obtain such a comprehensive approach to a single condition, one would have had to purchase a text devoted solely to that disease state.

In addition, the volume includes titles which to my knowledge have never been presented in an allergy text before. These include topics such as "NKT ligand conjugated immunotherapy," "Hypersensitivity reactions to nano medicines: causative factors and optimization," and "An environmental systems biology approach to the study of asthma."

It is not hard to see that this textbook is unique, offering the reader a means of obtaining a detailed review of a single highly focused subject, such as the neuropeptide S receptor, while also providing the ability to access a panoramic and remarkably in-depth view of a broader subject, such as asthma. Clearly it is intended primarily for the serious student of allergy and immunology, but can also serve as a resource text for those with an interest in medicine in general.

I find it most reassuring that even though we have surpassed the stage of the one-volume, single-author texts, because of the wonderful complexity of our specialty and its broadening scope that has evolved over the years, the reader can still obtain an all-inclusive and comprehensive review of allergy in a single source. It should become part of the canon of our specialty.

Phil Lieberman, M.D.

Foreword

When I started immunology under Professor Kimishige Ishizaka in the early 1950s, allergy was a mere group of odd syndromes of almost unknown etiology. An immunological origin was only suspected but not proven. The term "atopy," originally from the Greek word *à-topòs*, represents the oddness of allergic diseases. I would call this era "stage 1," or the primitive era of allergology.

Even in the 1950s, there was some doubt as to whether the antibody that causes an allergic reaction was really an antibody, and was thus called a "reagin," and allergens were known as peculiar substances that caused allergy, differentiating them from other known antigens.

It was only in 1965 that reagin was proven to be an antibody having a light chain and a unique heavy chain, which was designated as IgE in 1967 with international consensus. The discovery of IgE opened up an entirely new era in the field of allergology, and the mechanisms of the immediate type of allergic reaction was soon evaluated and described. At that point in time we believed that the nature of allergic diseases was a mere IgE-mediated inflammation, and that these could soon be cured by studying the IgE and the various mediators that induced the inflammation. This era I would like to call "stage 2," or the classic era.

The classic belief that allergic diseases would be explained by a mere allergen-IgE antibody reaction did not last long. People were dismayed by the complexity and diversity of allergic diseases that could not be explained by mere IgE-mediated inflammation. Scientists soon realized that the mechanisms involved in allergic diseases were far more complex and that they extended beyond the conventional idea of a pure IgE-mediated inflammation. A variety of cells and their products (cytokines/chemokines and other inflammatory molecules) have been found to interact in a more complex manner; they create a network of reactions via their receptors to produce various forms of inflammatory changes that could never be categorized as a single entity of inflammation. This opened a new era, which I would like to call the modern age of allergology or "stage 3."

The modern era stage 3 coincided with the discovery that similar kinds of cytokines and cells are involved in the regulation of IgE production. When immunologists investigated the cell types and cytokines that regulate IgE production,

they found that two types of helper T cells, distinguishable by the profile of cytokines they produce, play important regulatory roles in not only IgE production but also in regulating allergic inflammation. The advancement of modern molecular technologies has enabled detailed analyses of molecules and genes involved in this extremely complex regulatory mechanism. Hence, there are a number of important discoveries in this area, which are still of major interest to allergologists, as can be seen in the six volumes of this book.

We realize that allergology has rapidly progressed during the last century, but mechanisms of allergic diseases are far more complex than we had expected. New discoveries have created new questions, and new facts have reminded us of old concepts. For example, the genetic disposition of allergic diseases was suspected even in the earlier, primitive era but is still only partially proven on a molecular basis. Even the molecular mechanisms of allergic inflammation continue to be a matter of debate and there is no single answer to explain the phenomenon. There is little doubt that the etiology of allergic diseases is far more varied and complex than we had expected. An immunological origin is not the only mechanism, and there are more unknown origins of similar reactions. Although therapeutic means have also progressed, we remain far from our goal to cure and prevent allergic diseases.

We have to admit that while we have more knowledge of the many intricate mechanisms that are involved in the various forms of allergic disease, we are still at the primitive stage of allergology in this respect. We are undoubtedly proceeding into a new stage, stage 4, that may be called the postmodern age of allergology and hope this era will bring us closer to finding a true solution for the enigma of allergy and allergic diseases.

We are happy that at this turning point the editors, Ruby Pawankar, Stephen Holgate, and Lanny Rosenwasser, are able to bring out such a comprehensive book which summarizes the most current knowledge on allergic diseases, from epidemiology to mechanisms, the impact of environmental and genetic factors on allergy and asthma, clinical aspects, recent therapeutic and preventive strategies, as well as future perspectives. This comprehensive knowledge is a valuable resource and will give young investigators and clinicians new insights into modern allergology which is an ever-growing field.

Tomio Tada, M.D., Ph.D., D.Med.Sci.

Foreword

Allergic diseases represent one of the major health problems in most modern societies. The increase in prevalence over the last decades is dramatic. The reasons for this increase are only partly known. While in former times allergy was regarded as a disease of the rich industrialized countries only, it has become clear that all over the world, even in marginal societies and in all geographic areas—north and south of the equator—allergy is a major global health problem.

The complexity and the interdisciplinary character of allergology, being the science of allergic diseases, needs a concert of clinical disciplines (internal medicine, dermatology, pediatrics, pulmonology, otolaryngology, occupational medicine, etc.), basic sciences (immunology, molecular biology, botany, zoology, ecology), epidemiology, economics and social sciences, and psychology and psychosomatics, just to name a few. It is obvious that an undertaking like this book series must involve a multitude of authors; indeed, the wide spectrum of disciplines relevant to allergy is reflected by the excellent group of experts serving as authors who come from all over the world and from various fields of medicine and other sciences in a pooling of geographic, scientific, theoretical, and practical clinical diversity.

The first volume concentrates on the basics of etiology, namely, the causes of the many allergic diseases with epigenetics, allergens and risk factors. Here, the reader will find up-to-date information on the nature, distribution, and chemical structure of allergenic molecules, the genetic and epigenetic phenomena underlying the susceptibility of certain individuals to develop allergic diseases, and the manifold risk factors from the environment playing the role of modulators, both in enhancing and preventing the development of allergic reactions.

In times when economics plays an increasing role in medicine, it is important to reflect on this aspect and gather the available data which—as I modestly assume—may be yet rather scarce. The big effort needed to undertake well-controlled studies to establish the socio-economic burden of the various allergic diseases is still mainly ahead of us. The Global Allergy and Asthma European Network (GA2LEN), a group of centers of excellence in the European Union, will start an initiative regarding this topic this year.

In volume 2, the pathomechanisms of various allergic diseases and their classification are given, including such important special aspects as allergy and the bone marrow, allergy and the nervous system, and allergy and mucosal immunology.

Volume 3 deals with manifold clinical manifestations, from allergic rhinitis to drug allergy and allergic bronchopulmonary aspergillosis, as well as including other allergic reactions such as lactose and fructose intolerances.

Volume 4 deals with the practical aspects of diagnosis and differential diagnosis of allergic diseases and also reflects educational programs on asthma.

Volume 5 deals with therapy and prevention of allergies, including pharmacotherapy, as well as allergen-specific immunotherapy with novel aspects and special considerations for different groups such as children, the elderly, and pregnant women.

Volume 6 concludes the series with future perspectives, presenting a whole spectrum of exciting new approaches in allergy research possibly leading to new strategies in diagnosis, therapy, and prevention of allergic diseases.

The editors have accomplished an enormous task to first select and then motivate the many prominent authors. They and the authors have to be congratulated. The editors are masters in the field and come from different disciplines. Ruby Pawankar, from Asia, is one of the leaders in allergy who has contributed to the understanding of the cellular and immune mechanisms of allergic airway disease, in particular upper airway disease. Stephen Holgate, from the United Kingdom, has contributed enormously to the understanding of the pathophysiology of allergic airway reactions beyond the mere immune deviation, and focuses on the function of the epithelial barrier. He and Lanny Rosenwasser, who is from the United States, have contributed immensely to the elucidation of genetic factors in the susceptibility to allergy. All three editors are members of the Collegium Internationale Allergologicum (CIA) and serve on the Board of Directors of the World Allergy Organization (WAO).

I have had the pleasure of knowing them for many years and have cooperated with them at various levels in the endeavor to promote and advance clinical care, research, and education in allergy. Together with Lanny Rosenwasser as co-editor-in-chief, we have just started the new *WAO Journal* (electronic only), where the global representation in allergy research and education will be reflected on a continuous basis.

Finally, Springer, the publisher, has to be congratulated on their courage and enthusiasm with which they have launched this endeavor. Springer has a lot of experience in allergy—I think back to the series *New Trends in Allergy*, started in 1985, as well as to my own book *Allergy in Practice,* to the *Handbook of Atopic Eczema* and many other excellent publications.

I wish this book and the whole series of *Allergy Frontiers* complete success! It should be on the shelves of every physician or researcher who is interested in allergy, clinical immunology, or related fields.

Johannes Ring, M.D., Ph.d.

Preface

Allergic diseases are increasing in prevalence worldwide, in industrialized as well as industrializing countries, affecting from 10%–50% of the global population with a marked impact on the quality of life of patients and with substantial costs. Thus, allergy can be rightfully considered an epidemic of the twenty-first century, a global public health problem, and a socioeconomic burden. With the projected increase in the world's population, especially in the rapidly growing economies, it is predicted to worsen as this century moves forward.

Allergies are also becoming more complex. Patients frequently have multiple allergic disorders that involve multiple allergens and a combination of organs through which allergic diseases manifest. Thus exposure to aeroallergens or ingested allergens frequently gives rise to a combination of upper and lower airways disease, whereas direct contact or ingestion leads to atopic dermatitis with or without food allergy. Food allergy, allergic drug responses and anaphylaxis are often severe and can be life-threatening. However, even the less severe allergic diseases can have a major adverse effect on the health of hundreds of millions of patients and diminish quality of life and work productivity. The need of the hour to combat these issues is to promote a better understanding of the science of allergy and clinical immunology through research, training and dissemination of information and evidence-based better practice parameters.

Allergy Frontiers is a comprehensive series comprising six volumes, with each volume dedicated to a specific aspect of allergic disease to reflect the multidisciplinary character of the field and to capture the explosive growth of this specialty. The series summarizes the latest information about allergic diseases, ranging from epidemiology to the mechanisms and environmental and genetic factors that influence the development of allergy; clinical aspects of allergic diseases; recent therapeutic and preventive strategies; and future perspectives. The chapters of individual volumes in the series highlight the roles of eosinophils, mast cells, lymphocytes, dendritic cells, epithelial cells, neutrophils and T cells, adhesion molecules, and cytokines/chemokines in the pathomechanisms of allergic diseases. Some specific new features are the impact of infection and innate immunity on allergy, and mucosal immunology of the various target organs and allergies, and the impact of the nervous system on allergies. The most recent, emerging therapeutic strategies

are discussed, including allergen-specific immunotherapy and anti-IgE treatment, while also covering future perspectives from immunostimulatory DNA-based therapies to probiotics and nanomedicine.

A unique feature of the series is that a single topic is addressed by multiple contributors from various fields and regions of the world, giving the reader the advantage of being introduced to more than one point of view and being provided with comprehensive knowledge about a single disease. The reader thus obtains a detailed review of a single, highly focused topic and at the same time has access to a panoramic, in-depth view of a broader subject such as asthma.

The chapters attest to the multidisciplinary character of component parts of the series: environmental, genetics, molecular, and cellular biology; allergy; otolaryngology; pulmonology; dermatology; and others. Representing a collection of state-of-the-art reviews by world-renowned scientists from the United Kingdom and other parts of Europe, North America, South America, Australia, Japan, and South Africa, the volumes in this comprehensive, up-to-date series contain more than 150 chapters covering virtually all aspects of basic and clinical allergy. The publication of this extensive collection of reviews is being brought out within a span of two years and with the greatest precision to keep it as updated as possible. This six-volume series will be followed up by yearly updates on the cutting-edge advances in any specific aspect of allergy.

The editors would like to sincerely thank all the authors for having agreed to contribute and who, despite their busy schedules, contributed to this monumental work. We also thank the editorial staff of Springer Japan for their assistance in the preparation of this series. We hope that the series will serve as a valuable information tool for scientists and as a practical guide for clinicians and residents working and/or interested in the field of allergy, asthma, and immunology.

Ruby Pawankar, Stephen Holgate, and Lanny Rosenwasser

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The Allergy Epidemic: A Look into the Future

U. Wahn

Over the past decades, the increasing rates of allergic conditions among affluent societies have posed a heavy burden on healthcare systems. Cross-sectional studies such as the International Study of Asthma and Allergies in Childhood (ISAAC) have confirmed that atopic diseases such as atopic dermatitis, asthma, and seasonal allergic rhinoconjunctivitis represent major health problems in many countries, particularly in childhood [1].

During the past 2 decades, two general hypotheses have been proposed in the literature in connection with the observed increases of atopy and asthma in childhood:

New risk factors that were not known several decades ago might have become relevant in connection with nutrition, environmental exposure, and lifestyle.

Protective factors related to a more traditional lifestyles common in the past might have been lost, which could have led to increased susceptibility to atopic diseases.

The Atopic March

The term "atopic march" refers to the natural history of atopic manifestations, characterized by the typical sequences of immunoglobulin E (IgE) antibody responses and clinical symptoms that appear during a certain age period, persist over years and decades, and often show a tendency for spontaneous remission with time [2].

Prospective cohort studies have shown that sensitization to food allergens occurs usually during the first months of life with the antibody response to cow's milk and hen's egg occurring most frequently. Sensitization against inhalant allergens usually develops after the first 2 years of life. Most of these children will develop IgE responses to a wide array of environmental allergens such as house dust mites, animal dander, and pollen [2–7].

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_1, © Springer 2009

Specific patterns of atopic sensitization are associated with certain atopic illnesses. Atopic eczema is primarily related to IgE responses to dietary allergen, while individuals with allergic rhinitis tend to become sensitized to seasonal outdoor allergens. Specific IgE responses in asthmatic children are usually directed against perennial and indoor allergen such as house dust mites. Several studies have shown that early sensitization during infancy is a predictor for the persistence of childhood asthma until adolescence [8].

In the German Multicenter Allergy Study, food sensitization before age 1 to 2 years with or without concurrent inhalant sensitization was a strong predictor for the development of asthma and airway hyper-responsiveness until school age [9–11].

Our understanding of the determinants of the natural history of allergic diseases is limited. Although a strong genetic basis for atopy and asthma has been described and several genes have been identified, which are associated with different phenotypes [12, 13], a variety of modifiable environmental and lifestyle factors have been discovered in the past, which might offer future options for primary prevention.

Allergen Exposure

Exposure to environmental allergens is the most extensively studied potential risk factor for sensitization and manifestation of atopy and asthma. From a number of cross-sectional studies performed in children and adults, it has become obvious that there is a close association between allergen exposure, particularly in the domestic environment, and sensitization to that specific allergen. Longitudinal studies such as the MAS (Multicenter Allergy Study) study in Germany have clearly demonstrated that during the first years of life there is a dose–response relationship between indoor allergen exposure to dust mite and cat allergens and the risk of sensitization to cat and mites, respectively [14–20].

As far as the manifestation of atopic dermatitis and asthma are concerned, the situation is much less clear. Early studies performed by Sporik et al. [21] suggested that exposure of sensitized children to dust mite allergens determines not only the risk of asthma but also the time of the onset of the disease. More recent investigations by the same group, however, suggest that other factors besides allergen exposure are important in determining which children develop asthma.

In a comprehensive meta-analysis, Peat and Woolcock [22] and Peat et al. [23] evaluated several environmental factors said to be responsible for the incidence and severity of atopic diseases, particularly asthma. After comparing the strengths of the various effects, she concluded that on the basis of the literature, indoor allergen exposure is the environmental component with by far the strongest impact on the manifestation of asthma. In recent years, however, the paradigm that exposure induces asthma with airway inflammation via sensitization has been challenged. In several countries, the prevalence of asthma in children has been increasing independent of allergen exposure [22, 23].

Data sets obtained from the MAS birth cohort suggest that while domestic allergen exposure is a strong determinant for early sensitization in childhood, it cannot be

considered as a primary cause of airway hyper-responsiveness or asthmatic symptoms, since during the first 3 years of life the manifestation of wheeze is not related to elevated serum IgE levels or specific sensitization. Studies following up birth cohorts to adolescence have recently indicated that 90% of children with wheeze but without atopy lose their symptoms at school age and retain normal lung function in puberty (Fig. 1). By contrast, sensitization to perennial allergens (house dust mites, cats, and dogs) developing in the first 3 years of life was associated with a loss of lung function at school age. Concomitant exposure to high levels of perennial allergens early in life aggravates this process. Such exposure also enhances the development of airway hyper-responsiveness in sensitized children with wheeze. From these data, it can be concluded that impairment of lung function during school age is determined by continuing allergic airway inflammation beginning in the first 3 years of life [9].

A number of intervention studies to examine the effects of indoor allergen elimination on the incidence of asthma are currently being performed in cohorts followed prospectively from birth [24]. The results will have a strong impact on public health policies because they will determine whether considering indoor allergen elimination as an important element of primary prevention of various atopic manifestations is meaningful. Even if the result is that other factors play major parts in determining whether an atopic child will develop asthma, so that allergen elimination as a measure of primary prevention is inefficient, reduction of allergen exposure will still remain as a very important element in secondary prevention.

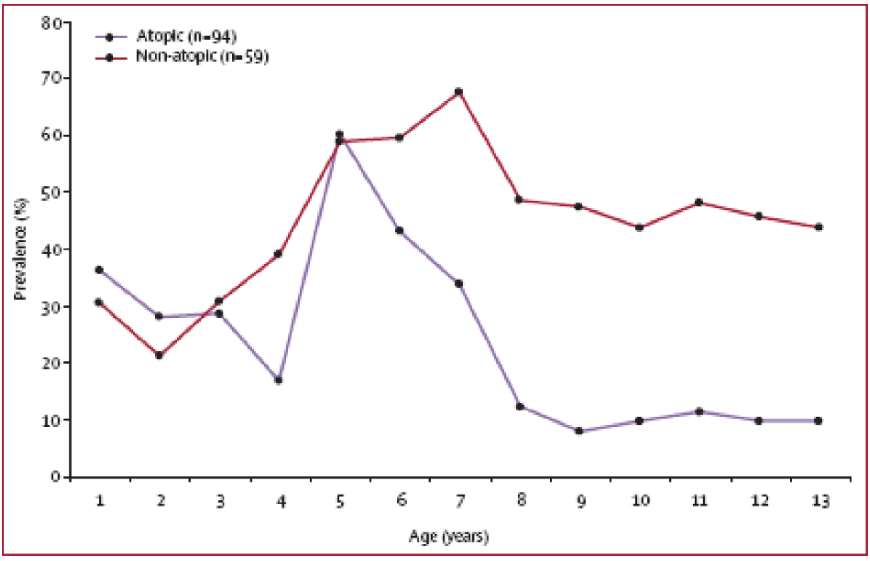

Fig. 1 School age (5–7 years), stratified for atopy at school age. Of the 178 children with wheeze at school age, 153 had measurements of immunoglobulin E at school age. Prevalence of current wheeze from birth to age 13 years in children with any wheezing episode

Pollutants and Tobacco Smoke

Other environmental factors have attracted the interest of epidemiologists and experimental researchers. Although they do not serve as allergens, these factors are capable of up-regulating existing IgE responses or leading to disease manifestation or aggravation of symptoms. Guinea pig and mouse experiments suggested an increase of allergic sensitization to ovalbumin after experimental exposure to traffic- or industry-related pollutants. A strong association between allergic rhinitis caused by cedar pollen allergy and exposure to heavy traffic was reported in Japan. Important sociodemographic confounders turned out to be problems in interpreting study results. Other investigators were unable to describe any relationship between traffic exposure and the prevalence of hay fever or asthma. The role of tobacco smoke, a complex mixture of various particles and organic compounds, was extensively studied.

Recent review studies consistently demonstrate that the risk of lower airway diseases such as bronchitis, recurrent wheezing in infants, and pneumonia is increased. Whether passive tobacco smoke exposure is causally related to the development of asthma is still disputed [25–28].

Until recently, data about the risk of sensitization have been lacking. The prospective birth cohort MAS in Germany suggests that an increased risk of sensitization is found only in children whose mothers smoked up to the end of their pregnancies and continued to smoke after childbirth. In this subgroup of the cohort, a significantly increased sensitization rate of IgE antibodies to food proteins, particularly to hen's egg and cow's milk, was observed during infancy. The effect of environmental tobacco smoke exposure is particularly strong in families with susceptibility for atopy [25].

Lifestyle

Obviously, a long list of lifestyle-related factors possibly associated with the apparent allergy and asthma epidemic of the late twentieth and early twenty-first centuries may have relevance to the atopic march in children.

Taking into account that the risk of atopic sensitization and disease manifestation early in life is particularly high in industrialized Western countries [29], and that within these countries concomitant variations in the socioeconomic status and the prevalence of atopy are evident [30], the question arises as to what factor related to Western lifestyle may be responsible for increasing the susceptibility to atopic sensitization? In a recent Swedish study, the prevalence of atopy in children from anthroposophic families was lower than in children from other types of families. This led the authors to the conclusion that lifestyle factors associated with *anthroposophy* (no vaccination, low exposure to antibodies, etc.) may lessen the risk of atopy in childhood [31].

Several studies focusing on differences between the former socialist countries and Western European countries reported lower prevalence rates for atopy in the former East.

The differences were particularly striking in the areas with few genetic differences such as East and West Germany where it was found that the critical period during which lifestyle mainly influences the development of atopy is probably the first years of life [32, 33]. These observations point in the same direction as studies reporting lower prevalence rates for children born into families that have few siblings. Recent observations from Germany suggest that within the population of an industrialized country with a Western lifestyle, high socioeconomic status must be considered as a risk factor for early sensitization and the manifestations of atopic dermatitis and allergic airway disease [29]. Turkish migrants living in Germany exhibited higher prevalences of atopy and asthma after cultural assimilation [34].

Differences in the intestinal microflora as a major source of microbial stimulation of the immune system in early childhood has been proposed as a possible explanation for this observation [35, 36]. The intestinal microflora have been shown to enhance Th1-type responses. The results of a comparative study of Estonian and Swedish children demonstrated differences in intestinal microflora. In Estonia, the typical microflora included more lactobacilli and fewer clostridia organisms that are associated with a lower presence of atopic disease. Intervention studies are needed to demonstrate the relevance of these findings and examine the effects of adding probiotics to infant formulas. In one study from Finland, which unfortunately was not blinded, infants with milk allergy and atopic dermatitis exhibited milder symptoms and fewer markers of intestinal inflammation if they were fed lactobacilli-fortified milk formula [37].

Few reports have described an association between the use of antibiotics during the first 2 years of life and increased risks of asthma. It seems too early to draw final conclusions from these publications.

Immunizations against infectious diseases do not appear to influence the risk of early sensitization or development of atopy. Physicians should therefore support successful immunization programs such as those targeting measles.

Early Exposure to Infections or Microbial Products?

One hypothesis that has attracted considerable interest is that a decline in certain childhood infections or a lack of exposure to infectious agents during the first years of life associated with smaller families in the middle class environments of industrialized countries may be causal for the recent epidemic in atopic disease and asthma [38]. Although this hypothesis is obviously very complex, various sources of information appear to support it. Studies from several countries provide indirect evidence for the hypothesis that early exposure to viral infections, although triggering lower airway symptoms during early life, may exert long-lasting protective effects. Children born into families with several siblings, especially

older siblings, have been found to have reduced risk of allergic sensitization and asthma at school age. Studies in children who attended day-care centers during infancy support this concept. Infections are known to produce long-lasting nonspecific systemic effects on the nature of the immune response to antigens and allergens. For example, recovery from natural measles infection reduces the incidence of atopy and allergic responses to house dust mites to half the rate found in vaccinated children [39–49].

Obviously, the fact that certain infections induce a systemic and nonspecific switch to Th1 cells may be responsible for inhibiting the development of atopy during childhood.

Observations from Japan suggesting that strong positive tuberculin responses in children predict a lower incidence of asthma, lower serum IgE levels, and cytokine profiles biased toward a Th1-type were supported by animal experiments demonstrating that IgE responses to ovalbumin in mice could be down-regulated by a previous infection with bacillus Calmette-Guerin (BCG).

Unfortunately, cohort studies from Europe were unable to describe any protective effect of BCG vaccination [50–52].

Although these observations on the relationship of immune responses to infectious agents, atopic sensitization, and disease expression are stimulating and challenging, conclusions regarding the relevance of the atopic march should be drawn with care. In different parts of the world, completely different infectious agents have been addressed in different study settings. It appears to be fashionable to join Rook and Stanford [53] who, in a recent review article pleaded "*Give us this day our daily germs*"—but which germ, at what time, under which circumstances, and at what price?

Farming Environment

In farming environments where animals such as cattle, pigs, and poultry are kept, microbial products are particularly abundant. Accumulating evidence indicates that children growing up on traditional dairy farms have a significantly lower prevalence of atopic sensitization, hay fever, and asthma when compared with children from the same rural areas but not raised on farms. Interestingly, no protective effect of a farming environment was seen for the prevalence of atopic dermatitis.

Contact with livestock and poultry was found to explain much of the relation between farming and atopy. Exposure to the farm environment during the first year of life or even before birth, and the dose and duration of exposure from the first to the fifth years of life were crucial for this protective effect. Children exposed to animal stables or unpasteurized milk in the first year of life, in contrast to later exposure, had a significantly reduced prevalence of asthma, whereas continued exposure was relevant for the protection from atopy and hay fever [54–58].

Endotoxin

Microbial exposures are abundant in these environments and microbial studies investigating stables report a large variety of gram-negative and gram-positive germs as well as a diversity of molds and fungi.

In addition, nonviable parts of microbes, such as endotoxin from the outer wall of gram-negative bacteria, are found in abundance in stables and also in elevated concentrations in indoor environments of adjacent farmhouses.

Endotoxins are a family of molecules called lipopolysaccharides (LPS) and are intrinsic parts of the outer membranes of gram-negative bacteria. LPS and other bacterial wall components are found in high concentrations in stables, where pigs, cattle, and poultry are kept engaged with antigen-presenting cells via CD14 ligation to induce strong interleukin (IL)-12 responses. IL-12, in turn, is regarded as an obligatory signal for the maturation of naive T cells into Th1-type cells. Endotoxin concentrations were recently found to be highest in stables of farming families and also in dust samples from kitchen floors and mattresses in rural areas in southern Germany and Switzerland. These findings support the hypothesis that environmental exposure to endotoxins and other bacterial wall components is an important protective determinant related to the development of atopic diseases. Indeed, endotoxin levels in samples of dust from children's mattresses were found to be inversely related to the rate of occurrence of hay fever, atopic asthma, and atopic sensitization [59, 60].

On the other hand, high exposure to endotoxins may only be a surrogate marker for other bacterial products such as nonmethylated cytidine-guanosine, dinucleotides specific for prokaryotic DNA (CpG motifs). Cell wall components from atypical mycobacteria or gram-positive bacteria, such as lipoteichoic acid, are known to affect immune responses in ways similar to endotoxin.

Primary Prevention: The Challenge of the Future

In an attempt to reverse the observed epidemiological trend, primary prevention strategies for decades aimed at avoiding risk factors and inhibiting their mechanism of action. More recently, attempts were initiated to promote protecting factors and stimulate their mechanisms of action.

Alimentary Ways to Protect

For numerous reasons, breast-feeding is the preferred method of infant nutrition; however, there is still controversy as to whether breast-feeding protects against the development of allergic diseases.

On the basis of the available data, an "Expert Group" of the "European Academy of Allergology and Clinical Immunology" recommends exclusively breast-feeding for 4 to 6 month irrespective of family history of atopy.

For a long time, primary prevention strategies for asthma were almost exclusively focused on allergen avoidance measures early in life, which were supposed to prevent primary sensitization to both food and inhalant allergens.

For several years, the use of hydrolyzed formula was recommended as an alternative for infants, for whom breast milk was not available and who were genetically predisposed to atopic diseases. Indeed, the German Infant Nutritional Intervention (GINI) Study demonstrated that extensively as well as certain partially hydrolyzed formulas compared to unhydrolyzed infant formulas resulted in a lower incidence of atopic eczema during the first 3 years of life. This study still represents the only large and well-designed trial when comparing different formulas in relation to primary prevention of atopic dermatitis and sensitization to food proteins [61–63].

More recently, new alimentary strategies to prevent allergic manifestations are being studied. These include supplementation with probiotics (e.g., lactobacilli) or prebiotics (oligosaccharides influencing the intestinal microflora). So far, the information from the initial studies on supplementation with probiotics is inconclusive. It will be interesting to see the outcomes of well-designed intervention studies focused on the efficacy of this approach [64, 65].

The Avoidance Concept

Since indoor allergen exposure was shown to be associated with allergic sensitization, which on the other hand was associated with childhood asthma, it was understandable that the first intervention studies aiming at primary prevention of early sensitization and the development of allergic airway disease have concentrated on indoor allergen avoidance [66, 67].

The earliest trial, the Isle of Wight study, showed that children at the age of 8 years tended to have less wheeze and a lower risk for mite sensitization following the avoidance of early house dust mite allergen contact [68]. In contrast, the Study of Prevention of Allergy in Children in Europe (SPACE) was not able to show a significant benefit in the intervention group (mattress covers) [69, 70]. In the Manchester Allergy and Asthma Study (MAAS), 291 infants—at high risk because both parents were atopic and there were pets in the home—were recruited, and a number of avoidance measures were instituted to decrease inhalant allergen exposure [71, 72]. The group was able to demonstrate that the avoidance measures were capable of achieving and maintaining a low dust allergen environment during pregnancy and for the first 3 years of these children. At age 3 years, children in the active group had less wheeze and a lower airway resistance; however, the sensitization rate to mites was higher than that in the control group [73].

In the Dutch Prevention of Incidence of Asthma and Mite Allergy (PIAMA) study, the intervention had a significant effect on mite allergen levels, but no effect was seen on respiratory symptoms, atopic dermatitis, or total and specific immunoglobulin E levels [74].

So far, we must admit that recommendations to families for primary prevention of asthma should be given with caution, as no single approach can definitively prevent children from developing asthma.

Perspectives

The challenge of primary and secondary prevention of atopy and asthma has stimulated a variety of prospective interventional trials that are currently ongoing all over the world (Table 1). Unfortunately, pharmacotherapeutic trials that aimed at long-term disease modification with an inhaled corticosteroid, or prevention of asthma in children with atopic dermatitis by giving an H_1-antihistamine such as cetirizine or levocetirizine, have failed to provide more than symptomatic relief during treatment. A long-term prevention study with a calcineurin inhibitor is currently underway.

On the basis of encouraging animal studies, avoidance studies including elimination of alimentary proteins as well as indoor allergens or tobacco smoke, and intervention with oral application of endotoxin, or exposure to mycobacteria or parasites are being conducted. Finally, trials aimed at nonspecific or specific induction of tolerance have recently been initiated.

Allergy immunotherapy has been based on antigen-specific stimulation of the adaptive immune system (by subcutaneous or sublingual specific immunotherapy) for a century. However, the most recent evolution modified our immune system in such a way that allergy is no longer the rare exception but is becoming increasingly prevalent. Factors once abundant in our environment that normally stimulated our innate immune system to protect us from allergy development are now missing more and more often. Several categories of new intervention strategies for allergy prevention are based on this concept: induction of immune functions that are able

Table 1 Possible preventative strategies under investigation in experimental animals and humans.

Avoidance of risks	Exposure to alimentary proteins (breast-feeding, hydrolyzed formulas)
	Exposure to indoor allergens
	Exposure to tobacco smoke
Providing protection	Exposure to endotoxin (LPS)
	Exposure to microbacteria vaccae
	Exposure to parasites/trichinosis suis
Nonspecific or specific induction of tolerance:	Modify intestinal flora
	Mucosal tolerance induction to specific allergens

LPS, lipopolysaccharides.

to down-regulate unwanted immune responses against allergens and suppress allergen-induced inflammation. These new preventive and therapeutic strategies are not limited to respiratory allergies, but involve food allergies as well.

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Is the Prevalence of Allergy Continuously Increasing?

Carlos E. Baena-Cagnani and R. Maximiliano Gómez

Health systems and investigators worldwide have been asking themselves for many years whether the prevalence of atopic illnesses has been increasing continuously. It is mandatory to consider studies using comparable methods to validate these results.

The Aberdeen study considered the presence of asthma diagnosis, wheezing, eczema, and rhinitis between the decades of 1960 and 1990, showing a significant increase in all of them, not attributable to a diagnosis fashion but to a truly change in prevalence, using the same methodology in two time points in 25 years [1]. In this population and throughout these years, the proportion of wheezing increased from 10% to almost double, diagnosis of asthma from 4% to 10%, rhinitis from 3% to almost four times, and eczema from 5% to more than double. All these variables increased particularly noticeable in boys.

Is the Prevalence of Asthma Continuously Increasing?

In Finnish young men, the incremental tendency of asthma diagnosis remained from 0.29% in 1966 to 1.79% in 1989. The possibility of confounding factors in the diagnosing is improbable, as the exemption of military service due to incapacitating asthma was correlated with the increase reported [2].

In another wider evaluation in the UK, from 1955 to 2004, several indicators of asthma such as primary care, prescriptions, hospitalizations, and mortality evidenced an increase until the 1990s, where the curve flattened and even decreased [3].

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_2, © Springer 2009

The opposing evolution of these effects compared to the sale of inhaled corticosteroids (ICS) is one explanation, since the recognition of the inflammatory component of asthma began in the 1980s.

However, also in the UK, an evaluation of prevalence in schoolchildren between 1991 and 2002 showed a significant increase in wheezing in the past 12 months, in severe speech-limiting episodes and night waking, but non-significant increase in medical visit because of wheezing. Here again, this last finding could be explained by the significant increase in steroids prophylactic treatment reported in this population [4]. This explanation will be reconsidered ahead.

Another trend study also evidenced a significant increase from 1990 to 2003 in doctor-diagnosed asthma, more evident in females (7.3–14.6%) than in males (7.8–9.4%), in all age groups but larger in people aged 55 and older [5].

Is It the Same in Low- and Medium-Income Countries (LMIC) in the Planet?

Some years ago, Faniran et al. [6] compared the prevalence of asthma and atopy in children between an affluent versus a non-affluent country, having a smaller prevalence of wheeze and persistent cough in Nigeria when compared to Australia (10.2% and 5.1% compared to 21.9% and 9.6%, respectively).

Anyway, a recent report from Aït-Khaled et al. [7] evidenced a wide range of atopic disorders prevalent all over Africa, not only with the highest presence of current asthma in urban areas with higher standard of living (concordant with the hygiene hypothesis) but also with a representative prevalence in endemic parasite and tuberculosis zones (opposed to the hygiene hypothesis).

In Latin America, protective factors to avoid having asthma seem not to play a role, and the non-allergic factors like pollution are not conditioning a higher prevalence of respiratory symptoms. However, this prevalence is similar to industrialized countries [8]. In a recent survey of rural Asian children, 16.1% of wheezing prevalence in the past 12 months was found, not different from other developing regions of the planet [9].

The former reports, the International Study of Allergy and Asthma in Children (ISAAC), utilized the same methodology of evaluation, having strength enough to make conclusions and to compare different cultures and latitudes.

However, scarce tendency data are available from LMIC since the possibility of having these tools for evaluation has become recently available. An example is the ISAAC Phases I and III in comparison with Brazil, where nocturnal cough and wheezing slightly but significantly diminished [10]; however, the generalization of these results is improbable when considering previous references.

Taken all together, we could conclude that globally, the prevalence of asthma is high and still demonstrates a slight increasing tendency, even though there is a lessening of differences.

What Is the Scenario of the Rest of Atopic Diseases?

Other than analyzing asthma, a European study (SCARPOL) that was conducted four times between 1992 and 2001, revealed evidence of stabilizing asthma and hay fever, but with a predominant increase in atopic eczema in girls that was stable in boys [11].

The same tendency was found in the Aberdeen evaluation when considered up to 2004 [12]. There, the three atopic illnesses demonstrated a stable prevalence that was a pattern in the past 10 years, with a continuous increase present in girls that makes no sex difference at the end (Fig. 1). As in the former study, when evaluating eczema, females were more prevalent.

However, an Italian evaluation demonstrated an increasing trend from 1994 to 2002 in wheezing, allergic rhinoconjunctivitis and atopic eczema in both 6- to 7-year-old and 13- to 14-year-old populations, except for wheezing in the last group (Fig. 2) [13].

A global time trend analysis of prevalence in rhinoconjunctivitis symptoms evidenced yet again a smooth increase, being more evident in LMIC and in the older age group, suggesting that environmental influences in the development of allergy may not be limited to early childhood [14].

Related to these asseverations, a recent evaluation in the tendency of aeroallergen sensitization for 25 years (from 1976–1977 to 1999–2001) evidenced a significant increase in the prevalence of sensitivity as well as in the mean age of allergic patients [15].

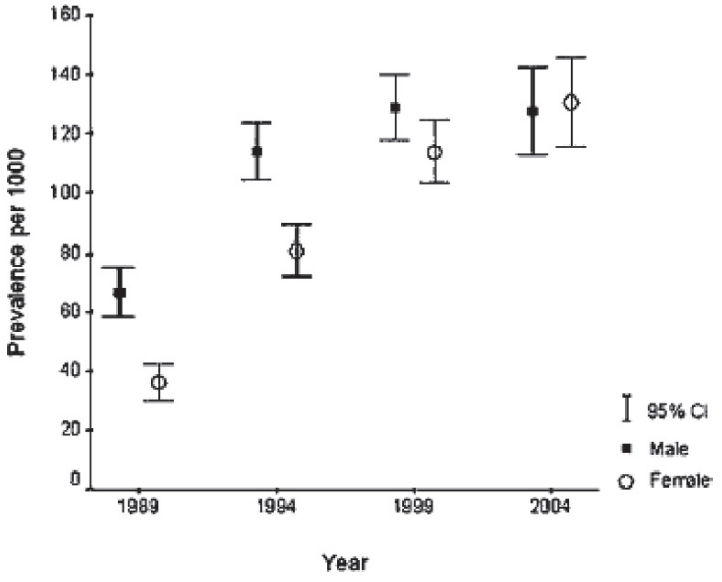

Fig. 1 Sex-specific prevalence rate for asthma reported by year of survey. (From [12], with permission.)

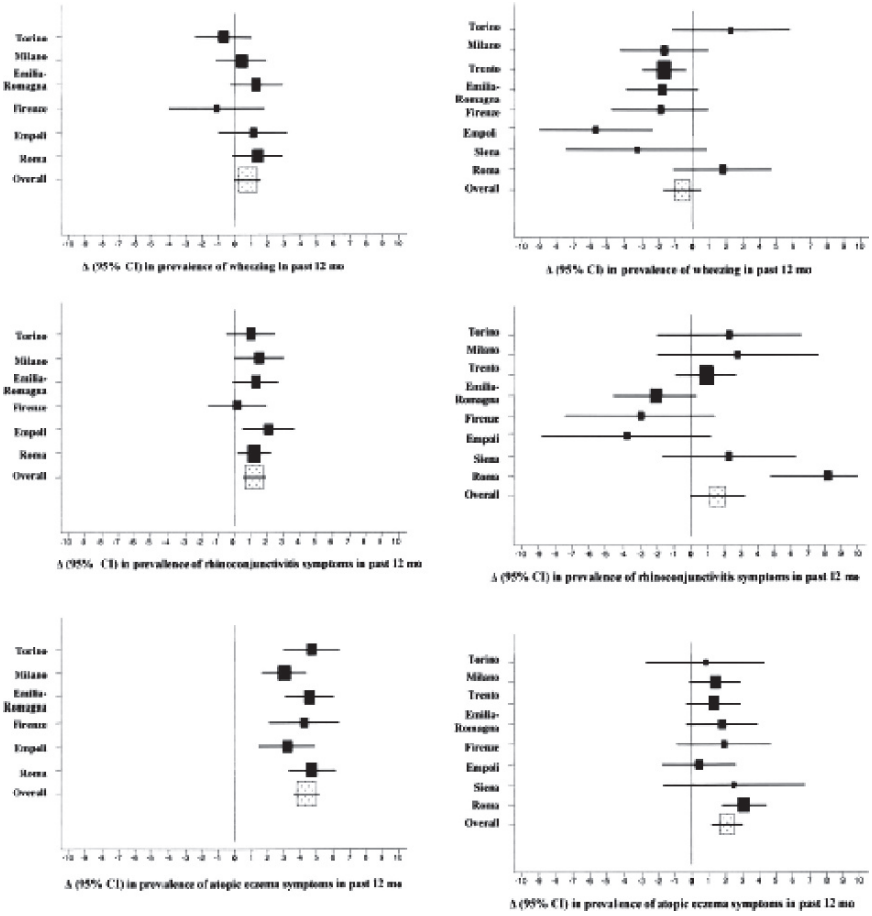

Fig. 2 Changes (*delta*) and 95% confidence interval in prevalence of wheezing, atopic rhinoconjunctivitis, and atopic eczema in the past 12 months, reported by parents of children 6–7 years of age (*left*) and by adolescents 13–14 years of age (*right*) in six areas of Italy. (From [13], with permission.)

Again, ISAAC is the option to have a global vision. A recent publication of a worldwide comparison of two phases in 6- to 7-year-old and 13- to 14-year-old populations, using the same methodology both times with a mean of 7 years of difference, allowed to evidence several projections of concern [16]: (a) In 6- to 7-year-old, an incremental tendency in asthma, rhinoconjunctivitis, and eczema was observed in Asia-Pacific, India, North America, Eastern Mediterranean, and Western Europe. (b) In 13- to 14-year-old, this augmentation was evidenced in Africa, Asia-Pacific, India, Latin America, and Northern and Eastern Europe. (c) In asthma at 6- to 7-year-old, more centers reported increase of prevalence, while in the 13- to 14-year-old group, almost equal centers reported up and down tendency. Those having larger prevalence in the first phase tend to have a decrease in the third phase and vice versa. (d) For allergic rhinoconjunctivitis, most centers at both ages

reported an incremental variation between phases. (e) For atopic eczema, the 6- to 7-year-old participants showed increased tendency in average, while in the 13- to 14-year-old samples, such tendency was not that evident. (f) Taking all disorders together, the younger group had an increase from 0.8% to 1%, and the older one from 1.1% to 1.2%.

We can then preliminarily conclude that globally, there is still a growing prevalence of atopic disorders, predominantly in developing regions of the planet.

Let us analyze the risk factors that could help to explain these phenomena:

Sex

In childhood, male sex has been considered to be a risk factor for having atopic diseases and asthma. Some years ago, this predominance was partially explained by an increased sensitivity to inhalant allergens [17]. However, we mentioned earlier that the increasing prevalence among girls equalized the male to female ratio recently, even being more prevalent when considering eczema [11, 12].

By the age of 11, male sex is still stronger when considering current wheezing [18]. As the age of the sample evaluated increases, the predominance reverses. In a cohort evaluation, male in childhood declined by adolescence and early adulthood, considering female sex as one of the major risk factors for having asthma [19]. It was also a predictive factor for persistence of asthma symptoms from childhood [20], but this conclusion needs to be reinforced in larger populations because the odds obtained revealed evidence of a wide confidence interval.

Not only the former but also allergic rhinitis shows similar transition from male in childhood to female in adolescence. Having those repeated observations reinforced by evaluations in large population samples, the fact that estrogen has pro-inflammatory and testosterone anti-inflammatory effects could explain this trend [21, 22].

Diet

Recently, Garcia-Marcos et al. [23] evaluated the relationship of the Mediterranean diet (vegetables, pulses, cereals, potatoes, pasta, and rice) with asthma and rhinoconjunctivitis in more than 20,000 children, adjusting for exercise and obesity, finding its protective effect against current severe asthma in girls. Also, seafood and fruit were protective against having rhinoconjunctivitis.

In the same direction, Wickens et al. [24] corroborated that fast food intake was related with asthma symptoms in a frequency-dependent manner. Takeaway consumption greater than once a week showed an increased (although not significant) bronchial hyper-responsiveness, but had no effect on atopy.

Not only animal fat consumption was implicated as a risk factor for atopic diseases expressions. Vegetable oils contain linoleic acid, an Omega 6 poly-unsaturated fatty acid (PUFA) precursor of arachidonic acid and consequently of eicosanoid metabolites, promoting the Th2 imbalance while decreasing interferon g (IFNg); omega 3 PUFA found in fish oil inhibits PGE2 formation, modulating the production of immunoglobulin E (IgE) indirectly [25]. However, the clinical relevance of adding fish oil in pregnancy diet demonstrated just a decrease in the severity of eczema in infants at high risk of atopy [26].

Feeding habits in the UK over the last decades, where atopic expressions grew, evidenced diminished saturated fat consumption [27]. This growing could then be attributed to a reduction of antioxidants in the diet, since only the fatty acids deregulations could oversimplify the frame. Anyway, more studies are needed in this field as interventional strategies have been disappointing as of date.

Could Diet Effect Be Related to Overweight?

As atopy, asthma, and obesity increased in the last decades, it was reasonable to speculate that maybe they are linked. When evaluating the effect of the Mediterranean diet [23], it was reported that obesity was a risk factor for current severe asthma in girls. A practical measurement of total body fat is the estimation of body mass index (BMI)–weight/height ratio [28].

However, controversies about the relationship of BMI with the presence of atopy and asthma is shown by a report from Australia [29], which states that increased BMI was a risk factor for cough, ever wheezing and atopy (predominantly in girls), but not for diagnosed asthma or bronchial hyper-responsiveness. Without these last two conditions, it is difficult to be conclusive, as gastro-esophageal reflux, sleep disorders, being unfit, or altered mechanical ventilation could explain symptoms, and all are associated with overweight.

So some meta-analysis was required to elucidate the real impact of overweight in the incidence of asthma, and 1 with a sample larger than 300,000, evidenced a dose–response increasing odds for incident asthma: odds ratio (OR) 1.38 for normal versus overweight comparison, and OR 1.92 for obesity; none of them was affected by sex [30]. These odds have a huge impact on populations like the USA, where more than 60% of adults are overweight/obese, and in consequence at risk of developing asthma.

Also considering a meta-analysis in children, the same evidence was reported. The relative risk (RR) of high birth weight on developing asthma later was RR = 1.2 (95% confidence interval (CI) 1.1–1.3), while the effect of overweight in middle childhood was RR = 1.5 (95% CI 1.2–1.8) [31]. Misclassification, diagnostic bias, and individual confounders are always doubts emerging from meta-analysis; however, the results from an enormous cohort study, from childhood to adulthood, are the only possibility to corroborate or contradict this evidence.

What About Environmental Pollution and Work Exposure?

The effects of air pollution have been described some years ago as significantly harmful in children with elevated IgE and bronchial hyper-responsiveness. Airborne particulate of a size of less than 10 ∝m (PM10), sulfur dioxide, black smoke, and nitrogen dioxide provoked lower airways symptoms in these patients (wheezing and dyspnea), as well as a decrease in peak expiratory flow greater than 10% while particulate amounts increased [32].

PM10, nitrogen dioxide, and carbon monoxide showed a considerable correlation with emergency assistance in children, but not in adults [33]. In children under 5 years, peak carbon monoxide level was predictive of hospitalization because of asthma attack [34].

Going from an epidemiological to a bio-immunological approach, one of the risk factors that could explain the increasing prevalence of atopic diseases in industrialized countries has been the exposure to diesel exhaust particles, recognized as enhancer of IgE-dependent allergic inflammation, and the consequent symptoms of asthma and rhinitis [35]. Once again, a recent revision cannot be conclusive in considering these particles as a significant risk factor for having atopic diseases [36].

About indoor pollution, there is no doubt that tobacco smoke constitutes the key factor to be considered, since it has been implicated in the development of asthma in children and non-smoking adults exposed [37]. About those smoking actively, the RR for incidental asthma was reported as high as 3.9 (95% CI 1.7–8.5) [38].

Work Exposure

With an obvious gap in concentration, some same outdoor pollutants could be found at working places. But time and dose exposure could promote the starting of irritant asthma, like sulfite mill workers in whom sulfur dioxide established a risk of four to six times greater for new-onset medical-diagnosed asthma [39]. Not only pollutants are capable of inducing asthma, instruments and surface cleaners, adhesives and latex particles have been implicated in that process within healthcare workers [40]. The list of demonstrated provoking agents, as well as mechanism involved, goes beyond the present analysis.

What About Infections and the Hygiene Hypothesis?

In 1989, Strachan [41] proposed that allergic diseases could be prevented by infections in early childhood, and the transmission of them by unhygienic contact with older siblings. Smaller family size, higher standard of living, and personal cleaning

reduced the chances of spreading "protective" infections, originating the *hygiene hypothesis*.

A recent comparison of two genetically related but cultural and socio-economic different populations (Russian and Finnish) evidenced higher specific IgE levels in Finnish but more total IgE and specific microbial antibodies in Russians. Enterovirus infection represented the strongest protective factor against allergen sensitization [42].

In this direction, farmers' children from a rural environment were evaluated for atopic symptoms (by questionnaire) and atopy (by skin test), as well as endotoxin measurement. Compared to non-farmers' children, they presented significantly fewer symptoms of current asthma (adjusted OR 0.67; 95% CI 0.49–0.91; $P = 0.01$) and rhinitis (OR 0.50; 95% CI 0.33–0.77; $P = 0.002$). If having unpasteurized milk also, a significant reduction of atopy (OR 0.24; 95% CI 0.10–0.53; $P = 0.001$) and current eczema symptoms were added (OR 0.59; 95% CI 0.40–0.87; $P = 0.008$), while reducing IgE ($P < 0.001$) and increasing IFNg ($P = 0.02$) [43]. Pasteurized milk, vaccinations, early use of antibiotics, and the westernized lifestyle with less exposure to infectious agents could contribute to this lack of stimulation, essential in the first years of life to change the initial Th2 profile toward a Th1 just not to favor atopy development.

Ten years ago the hygiene hypothesis was suggested, an extensive analysis was done to determine its current relevance, and the conclusions were [44]: (a) atopic diseases, but not necessarily asthma, are highly prevalent in smaller and more affluent families; (b) the postulate of protective infections against atopy is immunologically plausible; the reversal is inconclusive; (c) the modulating effects of antibiotic therapy and diet influencing intestinal flora need to be evaluated extensively; (d) The inverse association of family size and allergic sensitization could potentially help to discern underlying causes of the increasing prevalence of atopic diseases.

However, the Th1/Th2 paradigm and how it fits in the hygiene hypothesis must be analyzed. Table 1 considers how all these factors affect both Th2 and Th1 illnesses, and its scheme outlines factors influencing immune system development at different time points [45].

In this context, genetically inheritance should be the beginning, while the attributable genetic risk ranges from 30% to 80% depending on the disease considered. Then, susceptibility to multiple exposures will determine if "western and industrialized world" affects the development of atopic diseases in these individuals. There, developing countries with the objective of reaching a better quality of life increase their risk as shown by the increased atopic prevalence in people who migrated to developed regions and in urban cities when compared to rural [7, 45, 46].

As a conclusion, we do not need to go back in evolution, we must maintain the control over infections, but need to clarify the role of each microbial stimulus (especially at the gastrointestinal tract), in parallel with genetic background and every co-factor. Large longitudinal birth cohort studies, getting representative biological and environmental samples, will help us in the future.

Table 1 Discrimination of factors influencing Th1 and Th2 diseases; scheme below: factors that could manipulate immune system development, at different periods. (From [45], with permission.)

	Atopic disease	Auto-immune disease
Epidemiological findings		
Decreasing family size	⇑	⇑
Number of older siblings	⇑	⇑
High socio-economic status	⇑	⇑
Decreased day-care exposure	⇑	⇑
Evidence of cleaner houses		⇑
Evidence of previous oro-fecal infection (as a marker for poor hygiene)	⇓	?
Higher frequency of viral "cold" in early childhood (parentally reported)	⇓ ⇒	?
Environmental measurements		
High endotoxin exposure (e.g., on farms)	⇓	?
GI-flora		
Decreased Lactobacilli, Bifidobacteria	⇑	?
Supplementation with *Lactobacillus* CG	⇓	?
Increase in Clostridia (esp *Costridium difficile*)	⇑	?
GI-parasite infection		**?**
Active/chronic infection	⇓	
Treatment of parasite infection	⇑	

GI, gastrointestinal.

Is Atopy Per Se a Risk Factor for Having Atopic Diseases?

Taking the former proposal to consider longitudinal studies, to elucidate the attributable risk of different exposures, a cohort of more than 1,000 children was evaluated by their atopic status, and related to asthma, rhinitis, and eczema. Sensitization to dust mites was the strongest independent risk factor for having asthma (OR 8.07, CI 4.6–14.4), to grass pollen for having rhinitis (OR 5.02, CI 2.21–11.41), and to peanut for having eczema (OR 4.65, CI 1.02–21.34). Even though less than half of the original cohort was skin tested at the age of 4, some relevant tendencies were evident: the prevalence and severity of asthma correlated with allergen sensitization, the risk of all allergic diseases increased with the number of positive prick tests, there was a predominance of male sex at this age, but they conclude that only 30–40% of allergic diseases is attributable to atopy, and the rest to the affected organ or other factors [47]. A recent report suggests that asthma attributable to atopy could vary depending on allergen exposure and its modifications because of the environment such as climate [48].

But atopy alone does not explain much of the real life, where multiple factors could influence the development of atopic diseases, such as respiratory viral infections and the development of asthma. In a cohort of more than 2,000 children,

where the presence of current asthma at 6 years of life was correlated with atopy and respiratory tract infections in first year, concluded that both conditions were independently associated with a significant risk of having asthma by the age of 6 [49]. Also, maternal feeding evidences a protective behavior.

Another longitudinal study demonstrated the association of infantile chest infections with wheezing and asthma, and the importance of early life atopic status for the presence of wheezing, asthma, and bronchial hyper-responsiveness at 10 years of life [50]. Other conditions such as familiar asthma, early passive smoking, and having eczema at the age of 4 were also significantly associated with asthma and wheezing but not with bronchial sensitivity.

We must preliminary conclude that atopy per se is not enough, neither to express atopic diseases nor to justify the increased incidence of them.

But What Is the Natural History of Asthma and Allergy?

A prevalence of positive skin test ranging from 8% to 30% in general asymptomatic population has been described; from them, one to two out of three will develop an atopic respiratory disorder in the future [51]. Multiple risk factors associated with the development of allergy and asthma have been detailed.

Genetic polymorphism and their environmental interaction, premature aeroallergen sensitivity, exposure to tobacco smoke, presence of eczema and rhinitis, and lower respiratory viral infections are all risk factors for developing chronic asthma [50, 52]. Once asthma is present, several predictors have been detailed for persistence and severity of the disease *in children* [53]: (a) severe wheezing in preschool age, (b) the onset at school age, (c) familiar history of asthma and allergy, (d) elevated serum IgE levels, (e) early sensitization to aeroallergens, (f) early development of bronchial hyper-responsiveness, (g) frequency of respiratory infections, (h) lack of contact with older children, (i) familiar discrepancies with psychological involvement. For persistence and severity *in adults*, predictors described are [53]: (a) constant exposure to sensitized allergens (including occupational), (b) older age of the onset, (c) aspirin intolerance, (d) socio-economic status, (e) smoking, (f) coexisting pulmonary diseases provoking COPD (like bronchiectasis or aspergillosis).

Some absolutely relevant cohort studies allowed to discriminate phenotypes of asthma that can be grouped in: (a) intermittent wheezers associated with respiratory infections, (b) transient or persistent wheezers (the latter associated with atopy), (c) atopic and intrinsic asthma (invariably persistent), (d) occupational or drug-induced asthma (mainly adults with prognosis related to severity) [53–56]. This differentiation has important therapeutic implications as supposed.

Regarding the other atopic disorders, atopic march described that while in the first years of life the prevalence of food allergy and eczema is present but declines progressively, giving respiratory allergy the chance to persist [57, 58]. The first

atopic expressions being eczema and food allergy, maternal diet restrictions and food avoidance have both been recommended as primary prevention without conclusive and strong evidence [59, 60]. Indeed, a recent evaluation of the delay in solid food introduction could not demonstrate a protective effect against food or any allergen sensitization and/or eczema by the age of 2 [61].

Allergic rhinitis is undoubtedly an independent risk factor for having asthma; moreover, treating rhinitis with allergen immunotherapy reduced the risk of developing asthma [62]. Eczema (together with familiar history of asthma) was considered to be a major predictor for having asthma [63].

Has Any Therapeutic Intervention Been Demonstrated to Alter This Natural Course?

One of the most controversial issues to date is the use of ICS to alter the natural development of asthma, specifically when to begin its use and for how long. There is no doubt that persistent asthma must be treated chronically with ICS [64–66], and significant reduction in its impact is remarkable, in any case, considering hospitalizations or mortality [67, 68]. However, the convenience of early introduction of them in intermittent asthma and the regular versus intermittent use in mild persistent cases are not conclusive yet; robust evidence is needed to conclude that early introduction and permanent use of ICS prevent a significant decline in lung function in such a mild profile, with truly clinical relevance, and a strong risk–benefit ratio [69–74].

About primary prevention of atopic diseases, we mentioned that no concluding recommendations should be given regarding maternal diet and feeding of babies [59–61].

In clinically relevant aeroallergen sensitization, measures for avoidance of house dust mites may benefit in reducing symptoms only [75]. However, specific immunotherapy can prevent new sensitizations while maintaining an asymptomatic condition for many years; moreover, it has been demonstrated to prevent the onset of asthma in children with rhinitis [76–78]. Sublingual immunotherapy has an excellent safety profile while having same immunological effectiveness as subcutaneopus, emerging then as the only interventional option that can modify the natural course of allergic diseases [77, 78].

Concluding Remarks

1. The prevalence of allergic diseases is still slightly increasing, with different profiles in the developed world (stabilization) and the developing world (increasing). The direct implication must be analyzed in the context of the regions where population is growing.

2. Urgent global networks and programs must be implemented, to allow admission to all people for prevention, diagnosis, and treatment. This is the only possibility for reversing this trend.

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Allergy: A Burden for the Patient and for the Society

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Introduction

Allergic diseases and asthma represent some of the most common chronic pathological conditions prevalent all over the world [1, 2] that begin usually in infancy and persist throughout life [3]. They are most common in developed countries. Allergy may affect more than 50% of children. Moreover, the prevalence of allergic diseases and asthma has actually increased during the past three to four decades.

Asthma is the most frequent chronic disease in childhood, with increasing levels of morbidity in most of the countries worldwide [4]. Pediatric asthma represents a huge burden on the individual child, on the family, and on the society as a whole.

The incidence of food allergy is continuously increasing; it is potentially life threatening, and has a major impact on the lives of the sufferers [5]. Food allergy mainly affects children; however, more and more adults are also suffering from food allergies. Allergic diseases are also increasing in the developing countries [6].

The prevalence and severity of allergic diseases and asthma present a serious challenge to healthcare systems, the society, the patients, their care-givers, and families. Occupational allergy is another important medical and economical problem [7].

Allergic diseases and asthma seriously affect the social life of the patients. Asthma is a leading condition of school absenteeism and a major cause of work absenteeism [8]. Allergic diseases have also impact on cognitive functions [9]. Direct and indirect costs for allergic diseases and asthma have increased during the past 10 years [10].

In this chapter, the burden of allergies and asthma is evaluated on the basis of the current knowledge of the patient's perspectives and attitudes toward the burden of these diseases.

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_3, © Springer 2009

Allergic Rhinitis

Allergic rhinitis is probably allergic pathology with the highest prevalence among all allergic diseases [11], affecting an average of 24% of the population across Europe [12]. Its relationship with asthma and atopic dermatitis is relatively well established, with approximately 80% of asthma patients and 80% of children with atopic dermatitis [13] suffering from allergic rhinitis. In addition to high prevalence, many clinical trials have documented the severity of allergic rhinitis symptoms and their impact on quality of life [14]. Moreover, not long ago, a new classification of allergic rhinitis was proposed in the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines based on severity and duration rather than causality [2]. Complications of allergic rhinitis and concomitant diseases are also well documented. Poorly controlled allergic rhinitis may contribute to the development of acute and chronic sinusitis, recurrence of nasal polyps, otitis media/otitis media with effusion, hearing impairment, abnormal craniofacial development, sleep apnea, aggravation of underlying asthma, and increased propensity to develop asthma [15]. Daytime fatigue, learning impairment, decreased overall cognitive functioning, and decreased long-term productivity have also been attributed to allergic rhinitis [9, 16].

Despite the plethora of data collected to date pointing to the fact that allergic rhinitis, especially in its persistent form (persistent allergic rhinitis), is a serious debilitating disease, there is still generally doubt as to how serious rhinitis is especially among regulatory agencies and health payers. The use of over-the-counter drugs is extremely common among allergy sufferers [17], which ultimately means that patients assume increased responsibility for diagnosing their condition, selecting appropriate drugs, and using these drugs properly. As costs are being shifted away from insurers to patients, the potential risk of denying patients access to optimally effective, comprehensive, and physician-supervised disease management, is growing.

Patients' involvement in the management of allergic rhinitis becomes more important and frequent. The knowledge of patients' perceptions of their own disease and its consequences are scarce. The impact of allergic rhinitis on the lives of allergy sufferers across Europe and the success of its management, both in terms of treatment and common preventive measures, was evaluated by Valovirta et al. [17]. Owing to the fact that many allergic rhinitis sufferers are undiagnosed and unaware of their diseases [18], Valovirta et al. chose to survey self-reported allergic rhinitis patients who are members of European allergy patient organizations. With their activities in the fields of allergy information, education, peer contact, and financial support, they provide a platform for members to exchange experiences and a framework for education (http://www.efanet.org).

The results show that almost 50% of the responders reported symptoms lasting for more than a season. Persistent allergic rhinitis as defined by ARIA [2] was reported by 62% of the respondents. The triggers of persistent and intermittent rhinitis are largely similar. Preventive household adjustments are expensive, however, with little perceived benefit. Sleep and emotional life are considerably affected

by allergic rhinitis. The most distressing impact is the feeling of being worn-out and tired. Although most patients are satisfied with the current allergic rhinitis medications, at least one-fifth of them report dissatisfaction. Patients perceive that AR worsens other concomitant allergic diseases.

Considering the severity and persistence of allergic rhinitis, as well as its impact on daily life, the patients with allergic rhinitis deserve a long-term management with potent treatments such as medications, allergen-specific immunotherapy, proper avoidance of allergens inducing their symptoms whenever practical and possible, patient information, education and training, and a proactive follow-up [19].

Asthma

The prevalence of asthma continues to increase in many countries: the current estimate of 300 million people with asthma worldwide is expected to increase by 33% to 400 million by 2025 [20]. In addition to the economic burden of asthma, which is considerable, there are physical, emotional, and social effects, leading to reduced quality of life of patients and their families [21].

International surveys have been valuable for understanding and managing asthma. The International Study of Asthma and Allergies in Childhood, the European Community Respiratory Health Survey, and other surveys have provided much needed information about the global patterns of asthma prevalence from childhood to adulthood, and have generated new hypotheses for further testing and validation [12, 22–24].

An understanding of the needs and behaviors of asthma patients is also important in developing asthma-related healthcare policies. Holgate et al. [25] recently published a structured review of patient surveys on asthma. The primary objective of this structured review was to assess patient perspectives on key issues in asthma and its management, including diagnosis, treatment, control, and quality of life, as captured in patient surveys in Europe and in North America published between 1997 and 2003. Twenty-four surveys, including a total of 66,450 subjects from a total of 24 countries, were reviewed. Of this number, 57,817 were patients (including 11,875 children—generally classified as <16 years—and parents of children) and 8,633 were healthcare professionals. Table 1 summarizes the general findings from the survey review.

Findings of this structured review suggest that patients often understate their symptoms, tolerate poor symptom control, have low expectations of therapy, possess meager knowledge of correct drug usage, and display insufficient adherence to therapy. Among healthcare professionals, there is evidence of an inadequate understanding of disease etiology and poor or unstructured communication with patients, resulting often in inaccurate assessment of disease severity. With the increasing incidence of asthma, it is important to address these issues as a matter of priority.

Table 1 Summary of key review findings from asthma out of control? A structured review. (Modified from Holgate et al. [25]).

	Subthemes	Core findings	Key supporting data
Patient perceptions	Understanding of disease	In general patients (or caregiver) lack of knowledge of their asthma and its causes	Only 22% thought asthma therapy reduced inflammation
		Patients are aware of asthma symptoms, but are often willing to tolerate poor control or are unaware of the risks	92% of patients experienced limitations of activities due to asthma, and 48% had difficulty with sleeping
		Despite poor control, many patients still describe themselves as "well controlled"	>65% had symptoms during the last week although, >80% considered themselves to be "under control"
	Symptom control	Inappropriate use of available drugs may contribute to poor control	21.3% and 26.4% of patients with "some" and "severe" control limitations, respectively, actually used anti-inflammatory drugs
		More aggressive anti-inflammatory treatment can improve control	Addition of a LTRA improved sleep (87% of patients), early waking (80%), daily functionality (85%), and need for rescue medication (77%)
		Patients often do not realise asthma drugs have side effects	61% of parents of children with asthma did not realise ICSs had side effects
	Patient satisfaction	Patient satisfaction with their treatment is low	In general, these figures are under-statements and inference gives higher possibilities
		Patient satisfaction (and participation) with their management is often low	28% of patients did not tell their doctor in consultation about troublesome coughing, and 36% failed to mention difficulty in sleeping
		Admitted compliance with treatment is often poor, expressed both by lack of and by excessive use of prescribed treatment	45% of patients admitted using their medication excessively
	Compliance	Patients cited steroid use as a major reason for lack of compliance	One-third of patients expressed dissatisfaction with long-term steroid treatment

(continued)

Table 1 (continued)

	Subthemes	Core findings	Key supporting data
Lifestyle issues for patients and family	Control	Lack of control was mentioned as being associated with reduced QoL in a number of surveys	General comment
	Disease severity	Correlation between QoL and disease severity was suggested	General comment
	True impact	The impact of asthma on QoL is often understated	General comment
	Lifestyle restrictions	Patients reported significant lifestyle restrictions	Irrespective of disease severity, approximately 70% report significant lifestyle restrictions
	Families	The QoL of families of children with asthma is also clearly affected	20% of parents stated that their work attendance was affected, and 50% said their own lives were affected (20)
Child specific	Management	Generally children are better managed than adults despite some parental reservations about disease	Asthmatic children are significantly greater consumers of resources than asthmatic adults, despite having better initial asthma control
	Perceptions	As in adult asthmatics, there is a marked difference between perception and reality of symptom control in children (or by their caregivers)	65% of children with asthma or their carers considered their asthma to be well controlled although 37% had breathing difficulty, 34% had nocturnal waking, 29% had dry cough, and the ability to talk was affected in 29%
	Therapy understanding	Parental understanding of their child's medication (and compliance) can also be poor	33% of parents of asthmatic children did not understand the role of "controller" versus "preventer" therapies and only 38% of parents took their controller medication on a regular basis
	Treatment needs	There seems to be a particular demand for better treatments for children	70% of parents of asthmatic children were concerned about the effects of inhaled corticosteroids

(continued)

Table 1 (continued)

	Subthemes	Core findings	Key supporting data
Healthcare providers	Etiology	Some HCPs do not fully understand some of the recent advances in the understanding of asthma etiology	59% of physicians questioned considered allergy to be the main cause of asthma, with only 35% (and only 16% of pediatricians) citing the underlying inflammation. However, in the same survey, 92% of physicians understood that leukotrienes were important mediators of inflammation in asthma, and 80% understood that LTRAs were anti-inflammatory agents
	Treatment needs	Some of the surveys examined physicians' inconsistent use of anti-inflammatory agents in asthma among the suboptimal numbers of patients actually being treated	92% of physicians considered anti-inflammatories "essential" in asthma care, although only 21% of patients were receiving these agents
	Diagnosis	There was practical support for the need for improved diagnosis of asthma leading to improved management	The utility of decision-making tools and self-reporting questionnaires for assessing disease severity and optimizing therapy can measure and improve treatment compliance
Similarities and differences between HCPs and patients	Similarities	In most relevant studies, patients and HCPs generally agreed that better treatments with fewer side effects would be desirable	General comment
	Significant differences	HCPs and patients disagreed over symptom control	Only 1% of patients considered themselves symptom free when compared with 24% of their GPs
		HCPs and patients disagreed over compliance levels	HCPs believed that "all" their patients complied with treatment whereas only 60% of patients actually did according to HCP definition
		HCPs and patients disagreed over concern toward side effects	General comment

One encouraging message from these observations is that within the currently available parameters for asthma management, there is ample scope for improving the standard of care. In particular, care could be significantly improved by improving the education of patients regarding the nature of the disease (as one primarily of inflammation) and optimizing the use of existing medical systems and treatments. Such relatively simple measures would not require an enormous financial commitment, but would certainly improve the lives of many asthma patients and their families.

In Finland, the national asthma programme 1994–2004 succeeded by implementing new knowledge of asthma especially for primary care and educating patients for effective self-management to reduce the morbidity of asthma and its impact on individuals as well as on society [26]. When compensation for disability, drugs, hospital care, and outpatient doctor visits are taken into account, costs per patient have decreased 36% from €1611 to €1031 and, if related to the increase in gross national product, by 50% per year. In 1993, the total costs were €218 million which had fallen to €213.5 in 2003. Approximately 70% of all asthma in Finland is mild and may require only intermittent drug treatment. However, in both mild and moderate severe asthma, guided self-management is essential in preventing prolonged symptoms and exacerbations. Understanding and partnership are more important than compliance [27].

The Global Asthma Physician and Patient (GAPP) Survey [28] not only defines an unmet need in asthma treatment but also reveals that there is a direct relationship between the quality of physician–patient communication, the level of side effects, and the extent of patient compliance.

The limitations of severe asthma: the results of a European survey by Dockrell et al. [29] shows that severe asthma has a major impact on patients—restricting their activities, causing embarrassment, imparting fear—and is a major burden on healthcare systems. Despite studies indicating that severe asthma is still not adequately controlled, there continue to be inefficiencies in the management of this population; consequently, guideline objectives are not being achieved. Patient perceptions toward their asthma and expectations for the future management of asthma differ across Europe, and, understandably, many patients are not optimistic about the future for asthma management. On a positive note, patients are optimistic about the development of new medications to help control the debilitating symptoms of severe asthma. National healthcare investment in new strategies, improving surveillance across Europe, working with patients to understand their needs and the development of new treatments to facilitate the management of severe asthma will give patients the hope that they might one day live beyond the limitations of their asthma.

On 18 and 19 October 2006, leading asthma experts, EU policymakers, regulators, and patient groups gathered at the European Parliament to discuss current concerns relating to asthma management. The meeting was chaired by Liz Lynne MEP, suffering from severe asthma herself, and Professor Stephen Holgate, University of Southampton, UK.

The Summit's participants were challenged to the following: ensuring optimal safety and efficacy of treatment for patients; reviewing the data that together build a new evolved picture of asthma as a systemic inflammatory disease; agreeing with the urgent need for change in asthma management today and recognizing asthma as

an increasingly serious public health issue with human and economic impact; agreeing with the practical clinical and regulatory strategies to recognize current gaps in care, offer more options in primary care setting; and addressing future developments and catalyzing the process of change and immediate action.

The Summit culminated in the development and agreement of the Brussels Declaration (http://www.Summitforchange.eu), which outlines how and when changes need to be made to the way that asthma is managed in the European Union—ensuring optimum treatment for all patients.

As highlighted by the ARIA guidelines [2], asthma and allergic rhinitis are related conditions and should be considered together when treatment options are discussed with patients. The results of a recent survey [30] suggest that the worsening of allergic rhinitis symptoms in patients with asthma can be associated with worsening asthma symptoms, and that comorbid asthma and allergic rhinitis can cause substantial disruption in daily activities. Moreover, study respondents expressed concerns and difficulties with medications to treat asthma and allergic rhinitis.

Allergic rhinitis and asthma are most commonly managed in the primary care setting. Physicians treating patients with asthma or allergic rhinitis must remain vigilant to the possible presence of the other condition, must be aware of the risks posed by one condition for the development of the other, and must evaluate treatment options for improving symptoms of both conditions when present concomitantly. In addition, physicians must be aware of possible patient concerns about medications, particularly patient concerns about potential side effects of corticosteroids and using much medication for their asthma and allergies. More generally, there is a need to promote the use of combined therapies that are safe and effective for treating symptoms of both asthma and allergic rhinitis, and that address the inflammatory nature of these two conditions affecting the “one airway”.

A population-based study in the USA, assessed the impact of comorbid allergic rhinitis on medical costs in a cohort of 1,065 infants, children, and adults (below 65 years of age) with asthma. The investigators compared the costs for medical services (excluding medications) incurred after January 1987 by patients with versus those without concomitant allergic rhinitis. Data were available for 8,564 person–years of follow-up. Total medical-care charges were 34% higher with comorbid allergic rhinitis and asthma than with asthma alone ($P < 0.0001$). Charges for the office care of children and young adults with these comorbid conditions were 46% higher than in this subset with asthma alone.

A retrospective analysis [31] showed that effective treatment of allergic rhinitis in asthma patients decreased the use asthma-related healthcare services by 61% in a population of 4,944 asthmatic of 12 to 60 years of age.

Food Allergy

Food allergy, whether clinically diagnosed or self-perceived, represents a major health issue in Western societies and may have a considerably greater impact on society than was believed. It has been estimated that in the general population

approximately 4–6% of children and 1–3% of adults experience food allergy. There is some evidence to suggest that the prevalence of food allergy has increased over the past 10 years [32]. This is demonstrated by the increase in emergency room visits due to food allergy in the UK, which have increased by a factor of 6 over a decade, accompanied by an increase in the incidence of anaphylaxis caused by food allergy [33]. Another remarkable observation is that the prevalence of perceived food allergy seems to be much higher than verified food allergy, up to 22% of the adult population [34]. This may be related to inadequate diagnosis of food allergy, in part reflecting the lack of adequate provision of relevant health services. The social functioning of individuals with a food allergy, or activities in families with an allergic child or a family member, may be seriously disrupted by the need for continuous vigilance to avoid foods to which they are (or believe to be) allergic [35]. In the case of individuals with self-diagnosed food allergy, majority may be restricting their diet unnecessarily and consequently running the risk of nutritionally compromising themselves or becoming deficient in certain nutrients [36]. Furthermore, such dietary management disrupts social and family life, and could be costly to implement in time and money. However, the effects of food allergy are not only limited to individuals or households. The food industry may also experience an extra-burden of costs due to food allergy; in fact, this may be the case with every step of the food chain, retailing and catering. This may, for example, result from legislative changes aimed at improving consumer protection such as the new European Union legislation on food labeling that came into force in November 2006 [37].

At present, the potential social impact and economic burden costs of food allergy on the individuals, families, health-related services, and food industry are not well understood.

The social impact of food allergy has not been systemically investigated using validated instruments. EuroPrevall, a European multicenter research project funded by the European Union, http://www.europrevall.org, combining the information from studies on health-related quality of life with epidemiological data on prevalence will ultimately give some indication of the magnitude of the social impact of food allergy in Europe [38]. New instruments to assess the socioeconomic impact of food allergy are being developed in this project and their application in the clinical cohorts will allow, for the first time, an assessment to be made of the burden this disease places on allergy sufferers and their communities [39].

Communication, including the doctor–patient relationship and linking printed information with explanation, plays an important role in helping food allergic individuals manage their condition. Targeted information strategies may be the most resource-efficient way to effectively communicate to different stakeholders about food allergy. However, information channels best suited to a specific stakeholder needs remain to be investigated and explored [40]. Communication is also important in dealing with psychological distress and helping allergic individuals adopt the necessary treatment regimens. As allergy sufferers at present have to avoid symptom-inducing foods, often for the rest of their life, there is also a need for others involved in producing and serving safe food to become partners in the management of food. The responsibility for not eating the allergenic food is primarily that

of the patient, but to fulfill this task the patient has to be able to rely on information provided by the food manufacturers, retailers or catering staff [39].

Food allergies, particularly in children, also require constant vigilance, which can be stressful [41]. Allergic individuals and their families need to recognize the signs of inadvertent ingestion, including anaphylaxis, and they may need to learn how to provide emergency treatment. Parents of food allergic children need to monitor their child's diet and behavior more closely than parents of nonallergic children. Food allergy can sometimes impact on the family relationships. Meltzer [42] comments that siblings may be deprived of attention, which may lead to resentment toward the allergic brother or sister. Furthermore, parents may become anxious about, and overprotective of, the allergic child. They may feel even hostile toward the child, and subsequently feel guilty about those feelings.

Food allergies in children have a wider impact beyond the child, and extend to the child's family, other carers, friends, and staff and pupils at schools and other day care centers [43]. It has been observed that many aspects of quality of life (including daily activities, family relations, distress, and worry experienced by parents) can be impaired for the whole family [44]. In addition, in some countries children with severe allergies cannot stay at school for lunch and have to return home affecting parents' ability to work, while in others peanuts and nuts are banned from the school. It is evident that awareness in school of food allergies, in both teachers and catering staff, is often poor [45] and may compromise the safety of severely allergic children at school, adding to parental concerns and worries about their children when they are not in control. There are incidents of children suffering fatal reactions while in daycare nurseries because of insufficient vigilance by staff. It is also emerging that teenagers are especially vulnerable, with some evidence that adolescents and young adults are at greater risk of suffering fatal reactions [46]. In a recent review about food hypersensitivity and quality of life, Marklund et al. [47] reported that several domains of quality of life are affected, such as family and social activities, emotional issues, and family economy. Food allergic children are to a large extent limited in their autonomous social activities. Food allergic adolescents absent themselves for more weeks from school when compared with a control group, and a relatively high percentage of food allergic young adults do not participate in the labor market. Comorbidity has to be taken into consideration when assessing the quality of life in food allergic individuals.

The Patient Needs

Recently, health professionals have raised the question, hat do patients need? Although the answer to the question is very important, it is also equally important to look at the reason why healthcare professionals are interested in the patients' wants and needs [48].

Patients consult healthcare professionals, mostly physicians, because they want to become healthy again and continue their normal life. In chronic illness, such as

allergic diseases, this is not a realistic option and patients are aware of that. So, they will seek help to live as normal a life as possible [49]. Healthcare professionals want to cure the illness, or in the case of chronic illness, to diminish the complaints and symptoms as much as possible. They prescribe a treatment and communicate prescribed medication and lifestyle advice to the patient. Often they refer the patient to another healthcare professional or a patient group for further explanation or even education to ensure that the patient will understand and be compliant with the treatment advices.

A lot has been written about the communication between physicians and patients. Recently, more attention is given to the patient's own role in self-management, and the patient is considered to be an informed decision-maker.

It is now recognized that there are several distinct approaches to treatment decision making that doctors can use with their patients: the paternalistic, the shared, and the informed (or consumerist) approach. Each has different implications for the roles of doctors and patients in communicating information and for the type, amount and flow of information between the two.

In the paternalistic approach, doctors are unlikely to have much interest in discussing patient concerns expressed in the voice of the real world. They are more likely to want short descriptions of physical symptoms that they can translate into diagnostic categories. In the pure paternalistic type, doctors can make a treatment decision that they think is in their patients' best interest without having to explore patient values and concerns.

In the informed approach, patients are accorded a more active role both in defining the problem for which they want help and in determining appropriate treatment. In the pure type of this approach, the doctor's role is limited to providing relevant research information about treatment options and their benefits and risks so that the patient can make an informed decision.

Only in the shared approach do doctors commit themselves to an interactive relationship with patients in developing a treatment recommendation that is consistent with patient values and preferences. To enable this to happen, the doctor needs to create an open atmosphere in which patients can communicate all their agenda items. In this approach, information exchange helps the doctor to understand the patient and ensures that the patient is informed of treatment options and their risks and benefits. It also allows patients to assess whether they feel that they can build a relationship of trust with their doctor [50].

Patients want and need to be taken seriously; a physician should look at his/her patient as a person and not as a sum of symptoms or spare parts. Their illness influences their daily life and so do their symptoms. This cannot be treated by medical technical treatment alone. Patients come to consult the physician to discuss their entire problem, not only the organ concerned. This is especially important in the allergic disorders. Patients need help in solving their problems; they need advice that takes into account their daily living patterns. In relation with therapy they want to choose between alternatives and to do so they need to be informed. And finally, they want their decision to be taken seriously [51]. Patients need to be a partner in care.

Conclusion

Allergy is a growing global public health problem that greatly impacts on the day-to-day life of patients, and on their families, school, professional, and social life. Allergic diseases are a continuum from atopic eczema and allergic rhinitis to asthma; in certain cases food allergy is also a risk factor for the development of asthma. This "allergy march" is a challenge for healthcare systems because there is a need for continuous control of patients with these diseases and also of those at risk of developing them.

Institutions and public opinion are often unaware of the impact of these diseases on individuals and on society as a whole. Allergy is often underestimated, underdiagnosed, and undertreated, despite its high prevalence and its effect on the quality of life of affected people, their families and caregivers. It is a chronic condition that accompanies the patient throughout life. Reactions vary from mild to severe and even fatal. The social and economic burden is very high for families and for social security and healthcare systems.

According to the World Health Organisation, allergy, defined as immunologically mediated hypersensitivity, is increasing and it is estimated that more than 20% of the world's population suffer from IgE-mediated allergic diseases, such as allergic asthma, allergic rhinitis, allergic conjunctivitis, atopic eczema/atopic dermatitis, urticaria, angioedema, venom allergies and anaphylaxis. Allergy affects all age groups, from infancy to childhood, from adolescence to adulthood up to the elderly.

Scientific societies have drawn up international guidelines and position papers regarding the diagnosis, treatment, and management of these common conditions. However, there is a need for more research in the different fields of allergy. Moreover, important new results are often slow in reaching healthcare professionals. Patients should be helped to understand their condition, to comply with their doctor's prescriptions and recommendations to improve their disease control and hence their quality of life.

Allergy knows no boundaries. Hence, there is a call for a global strategy for European and national programmes, as well as global, and actions aimed at translating into daily life the scientific data that will help counteract the increase of allergy.

Because of the extent of the problem, allergy should be a part of the national political agenda. The EFA, European Federation of Allergy and Airways Diseases Patients Associations, Allergy Manifesto http://www.efanet.org, urges the European and national institutions, healthcare professionals, and policy decision makers in Europe to work together to create the conditions for early diagnosis, correct treatment, and control of allergic diseases as well as for the application of preventive measures including the elimination of social and environmental barriers.

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Epidemiology of Asthma and Allergic Rhinitis

Deborah Jarvis, Seif Shaheen, and Peter Burney

Introduction

Asthma and allergic rhinitis are common chronic conditions in children and adults in many parts of the world and the prevalence of both increased substantially during the twentieth century. Many people suffer from both asthma and allergic rhinitis and this is usually attributed to a shared link with IgE sensitisation to common environmental allergens. Despite extensive research into the environmental and lifestyle causes of asthma and allergic rhinitis no single factor has been identified to explain the marked geographical variation or the time trends in disease prevalence.

In this chapter, we will review the burden of both diseases, consider the risk factors that have been implicated in their aetiology and comment on the pattern of association of the two conditions.

Definition of Disease

Asthma

Attempts were made to standardize the definition of asthma as long ago as 1958, when the CIBA Guest Symposium defined asthma as "the condition of subjects with widespread narrowing of the bronchial airways which changes in severity over short periods of time either spontaneously or under treatment" [1]. There has been little improvement on these definitions despite several further attempts [2–4].

In epidemiological studies, asthma has been identified by symptoms suggestive of disease, by diagnosed disease and by physiological measures of airway responsiveness including the bronchial response to histamine, methacholine or exercise, serial measurement of peak flow and response to bronchodilators.

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_4, © Springer 2009

Two large international studies, the European Community Respiratory Health Survey (ECRHS) [5] and the International Study of Asthma and Allergy in Children (ISAAC) [6], have developed standardised questionnaires for the assessment of asthma and asthma-like symptoms. These questionnaires have been widely adopted by other groups. More recently there have been calls for greater recognition and better definition of the different patterns of disease ('phenotypes') and this may prompt further work in the development of standardised questionnaires [7].

Despite recommendations for standardisation of bronchial reactivity [8, 9] variations in protocols between studies are common [10]. The relationship of bronchial hyperresponsivenss (BR) to clinical disease may differ depending on the agent used [11] and BR to histamine and methacholine is not specific for asthma, being independently associated with age, atopy and smoking[12].

Rhinitis

Rhinitis is 'inflammation of the nose', which occurs in response to several agents including infection and environmental allergens. The Allergic Rhinitis and its Impact in Asthma (ARIA) initiative has defined allergic rhinitis clinically as 'a symptomatic disorder of the nose induced by an IgE-mediated inflammation after allergen exposure of the membranes lining the nose' with symptoms including rhinorrhea, nasal obstruction, nasal itching and sneezing [13]. Many epidemiological surveys, however, ask directly whether subjects have 'hay fever' or 'nasal allergies' or whether nasal symptoms are present 'when you did not have a cold or the flu', sometimes with questions on the seasonality of symptoms.

Prevalence of Disease and Geographical Variation

Asthma

There is no single figure that can be used to describe the prevalence of asthma. As suggested above, the figure will depend on the definition used but it will also depend on the year of the survey and the population under study.

The ECRHS has shown large geographical variations in reported asthma symptoms [14] and in bronchial reactivity [15] in adults. The magnitude of these variations can be seen in Table 1. Some other epidemiological studies have used a similar methodology to the ECRHS and geographical variation in the 12-month period prevalence of asthma has been presented in the Global Initiative for Asthma Burden of Disease report [16]. This confirms previous observations that the prevalence of disease tends to be higher in countries in which English is the main language. It also shows a lower prevalence in the developing nations. The ISAAC study covers a much wider geographical area than ECRHS and also shows a higher

Table 1 Variation in prevalence (%) of asthma, asthma-like symptoms and hay fever in the European Community Respiratory Health

Survey (conducted 1990–1992)

In the last 12 months	No of centres	Min	25th Centile	Median	75th Centile	Max
Wheeze with breathlessness	46	1.4	7.7	9.8	13.9	16.3
Wheeze in the absence of a cold	46	2.0	9.3	12.7	16.2	21.6
Waking with breathlessness	47	1.5	4.7	7.3	8.9	11.4
Attack of asthma	48	1.3	2.6	3.1	4.5	9.7
Current treatment for asthma	47	0.6	2.4	3.5	5.0	9.8
Hayfever or nasal allergy	45	9.5	16.6	20.9	28.2	40.9

asthma prevalence in English-speaking countries with a lower prevalence in many parts of the developing world [17].

For some conditions, mapping variation in mortality can be a useful proxy for mapping variation in disease prevalence. The European Community Atlas of Avoidable Deaths 1985–1989 showed substantial variations in mortality from several diseases, including asthma, across Europe. High asthma mortality was observed in northern Europe compared with the south [18]. Making these comparisons is highly dependent on similar methods for deciding what conditions are entered on death certificates and also require that there is no significant variation in case-fatality rates. For asthma, these assumptions may not hold. In children and young adults, asthma as the primary cause of death on a death certificate is both sensitive and specific [19–21] for what clinicians would agree was fatal asthma, but in older adults, diagnostic preferences between chronic obstructive lung disease and asthma may influence what is written on the certificate [22].

Health care utilisation data have also been used to describe the burden of asthma in communities and to consider geographical variation in disease prevalence. Such data are highly dependent on health-seeking behaviour, access to health care resources, the way in which health care services are organised and on the information technology used to capture events. Great Britain has good information on health service utilisation for asthma as health care services are state-run with general practitioners acting as gatekeepers to services. Less than one in ten asthmatics will ever be admitted to hospital for their disease and hospital admission rates are not interchangeable with prevalence of disease. However, variations in hospital admission rates in the UK may reflect variation in disease prevalence [23].

Rhinitis

Both ECRHS and ISAAC have shown substantial variations in the prevalence of ‘hay fever and nasal allergies’ [14] (see Table 1) and allergic rhinoconjunctivitis [24, 25]. In general, higher levels of hay fever are observed in communities with

higher levels of asthma but such a sweeping generalisation masks some important exceptions. For example, in ISAAC, the Nigerian sample of children had one of the highest prevalences of reported allergic rhinoconjunctivitis while the prevalence of reported asthma symptoms was relatively low.

In many countries, many people with rhinitis can be self-treated with over-the-counter medications and do not seek medical consultation. Health care utilisation data for rhinitis are therefore of little or no value in assessing disease prevalence.

Time Trends

Asthma

From the middle of the twentieth century up to the mid-1990s, almost all studies that measured prevalence in the same population at different times showed an increasing prevalence of asthma and wheezy illness [26]. This amounted to an approximate doubling of disease every 14 years. There is some evidence that this trend may now be changing. The largest and most recent study of time trends in childhood asthma is the repeat ISAAC survey [27, 28]. This showed that over the previous decade the prevalence of asthma in 6–7- and in 13–14-year-olds had increased in some parts of the world and decreased in others. Furthermore, the pattern of change in older children did not mirror the pattern of change in the younger children [27] (see Fig. 1). In the adult populations taking part in the ECRHS, the prevalence of asthma and treatment for asthma increased over an 8-year follow-up, although the prevalence of wheeze remained relatively stable [29].

Studies that examine change in objective, rather than subjective, markers of asthma are quite limited [30] but increases in exercise-induced bronchoconstriction have been reported in South Wales in the late 1980s [31]. However, when the study was repeated in 1998, reported asthma symptoms had increased while there had been a decrease in exercise-induced bronchoconstriction [32]. This latter observation might be explained by the more widespread use of inhaled corticosteroids amongst the children. Increases in BR have been noted in children living in New South Wales, Australia [33], but when adults living in a coastal area of Western Australia were surveyed in 1981 and 1990, the prevalence of wheeze increased (17.5–28.8%) without any associated increase in the prevalence of bronchial reactivity [34]. In Belgian conscripts, the prevalence of asthma at medical examination increased from 2.4% to 7.2% between 1978 and 1991, while the proportion of asthmatic individuals with measurable BR to methacholine remained constant, providing evidence that the increase in asthma had been genuine and not related to increased reporting of symptoms or changes in labelling of disease [35].

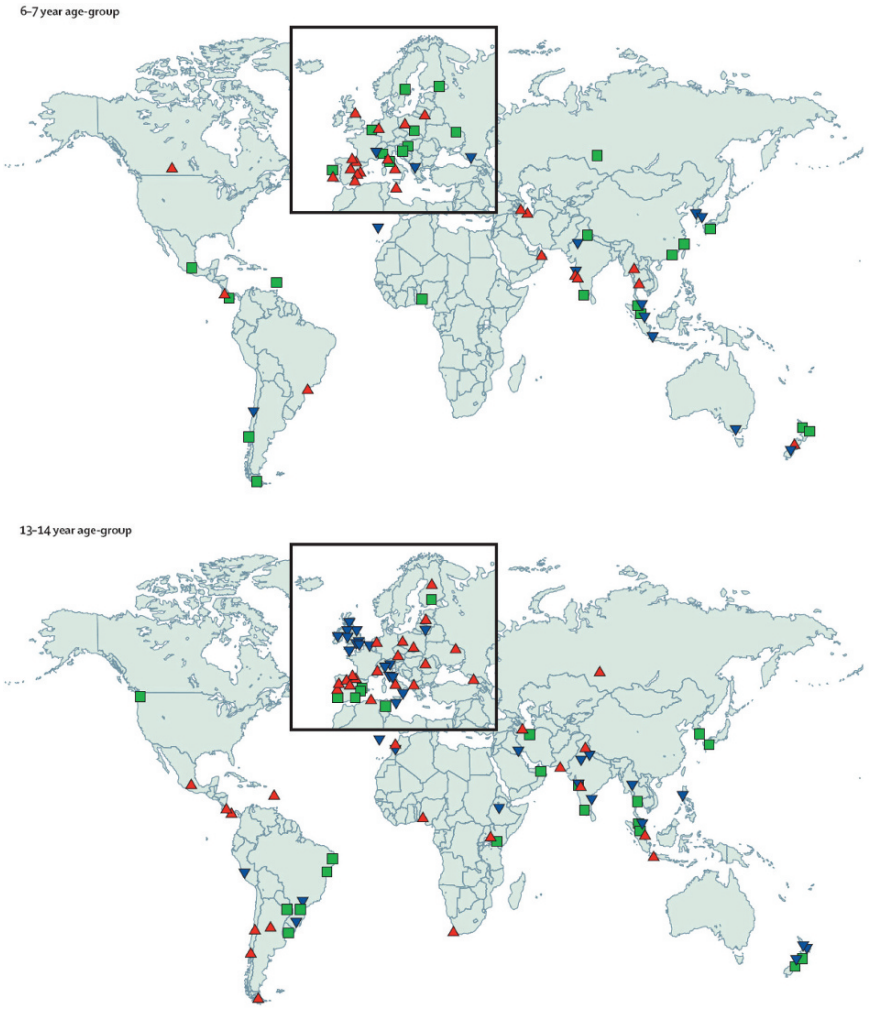

Fig. 1 World map showing direction of change in prevalence of asthma symptoms in children from 1995/6 -2002/3 as demonstrated in the International Study of Asthma and Allergies in Childhood. Reproduced with permission by Lancet

Further indirect evidence for increases in asthma comes from health service utilisation data. Hospital admission rates for asthma showed a steady increase during the 1980s [36–39] as did general practice consultations [40] although rates, at least in England, may have fallen over the last decade [41, 42].

Rhinitis

Many of the large studies that have shown increases in asthma prevalence have also shown increases in hay fever [43, 44]. The ISAAC study has described these changes in many centres across the world in both 6–7-year-old children and 13–14-year-old children [27]. In this latter age group, centres that had a higher rate of change for rhinitis symptoms tended to be the centres that ranked highly for changes in asthma symptoms prevalence.

Natural History

Asthma

Although it is a common cause of morbidity in adults, asthma is widely perceived as a disease of childhood. The incidence and period prevalence of wheeze and asthma is higher in children than adults [45–47] but this is, in part, because those born more recently have experienced a high incidence of disease, the so-called birth-cohort effect.

There are several studies showing that boys have more wheeze and asthma than girls, a difference that seems to become less apparent as the children get older, and which may even reverse after puberty [45]. This difference may be due to an increased incidence of asthma in girls during the adolescent years compared to boys, rather than an increased resolution of symptoms in boys with asthma [45, 48]. However, some caution should be exercised in the interpretation of these data, as wheeze in early childhood may be a manifestation of lung size, and boys may have smaller lungs than girls at birth [49].

The heterogeneity of wheezing in childhood and the different risk factors and prognosis associated with each has been reported. In the Tucson study, Martinez and colleagues proposed three patterns of wheeze in children up to the age of 6 years: transient early childhood wheeze, wheeze starting after the age of 3 years and persistent wheeze [50]. Certainly not all wheezing children will go on to have asthma in adult life but remission of symptoms may not be permanent [51] and is unlikely after the age of 30 years [52]. Follow-up of the 1970 British birth cohort showed that, of those who had reported wheeze at the age of 5 years, only 15% had wheeze that persisted to 16 years [53]. In the 1958 British birth cohort, a quarter of those who had a history of asthma or wheezy bronchitis by the age of 7 years reported wheeze in the past year at the age of 33 years. Recurrence of wheeze after prolonged remission was associated with the presence of other allergic diseases and cigarette smoking [54]. However, loss of symptoms may not be permanent. In New Zealand, 12.4% of children with symptoms of wheeze at the

age of 9 years, which disappeared during early adolescence, had recurrence of symptoms by age 26 years. Risk factors for relapse following remission were sensitisation to house dust mite, bronchial reactivity and an earlier age of onset of symptoms in childhood [55].

There is limited evidence that people with asthma experience higher mortality rates than those without, the excess mainly being explained by excess deaths from respiratory disease [56]. In adults with asthma, poor lung function is associated with an increased mortality [57] as it is in the general population [58], but the influence of asthma on lung function development and decline is not well understood.

When assessed at the age of 35 years, subjects in the 1958 British Birth cohort with current wheeze had lower forced expiratory volume (FEV_1) and forced expiratory capacity (FVC) than their non-wheezing peers, the difference persisted after inhalation of salbutamol and lung function measures were worse in those who wheezed earlier in life [59]. As no childhood measures of lung function were available for the cohort under study, it was not known whether this observation reflected failure to attain maximal lung function in those with asthma in childhood, or greater lung function decline in people with asthma during adult life. However other work suggests that children with asthma have lower lung function [60,61] and that this persists during adolescence even though lung growth rates in those with and without asthma may be similar [62]. With the advent of widespread and prolonged use of inhaled steroids during childhood, there are reports that there are no differences in lung function in treated children with and without asthma [61, 63]. Whether post-bronchodilator lung function would remain similar in the two groups if those with asthma ceased taking their steroids is not known.

It is also possible that having achieved maximal lung function, people with asthma experience a more rapid decline in FEV_1 [64, 65] of the order of 15 mL per year. Recent observational studies suggest that the decline in FEV_1 in adults with asthma may be diminished by regular use of inhaled steroids [66] particularly in those with high total IgE [67].

Ideally, randomised controlled trials would be used to assess the effect of treatment on lung function decline, but the duration of such trials is usually too short, with primary outcome measures being related to symptom control. Interpretation of data from observational studies may have to suffice.

Allergic Rhinitis

Hay fever is generally thought to be uncommon before the age of 5 years [68, 69] and from the limited information available, the peak incidence of rhinitis may be between 17 and 22 years [70]. Disease resolution may occur. In the 1958, British Birth Cohort less than 70% of those with hay fever at the age of 11 years or those with hay fever at the age of 16 years reported symptoms at the age of

23 years [71]. However, even though cross-sectional surveys show a higher prevalence in the younger population than the older population (see Fig. 2), the differences with age observed in cross-sectional studies is largely explained by cohort-related increases in disease prevalence, rather than disease resolution in those who are older. Although Broder et al. [46] reported more hay fever in boys than girls, others have found little difference between males and females [70].

Up to 70% of people reporting asthma who took part in the ECRHS also reported hay fever, and in all centres hay fever was strongly associated with having asthma [72]. This association existed even in those who had no serological markers of IgE to common allergens and in those with low total IgE.

When two conditions often coexist, have poorly defined time of onset and share risk factors, it is not easy to determine their precise relationship. However, some longitudinal studies have suggested that incident asthma is more common in those with a history of rhinitis, with greater risks seen in those with hay fever of the longest duration and greatest severity, and in those with both sinusitis and rhinitis [73]. Chronic sinusitis has also been associated with the onset of cough and wheeze [74].

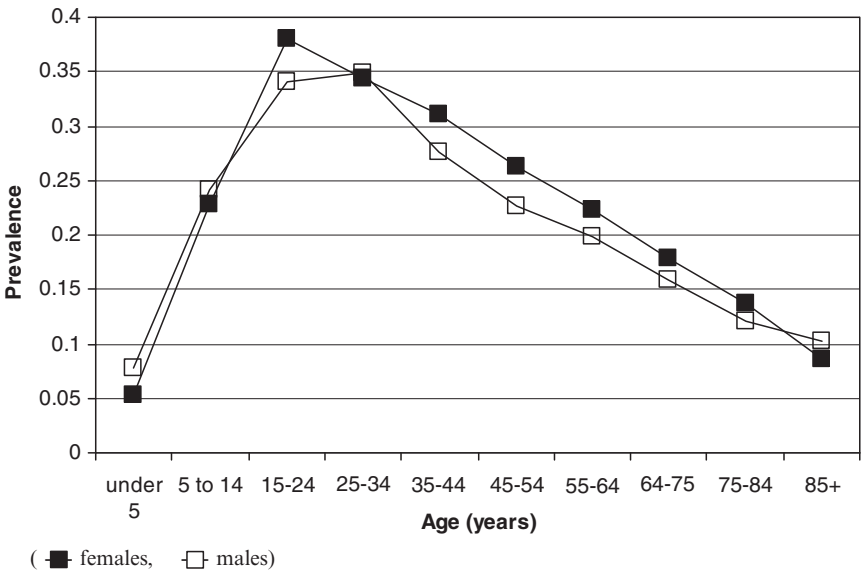

Fig. 2 Lifetime prevalence of 'hayfever or nasal allergies' in England (n=16648) (Data from Health Survey for England 2001)

Race

Asthma

There are inconsistent reports of racial differences in the prevalence of asthma, and where differences have been observed, it is difficult to determine whether they reflect differences in genetic predisposition, exposure to environmental risk factors or cultural attitude to disease [75]. Studies to examine racial differences have been conducted in the USA [76, 77], Africa [78], New Zealand [79], Australia [80, 81] and the UK [82].

Rhinitis

Less is known about ethnic and racial differences in the prevalence of hay fever although a large study in the USA showed Asians were at an almost 50% greater risk of reporting hay fever than the White population, but had a similar risk of asthma. The prevalence of asthma was similar and the prevalence of hay fever was slightly higher in the Black population compared to the white, but the Black population was more likely to report 'asthma without hay fever' [83].

Socio-economic Status

Asthma

The prevalence of asthma in children is higher in wealthy countries [84], but in the West, the relation of social class (a marker of personal wealth, at least in the UK) to asthma appears to have changed over time, asthma having once been a disease of the more advantaged and becoming more a disease of the disadvantaged [85]. Socio-economic status may be important in disease aetiology, disease severity or labelling and treatment of disease [86].

Rhinitis

Rhinitis is also more common in wealthy countries but at an individual level its association with socio-economic status is not certain and, as for asthma, may be changing. In one British birth cohort, hay fever was more common in those from higher social classes but by adulthood was more related to father's social class than

own social class [71]. Labelling and diagnosis of symptoms and signs suggestive of hay fever may vary between socio-economic groups [87, 88].

Family Structure

In 1989 Strachan reported a strong negative association of birth order with the prevalence of hay fever at ages 11 and 23, and proposed that exposure to older siblings led to an increased level of infections in early life, which in turn decreased the likelihood of allergic disease [89]. This hypothesis was termed the 'hygiene hypothesis'. Studies published up to the year 2000 that examined the relationship of asthma and hay fever with family size have been included in a systematic review [90]. There is overwhelming evidence that hay fever is negatively associated with family size but the associations with asthma are less consistent, and when seen are not as strong [90, 91]. Older siblings or day care attendance may protect against later wheezing (generally thought to be associated with IgE sensitisation), but may increase the risk of early childhood wheeze, much of which is related to acute viral infections [92].

The hypothesis that the protection from hay fever by large sibships is due to infection is strengthened by the observation that children from small sibships, who attend child care facilities early in life, are similarly protected [93]. However, a possible alternative explanation has been proposed based on the observation that women who have had more children have less atopy [94]. Longitudinal studies to test this have produced conflicting results [95, 96] and studies assessing fertility in atopic women show no association of fertility with atopy [97,98]. Even if pregnancy does alter the maternal immune system, it cannot explain the observation that having younger siblings is also protective for hay fever, independently of older siblings [89–100].

Factors Linked to the Hygiene Hypothesis

Viral Infections in Infancy

The hygiene hypothesis initially proposed that children exposed to poor hygiene and increased infections in early life had lower levels of IgE sensitisation and allergic disease. However, in a large study in Sheffield, England, no association of symptoms of neonatal infectious disease or infectious disease in the child's family, and hay fever was observed [101].

Extensive work by Matricardi et al. suggested that orofaecal infections were of interest for protection against asthma and hay fever and IgE sensitisation, but he also showed that the virus herpes simplex 1 (which unlike herpes simplex 2 is

acquired in early life), was associated with lower levels of IgE sensitisation. Reported associations of less IgE sensitisation with measles in Guinea-Bissau, West Africa, may be explained by survivor bias, with fewer atopic children being likely to have survived severe infection [102].

Enteric Infections

Children and adults living in large families are likely to experience higher levels of oro-faecally transmitted infections, including hepatitis A. Italian military recruits who had evidence of previous hepatitis A infection had a lower prevalence of IgE sensitisation [103] and further work showed negative associations of 'allergic asthma' and allergic rhinitis with hepatitis A, *Toxoplasma gondii* and *Helicobacter pylori* [104].

The analysis was repeated using data from the National Health and Nutritional Examination Survey III conducted in the United States, and a lower prevalence of both hay fever and asthma were seen in those with serological evidence of past infection with *T. gondii* and Hepatitis A [104]. However, other studies in the UK have not replicated these observations [105, 106].

Bowel Flora

There has been some interest in the role of bowel flora in atopy and allergic disease [107]. Conduct of studies that require examination of multiple stool specimens is not easy and relatively few observational studies have been conducted. The presence of *Clostridium difficile* in stool samples collected at 1 month has been associated with recurrent wheeze at the age of 2 years and other markers of allergy (eczema and serum IgE) [108].

Randomised controlled trials of the use of probiotics to alter bowel flora and reduce allergic disease have shown a benefit for eczema but not for asthma or hay fever [109, 110]. Use of antibiotics, which is known to be associated with changes in bowel flora, has been linked to higher rates of asthma and hay fever in some studies [111]), but not others [112]. It seems likely that the link between antibiotic use and asthma reflects reverse causation, with atopic children possibly having more severe respiratory illness and being more likely to be prescribed antibiotics for repeated respiratory infections [113].

Anthroposophic Lifestyle

In Sweden, children following an anthroposophic lifestyle are likely to have a high intake of products containing lactobacilli. They also have lower rates of IgE

sensitisation, asthma and hay fever [114].The differences in disease prevalence between Steiner and non-Steiner children was not so marked in the Prevention of Allergy Risk factors for Sensitisation in Farming and Anthroposophic Lifestyle (PARSIFAL) study and PARSIFAL suggested that a lower use of antibiotics and of paracetamol was associated with a decreased risk of IgE sensitisation [115]. Frequent paracetamol use has been associated with asthma in adults both cross-sectionally [116, 117] and prospectively [118], and in a population-based birth cohort study, frequent use of paracetamol in late pregnancy was associated with an increased risk of asthma in the offspring [119]. The explanation for the low levels of asthma and hay fever in Steiner school children is therefore likely to be more complex than originally hypothesised.

Farming and Proximity to Animals

Animals harbour a range of infectious agents that may be passed to humans. Children who are brought up on farms have a lower prevalence of IgE sensitisation, wheeze, asthma and hay fever than those who are brought up in the countryside but not on farms [120, 121]. This association may, in some part, last into adult life [122].The association has been variably associated with regular drinking of unpasteurised milk [121, 123], going into animal sheds [121], exposure to pigs, feeding silage on the farm and the child's involvement in hay making over prolonged periods [124], but as yet there is no evidence that one of these exposures, any microbial exposures or any other specific contaminants, explain the apparent protection afforded by growing up on a farm.

Vaccination and Tuberculin Sensitivity

Concerns have been raised that the increase in asthma is related to the current extensive vaccination programmes. Observational studies in many parts of the world are complicated by the high population coverage of vaccination with a relatively small, highly selected proportion of individuals who have not received vaccinations. This leads to confounding by factors including family history of allergy or social class. The few randomised controlled trials that have been conducted show little evidence of an important effect [125] and the public should be reassured that vaccines are safe.

In contrast, there has been interest in the possible protective effect of early administration of BCG [126–128]. However, work conducted in Sweden [129], Greenland [130] and the UK [131] have not supported these observations.

Exacerbations due to Acute Viral Infections

Even though the hygiene hypothesis suggests infections in early life may be protective for disease, there is overwhelming evidence that viral upper respiratory tract

infections cause exacerbations of asthma in children [132], particularly at the beginning of school term [133], and in adults [134]. In children, infection is associated with wheeze, particularly in children with small lungs [50] and many first episodes of wheeze are associated with an acute infection [135] possibly due to 'unmasking' of asthma in susceptible individuals rather than direct causation. Infection with rhinovirus in the first year of life has been associated with the onset of asthma by the age of 3 years and may induce inflammatory mediators that influence airway remodelling and adversely affect lung development [136]. There is some evidence that exposure to allergen may make asthma symptoms worse in the presence of infection [137, 138].

Parasites

Observations that asthma and allergic disorders are less common in rural African communities have led to investigations of the role of parasitic infection. A recent systematic review suggests that different parasites have different effects, concluding that Ascaris infection was associated with an increased risk of asthma, but the opposite was true for hookworm infestation [139]. A randomised controlled trial in which children were treated with albendazole showed no difference in IgE sensitisation or allergic diseases between the treatment and placebo groups after 12 months [140] but the prevalence of hookworm infestation (*A. duodenale),* was relatively low in comparison to other infestations. Although *Schistosoma haematobium* may influence the allergic response [141], there is no clear evidence that infection with Schistosoma is protective for asthma or hay fever.

Genetics

The current evidence regarding the inheritance of asthma and allergic rhinitis will be discussed in depth elsewhere in this book. Rapid advances in the technologies for genotyping mean that samples can be rapidly analysed and hundreds of thousands of single nucleotide polymorphisms can be examined at once. Although several genes have been identified as being associated with disease, many initial findings for asthma have not been replicated [142].

If the function of a gene is known, and is considered likely to alter the body's response to a particular lifestyle or environmental exposure, individuals with the relevant genotype may be at substantially increased or decreased risk of disease compared to others. Finding such gene–environment interactions can strengthen the evidence for inferring causal associations between environmental exposures and disease. For example, glutathione *S*-transferase polymorphisms may modify the effect of tobacco smoke exposure on risk of childhood asthma [143, 144] and the effects of antioxidant supplementation on lung function in asthmatic children [145].

It seems likely that the genetics of asthma and related traits is complex, involving hundreds of genes, each with small effects (relative risks rarely exceeding 1.5). Adequately powered genetic epidemiologic studies with sample sizes of several thousand subjects are required to detect gene–gene and gene–environment interactions and to avoid false-positive results.

Exposure to Allergen

Geographical Variation and Time Trends

There is marked geographical variation in exposure to common allergens such as house dust mite [146] and cat allergen [147] but relatively inconsistent evidence for whether these levels have increased over the past 30 years [148, 33]. The pollen season in London and its immediate surroundings has decreased in length and in severity during the late twentieth century [149] and in the UK, there is little evidence that the number of pets has increased during the past 30 years [150]. However, features of modern-day living, with large proportions of time spent in the indoor environment, may have resulted in increased personal exposure to house dust mite and the allergens shed by indoor pets, even if there has been no measurable increase in allergen levels.

Asthma

Studies of exposure to house dust mite have suggested that exposure to high levels of allergen not only increases the risk of sensitisation but also increases the risk of clinical disease [148]. This is not seen in all studies [151–154], and two Cochrane Reviews of randomised controlled trials that attempted reductions in indoor allergen exposure as a means of secondary prevention of asthma concluded that, as yet, there was no evidence for beneficial effects of reduction in house dust mite allergen [155] or cat allergen [156]. However, people with asthma often attribute their symptoms to exposure to allergen (see Table 2).

Exposure to outdoor allergen may be an important determinant of severity of disease in asthmatic individuals. Morbidity [157] and mortality [158, 159] from asthma in young adults increase in the pollen season in the UK (see Fig. 3) and the USA, a seasonal pattern that is not observed in older adults. In the USA, seasonal variation in attendance at a medical centre with asthma associated with specific IgE to rye grass [160] occurs, although sensitisation and exposure to other allergens are also important [161].

Epidemics of asthma have occurred in response to high levels of allergen in the air. In Barcelona, these followed the release of soybean particles during unloading of soybean cargo at the docks. Case–control studies showed that cases had an

Table 2 Proportion of people with asthma who report that exposure to the agent makes their asthma worse. (Health Survey for England 2001)

Agent:	<16	16–45	46+
Dust	11.6	30.0	25.2
Pets	6.7	27.3	15.0
Feathers	1.8	10.5	8.8
Pollen	12.4	28.2	31.3
Grass	5.8	14.3	20.4
Any of the above	24.0	55.5	47.6
Infections	50.7	46.6	46.8
Excited or upset	9.8	13.9	15.6
Cold air	12.0	17.2	21.8
Exercise	24.4	21.4	10.8
Excited/cold air or exercise	37.3	41.2	36.7
Foods or drinks	3.1	5.5	4.7
Traffic fumes	2.7	10.5	18.4
Tobacco smoke	13.8	18.5	28.6

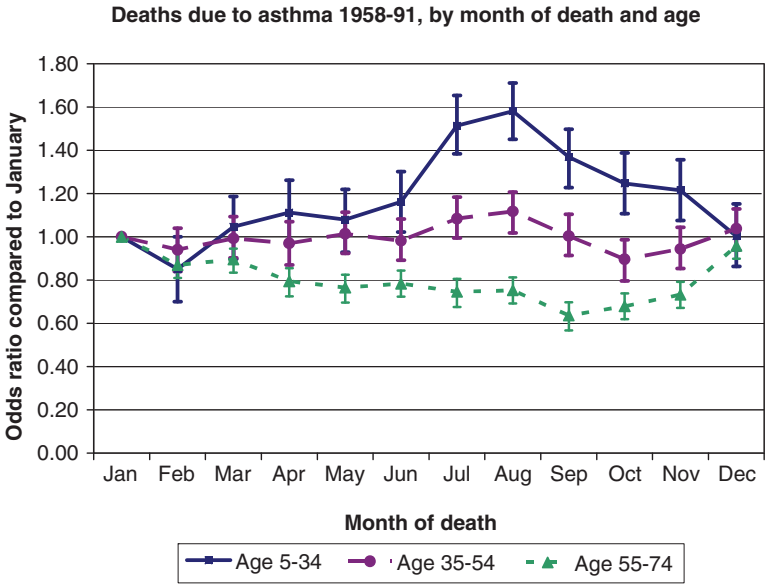

Fig. 3 Variation in asthma mortality with month by age group

increased risk of exposure to high levels of soybean [162] and appropriate changes to the unloading procedures resulted in cessation of epidemics [163]. Exposure to castor bean has also been implicated in asthma epidemics [164–166] and 'thunderstorm asthma' is a well-documented phenomenon [167–169]. Exposure to *Alternaria alternate* in sensitised individuals has been associated with near fatal asthma [170].

Hay Fever

Although some studies have suggested that infants born at the time of the birch and grass pollen season in Finland [171–172] are more likely than those born at other times of the year to become sensitised to birch and grass, there are inconsistent reports that the prevalence of hay fever varies by birth month [173, 174] and other studies looking at early exposure to allergen do not consistently suggest that this leads to hay fever [175–177]. Health service utilisation data for hay fever show clear seasonal patterns with increases during the pollen season.

Diet

Observational studies, mainly cross-sectional, have suggested that a low intake of fruit and vegetables, fish, butter and dairy fat, antioxidants (vitamins C, E, beta-carotene and selenium), magnesium, and *n*–3 fatty acids, and a high intake of sodium, margarine and *n*–6 fatty acids may be associated with increased risk of asthma, although evidence is conflicting [178]. However, *n*–3 fatty acid supplementation does not appear to benefit children or adults with established asthma [179], and recent trials in asthmatic adults of vitamin C plus magnesium, vitamin E, and selenium, have been disappointingly negative [180–182]

Nutrition in early life may be of importance in the inception of asthma [183] and birth cohort studies have suggested that low prenatal selenium status [184, 185] and low maternal intakes of vitamin E and zinc [186, 187], and vitamin D [188, 189],in pregnancy may increase the risk of wheezing in early childhood. No convincing association of maternal or cord blood *n*–3/*n*–6 fatty acid levels with early wheezing or eczema has been demonstrated [190].

The role of breastfeeding in atopic disease remains controversial, with some observational studies suggesting that it reduces the risk of asthma and others suggesting that it may increase risk [191]. There is a paucity of data on diet and rhinitis, but there is some evidence that rhinitis may be associated with higher intakes of margarine [192] and oleic acid.

Smoking

Personal Smoking

There are several methodological problems in the identification of associations of smoking with asthma, including the ‘healthy smoker’ effect, the tendency for those with disease to avoid smoking [193]. Smoking has been associated with total IgE and IgE sensitisation to environmental [194] and occupational allergens [195–197].

However, the association of specific IgE with smoking may depend on the allergen, with current smokers having more IgE to house dust mite and markedly less specific IgE to cat and to grass [194, 198]. Cross-sectional studies report lower levels of hay fever in those who smoke [199].

It is well established that smoking is related to chronic bronchitis and to fixed airways obstruction but whether smoking causes asthma remains highly controversial, with some arguing that there is a non-causal association [200] and others arguing that smoking causes increases in asthma severity rather than causing asthma to develop [201]. Adults who start to smoke or continue to smoke have greater increases in BR as they age [202].

Passive Smoking

In 1997, a systematic review and a series of meta-analyses were conducted to assess the health effects of passive smoking on children's health [203]. There is a very consistent picture of increased respiratory illnesses and symptoms in the children of those who smoke, the risks being greater in young children than older children, probably because as children grow up they spend less time in the home with their mother. The associations with symptoms may in part be a consequence of *in utero* exposure to maternal smoking influencing lung growth and making a child more susceptible to wheeze with infection. As most mothers who smoke in pregnancy continue to smoke after the child has been born, this effect is difficult to disentangle from the effects of postnatal exposure to tobacco smoke.

Cross-sectional studies show that adults reporting more exposure to other people's tobacco smoke, particularly in the workplace, have more symptoms suggestive of asthma and more bronchial reactivity [204].

Sex Hormones

The incidence and prevalence of asthma is higher in boys than in girls and this difference is less apparent, if not reversed, during the reproductive years [45]. While such differences might be related to different reporting, labelling and treatment of disease in men and women, other epidemiological observations suggest these differences may be related to sex hormones. Asthma severity varies during the menstrual cycle, in pregnancy and hormonal treatments have been associated with an increase in asthma in older women in cross-sectional and longitudinal studies. However none of the reported associations are consistent with a particular serum hormonal profile [205]. Although there is limited evidence that women with asthma have an increased prevalence of some forms of gynaecological disease [206], the explanation for this is uncertain, may not be related to sex hormone levels [205] and does not appear to influence fertility rates [97].

Hormonal 'rhinitis', however, is a recognised condition in allergy clinics and changes in nasal congestion and markers of rhinitis have been reported with the menstrual cycle [207]. In a birth cohort of more than 30,000 women, those who had an early menarche had more allergic rhinitis than those who started menstruating after the age of 13 years [100].

Air Pollution

The prevalences of asthma and hay fever have increased during a period when air pollution has changed from that largely due to domestic coal burning to that related to vehicle emissions. Emissions from traffic may influence allergic responses [208]. However, relatively few studies have assessed objective measures of atopy and traffic-related air pollution at a high level of resolution, and the results so far are not wholly consistent [48, 209, 210] or suggest complex associations [211, 212]. Air pollution may also affect the airways, altering the expression of all forms of airway disease including asthma and it is not clear whether the symptoms are worse in those with atopy or atopic symptoms [210–214].

Time series studies of asthma mortality and admissions have shown associations with levels of pollution, though there is a great deal of uncertainty about the specific exposures that are important and there are important discrepancies in the evidence. In London, for instance, variations in ozone levels have been associated with daily general physician (GP) consultations for asthma [215],visits to accident and emergency rooms with respiratory complaints [216], and admissions for asthma [217], as well as total respiratory mortality. However, neither of the two European multi-centre studies of acute air pollution effects using routine health data, Air Pollution and Health a European Approach (APHEA) I [218], nor APHEA II [219], found an association between hospital admissions from asthma and ozone levels. APHEA I found associations only with NO_2 and APHEAII found associations with small particles. Panel studies have been even less convincing. The largest of these to date, the PEACE study, was a well-conducted international study that had good power to detect effects but was unable to do so [220]. Finally, there have been major episodes of air pollution that have been identified as having no clear effect on asthma, including the London smog of 1952 [221] and the last major smog epidemic in Europe [222].

The Southern California Children's Health Study showed that exposure to some pollutants may influence lung growth [223, 224] but new diagnoses of asthma were associated with high pollutant areas only in children who played three or more team sports [225]. In spite of the inconsistency of the findings, there is some indication that the underlying mechanism relates to oxidative stress [145, 226, 227].

Given the strong biological plausibility that air pollution may affect both atopic disease and asthma, the question arises why the quantification of these effects is so problematic in epidemiological studies. This may be due to the effects being very small (but, given the almost universal exposure, none the less potentially important), difficulties of assessing exposure of individuals accurately and the large potential

for confounding or effect modification. One specific confounder in the studies of short-term exposure is airborne allergen. This can have a very large effect, as has been shown in a number of epidemics of asthma associated with release of castor bean or soy bean allergen into the air [162, 164, 166]. Some studies have attempted to deal with this problem by adjusting results for pollens and moulds in the air, but this is at best only a partial solution to the problem.

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Epidemiology of Pediatric Asthma

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Introduction

Childhood asthma is one of the most common chronic respiratory disorders affecting up to 30% of school children in the developed world. Many epidemiology studies have consistently documented an increasing trend of asthma over the past three decades [1]. A few recent reports suggested that there is a plateau or even decline in the trend of asthma prevalence [2–4]. Due to the lack of precise definitions of asthma and validation of survey instruments, reliable comparison of reported prevalence studies from different regions are very difficult. A number of large multicentered epidemiological studies using standardized instruments have been performed revealing important information on the prevalence and burden of asthma around the world [5–7]. They have also provided a framework to investigate the possible determinants of asthma. Studies in the rural areas have consistently showed a lower prevalence of asthma in children who have been brought up in a farming environment [8–11]. This chapter reviews the existing data on asthma epidemiology and summarizes the recent important findings from studies of the environmental determinants of childhood asthma.

Asthma Epidemiology

Many published epidemiological studies of asthma used different methodologies such that meaningful comparisons between countries are extremely difficult. The increasing awareness of asthma is likely to affect the responses of the parents or the subjects. Studies of young children with asthma are even more problematic as there

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_5, © Springer 2009

are different phenotypes of wheezing disorders in young children, reflecting different etiologies and pathogenesis [12]. "Wheeze" is the most widely recognized symptom of asthma and different standardized questionnaires usually ask for the presence and the frequency of this symptom [5]. However, in some languages such as German and Chinese, there is no equivalent term for "wheeze" such that the true prevalence of wheezing will be underestimated by the translated questionnaires [13]. In addition, validation of the survey instrument is difficult because of the lack of a universally accepted "gold standard" of diagnosing asthma [14]. Therefore, depending on the subjects' or the parents' understanding of the disease, access to medical care, physicians' use of diagnostic labels, translations of the survey instrument, and cultural differences, the responses of the subjects or their parents will be affected.

In order to circumvent the linguistic problem of interpretation of the written questionnaire, the International Study of Asthma and Allergies in Childhood (ISAAC) includes a video questionnaire in addition to the written questionnaire. The video questionnaire shows children and adolescents with different asthma symptoms and asks the respondents whether they have similar symptoms and the frequencies of such symptoms. Some investigators have included additional "objective" markers of asthma to define asthma phenotype. Bronchial hyperresponsiveness (BHR) has been considered to be one of the best objective measures of asthma, but there has been a continuing debate of whether such measurement would have greater validity than symptom questionnaires alone [14,15]. Therefore, the interpretation of any epidemiological study of asthma must take into account the case definition of asthma as well as the methodology used in data collection.

Prevalence of Childhood Asthma

The results of the ISAAC surveys, which used standardized methodologies, have been most interesting as they clearly showed dramatic variations of prevalence of asthma symptoms across different countries and racial background. The highest prevalence rates were found among the English-speaking countries such as British Isles, New Zealand, and Australia while lower rates were in Eastern Europe and some Asian countries [5]. The ISAAC study is the largest, epidemiological study of asthma and it has been carried out in different phases. ISAAC Phase One was carried out between 1994 and 1995 and to study the prevalence and severity of asthma in random samples of schoolchildren of two age groups (6–7 and 13–14 years) from 155 collaborating centers in 56 countries around the world [5]. The prevalence of reported wheeze in 13–14-year-old varies widely across different regions, with the highest prevalence of 32.2% in the UK and the lowest of 1.6% in Akola, India. Figure 1 shows the 12-month prevalence of self-reported asthma symptoms of 13–14-year-old children in selected countries. Table 1 shows the prevalence of wheeze in the past 12 months in the 13–14-year-old children as documented by the written and video questionnaires from ISAAC Phase One. Table 2 shows the 12-month prevalence of wheeze in the 6–7-year-old children from ISAAC Phase One and Three. ISAAC Phase Two was designed to assess the variation in the prevalence and severity of asthma

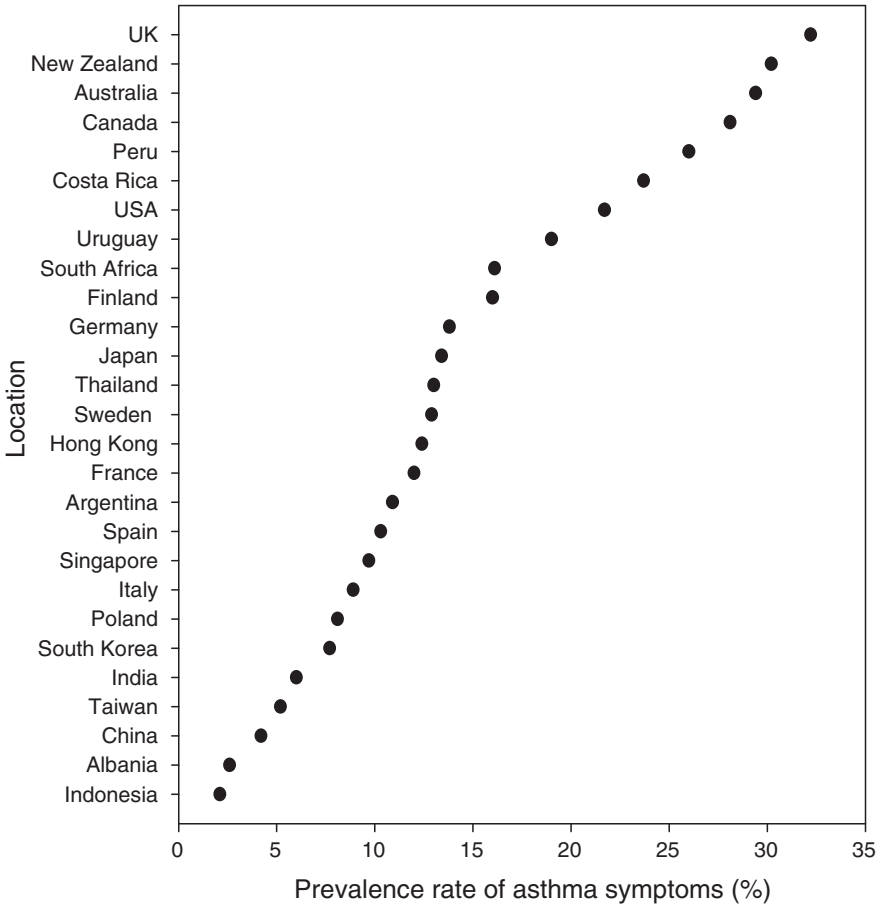

Fig. 1 Twelve-month prevalence of self-reported asthma symptoms from written questionnaires: ISAAC Phase One results in 13–14 year-old subjects

symptoms and objective markers [16]. Interestingly, the well-known association of atopic sensitization and wheeze was very strong in affluent Western countries but such association was much weaker in less developed countries like Brazil and Estonia. Further studies are needed to evaluate the role of atopy in the pathogenesis of asthma, especially in children from developing countries.

Secular Trends of Asthma Prevalence

Although many epidemiological studies have shown an increasing prevalence of asthma in Western countries, many of these studies used only questionnaire surveys in estimating the prevalence [1,17,18]. Increased community awareness of asthma

Table 1 Twelve-month prevalence of asthma symptoms in 13–14-year-old children: ISAAC Phase One results

	Written questionnaire			Video questionnaire		
Location	Wheeze	Wheeze disturbs sleep	Severe wheeze limiting speech	Wheeze	Wheeze disturbs sleep	Severe wheeze
New Zealand	30.2	3.2	8.0	18.4	11.7	12.4
Australia	29.4	3.0	8.3	17.6	11	18.7
Canada	28.1	2.1	8.1	12.0	6.5	8.5
USA	21.7	4.2	10.0	13.0	4.6	10.6
Kuwait	17.0	5.7	10.6	13.3	9.4	9.1
Finland	16.0	0.5	3.1	5.8	1.5	2.6
Germany	13.8	1.2	5.7	5.3	3.6	3.6
Japan	13.4	0.6	2.1	10.2	3.7	5.3
Hong Kong	12.4	0.5	2.4	10.1	3.8	6.9
France	12.0	1.5	2.6	8.3	4.4	4.6
Argentina	10.9	2.1	3.8	8.3	4.7	5.5
Poland	8.6	1.2	1.8	3.5	1.5	2.3
Malaysia	9.6	0.8	1.8	5.9	2.5	3.6
South Korea	7.7	0.2	2.7	3.7	0.5	1.9
India	6.0	1.1	3.0	2.9	2.3	2.5
Mainland China	4.2	0.3	0.7	2.0	0.6	1.2

Table 2 Comparison of ISAAC Phase One and Three results: 12-month prevalence of wheeze (written questionnaire) of 6–7-year-old children

Location	Phase 1	Phase 3	Year between phases
Australia	27.2	20.0	9
New Zealand	23.6	22.2	9.5
Brazil	21.3	24.4	7
Japan	17.4	18.2	8
Canada	14.1	18.2	9
South Korea	12.4	5.6	5
Poland	10.9	13.6	6
Sweden	10.3	10.2	8
Taiwan	9.6	9.8	7
Estonia	9.3	9.6	7
Hong Kong	9.1	9.4	6
Mexico	8.6	8.4	8
Thailand	8.2	11.9	6
Austria	7.8	7.4	7
Italy	7.5	7.9	8
Spain	6.2	9.5	7
India	6.2	6.8	7.5
Iran	5.4	12.0	6

is likely to contribute to the increase in disease prevalence documented by these questionnaire surveys. Some researchers have included the more "objective" markers of asthma such as the measurement of total IgE and specific IgE, skin-prick test, and measurement of BHR [19,20]. Burr et al. carried out two surveys using the same questionnaire along with exercise provocation test in schoolchildren from England in 1973 and 1988. The prevalence rates of asthma and BHR were found to have doubled over the study time period [21]. In another study conducted in primary schoolchildren in two Australian towns over the years 1982–1992, the prevalence of wheeze within the past 12 months has doubled while the prevalence of BHR has also increased by 1.4- to 2-fold [19]. In contrast, von Mutius et al. [20] performed two surveys 5 years apart in former East Germany using questionnaire assessment, skin-prick test, and measurement of BHR in 9–11-year-old children. The prevalence rates of symptoms of hay fever and atopic sensitization have increased by 2- and 1.5-fold, but there was no significant change in the prevalence of asthma or BHR. The existing data do suggest that asthma prevalence would increase with increasing economic development and westernization. The challenge is to find out what are the factors responsible for the increase in asthma while the society is undergoing "modernization" or "westernization."

Increasing trends of asthma prevalence have also been observed in Chinese and Japanese children [22–25]. The prevalence rates of asthma and wheeze have increased to 11.2% and 12.4% in 13–14-year-old children as shown by Phase One ISAAC study conducted in 1994–1995 [23]. Nishima studied children using the same Japanese translation of the American Thoracic Society (ATS) questionnaire to study and found that asthma prevalence has increased from 3.5% in 1982 to 4.6% in 1992 [22]. Migrant studies have also provided important insights into the possible environmental determinants important in the pathogenesis of asthma. Several large comparative studies in the Chinese population showed disparity of asthma prevalence within the same ethnic group [23–25]. The 12-month prevalence of wheeze in 13–14-year-old schoolchildren as identified by the ISAAC video questionnaire was 10.1% in Hong Kong, while the average rate in mainland China was only 2%, with the highest rate of 3.3% in Beijing and the lowest of 1.3% in Chongqing. Environmental exposure is likely to be the key in explaining the observed difference within the Chinese population. The ISAAC Phase Two protocol included "objective" markers of atopic disorder such as skin-prick test and bronchial challenge test [16,24]. Interestingly, the rate of allergic sensitization was significantly higher in Guangzhou (30.8%) than in Beijing (23.9%) but the prevalence of asthma and wheeze was similar in these two cities. Therefore, the difference in the prevalence rates of atopic sensitization among the three cities cannot explain the higher prevalence of asthma in Hong Kong. Factors other than those related to atopic sensitization must also be important in the pathogenesis of childhood asthma.

The ISAAC Phase Three study was planned to evaluate time trends in the prevalence of asthma and related atopic disorders by repeating the cross-sectional study after at least 5 years. Almost half a million children in the two age groups have participated in the Phase Three studies. Interestingly, children of the younger age groups were more likely to show increasing prevalence of allergic rhinoconjunctivitis

and eczema but not asthma. The exact reasons for such pattern remained to be explored. Among the centers with relatively high prevalence as documented in Phase One, the data from Phase Three either showed a plateau or even a declined in asthma prevalence [26]. Recent data from England also showed a decrease of prevalence of current wheeze from 33.9% in 1995 to 27.5% in 2002 [3]. Similar findings were documented in Australia, Switzerland, and Hong Kong [2–4]. Further studies are necessary to reveal the possible factors responsible for the recent decrease in asthma prevalence in these countries.

"Hygiene Hypothesis" and Asthma

It has been almost 20 years since Strachan described the phenomenon that infection in early childhood, transmitted by unhygienic contact with older siblings or acquired prenatally from the mother by contact with her older children, may reduce the development of asthma and related atopic diseases [27]. As early exposure to various infections maybe an important factor, many studies have been conducted to investigate the role of early microbial exposure in the subsequent development of asthma. Early exposure to various infections may alter the cytokine response and Th-1 and Th-2 balance of an individual, thereby affecting the subsequent risk of asthma [28]. Furthermore, microbial exposure may also enhance the activity of T regulatory cells, resulting in immune suppression and a subsequent downregulation of both Th-2 and Th-1 immunity [29]. Therefore, frequent early infections or exposure to microbes or their components most likely program the immune system and reduce future development of asthma and related allergic diseases.

Farming Exposure and Asthma Epidemiology

In line with the "hygiene hypothesis," children brought up in a farming environment have been found to have less asthma and allergies [8–11]. Brau-Fahrlander et al. reported a study of Swiss children aged 6–15 years showing farming as a parental occupation was significantly associated with lower rates of symptoms of allergic rhinitis and atopic sensitization [8]. In another large study of German children aged 5–7 years, the prevalence rates of hay fever (OR 0.52; 95% CI 0.28–0.99) and wheeze in the past year (OR 0.55; 95% CI 0.36–0.86) were significantly lower in farmers' children when compared with children not living in a rural farming environment [9]. Several other studies performed in Canada, Austria, Finland, and the United States corroborated these findings [10,11].

A variety of microbial components have been tested to determine if there are the important factors associated with the protection against asthma and allergies in the rural setting. Perhaps one of the most extensively studied factors is endotoxin.

The results of the ALEX study showed that early exposure to stables and consumption of unpasteurized farm milk were the two major factors associated with the lowest frequencies of asthma and atopic sensitization [30]. There was an inverse relationship between the levels of endotoxin level in the samples of dust from the subjects' mattress and the prevalence of atopic asthma and atopic sensitization. In addition, stimulated cytokines production of peripheral blood lymphocytes including tumor necrosis factor α and interleukin-10 showed an inverse relationship with endotoxin level. Therefore, exposure to high level of endotoxin may downregulate the immune response. The investigators also examined the relationship between allergies and the level of *N*-acetyl-muramic acid in the dust samples. *N*-acetyl-muramic acid is a major component of bacterial peptidoglycan found in the bacterial cell wall [31]. An inverse relationship of mattress dust muramic acid and prevalence of asthma was also found even after adjustment for the endotoxin concentration.

Risk Factors for Childhood Asthma

Allergens and Atopy

Allergen exposure is a well-known factor associated with sensitization while allergic sensitization is a strong risk factor for asthma [32]. Several birth cohort and intervention studies have been performed in the past decade to investigate the relationship of allergen exposure, allergic sensitization and subsequent development of asthma [33–38]. The German multicenter allergy study (MAS) was a prospective birth cohort study from five German cities [33]. At 7 years of age, asthma and symptoms of wheezing were ascertained by parental questionnaire. Subjects found to have early and persistent sensitization were 10 times more likely to have asthma at 7 years of age when compared to those subjects who had never been sensitized. However, a persistent pattern of atopic sensitization was not associated with increased risk for asthma in the absence of family history of atopy or asthma. There was no relationship between the level of allergen exposure and subsequent development of wheezing or physician-diagnosed asthma or the degree of BHR. Clearly, these studies do not support the hypothesis that exposure to environmental allergens causes asthma, but rather that the ability to mount specific IgE responses whereas the development of asthma is controlled by other independent determinants. Another birth cohort of from UK was performed to evaluate the role of early allergen exposure and later development of asthma [37]. At 5 years of age, there was no relationship was between allergen exposure and subsequent sensitization and asthma.

Prospective intervention studies have also been designed to test whether reduction of allergen exposure might alter the subsequent risk of asthma [34,36]. The Manchester Asthma and Allergy Study is a prospective, prenatally randomized

study to evaluate the development of asthma and atopy in a cohort of high-risk infants [34]. Subjects were randomized to a series of allergen avoidance measures or to a normal regime. At 3 years of age, symptoms suggestive of allergic disorders were generally lower in the active group, but the differences did not reach statistical significance. Paradoxically, the prevalence of atopic sensitization to at least one allergen was significantly higher in the active group (34.7% vs. 25.5%). The reason for this observation is unclear, but the stringent intervention might have reduced the exposure to a protective factor, such as endotoxin. Reassessment of the subjects at an older age will be needed to determine the true effects of the prescribed stringent measures of allergen avoidance at an early age. The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) Study also did not find any effect of using mite-impermeable mattress on other respiratory symptoms, atopic dermatitis, or serum IgE level [36]. The major limitation of these intervention studies is the short duration of follow-up. Detailed evaluation of the subjects in these cohorts including lung function and BHR testing at school age will be needed to determine the possible long-term effects of such intervention. Summing up the current evidence, it appears that allergic sensitization is most likely is a marker rather than a causative factor for subsequent development of asthma. Allergen avoidance does not appear to be an effective primary preventive strategy. Nevertheless, objective and detailed assessment of the subjects from these cohort studies when they reach school age will shine more light on the relationship between allergen exposure and asthma.

Infection

Daycare attendance has been well documented to be associated with increased respiratory infections early in life [39]. In a cross-sectional study of German schoolchildren, children from small families (less than four members) who entered day nursery at age 6–11 months had significantly lower prevalence of atopic sensitization compared to those who entered at an older age [40]. The prospective Tucson children's respiratory study also revealed that children who attended daycare during the first 6 months of life or had one or more older siblings had a lower risk of wheeze when they reached school age [41]. The inverse relationship between infections and allergic disorders has also been found in many studies. Among Italian military students, lifetime prevalence of asthma or allergic rhinitis was significantly lower in hepatitis A seropositive individuals when compared with seronegative subjects [42]. There were also studies showing a protective role for other infections including tuberculosis and measles [43,44]. Taken together, exposure to a variety of infections maybe an important contributing factor conferring protection against asthma in developing countries. However, the total load of microbial exposure is likely to be more important than a specific infection alone in modulating the immune system in early childhood such that future development of asthma maybe reduced.

Summary

Epidemiological studies of childhood asthma performed in the past two decades have provided us important information with respect to the occurrence of asthma in different regions of the world. Multicentered surveys using standardized methodology have provided accurate information of the trends of asthma prevalence. These studies have also generated several hypotheses relating to the development of asthma and associated allergic conditions. Comparative studies from different regions of the world have provided new information on the many possible genetic and environmental factors for asthma. The results from prospective and intervention studies have challenged the role of allergen exposure as a causative factor for asthma while studies from the rural and farming environment have provided insights into the role of microbial exposure in modulating the young immune system. Clear understanding of how various genes may interact with the protective determinants in the pathogenesis of asthma may lead to future development of primary preventive strategies for asthma.

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Epidemiology of Occupational Asthma

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What is Occupational Asthma?

Occupational asthma is asthma induced by an agent inhaled at work. This definition implies that an agent inhaled at work has caused or initiated the process of airway inflammation, which is characteristic of asthma (desquamative eosinophilic bronchitis); it distinguishes agents which cause occupational asthma from those which provoke asthma, or cause exacerbations, in those with pre-existing disease. Inducers of occupational asthma can cause asthma [1] through direct injury to the airway epithelium by a respiratory irritant inhaled in toxic concentration (irritant-induced asthma); or [2] as the outcome of an acquired specific hypersensitivity response to (a) an inhaled protein allergen or (b) low molecular weight chemical sensitiser (hypersensitivity-induced asthma).

What is Epidemiology?

Epidemiology is the study of the distribution of disease and its determinants in populations. Knowledge of the distribution of disease provides the building blocks for public health. Differences in the distribution of disease in different populations can form the basis for hypotheses about the determinants (or causation) of disease, which can be investigated in studies, which compare disease frequency in different populations or groups (e.g., within a workforce). Clinical epidemiology addresses the determinants of disease outcome.

A key characteristic of epidemiological study is the measurement of disease occurrence (numerator, e.g., the number of cases of asthma) in relation to an appropriate population at risk (denominator, e.g., a detergent factory workforce) to provide a measure of disease frequency. The essence of analytical epidemiology is well-designed observational studies, which compare "like with like" to test specific

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_6, © Springer 2009

hypotheses. These investigate questions not amenable to randomised controlled trials (RCT). While lacking the high internal validity of the RCT, the accumulation of consistent results from different studies, undertaken in different ways in different populations can provide strong evidence of cause and effect.

In principle, the ability of an agent inhaled at work to cause hypersensitivity-induced asthma can be demonstrated in the individual case by an inhalation test with the specific causal agent. Exposure to allergens, such as enzymes and flour dust, or low molecular weight chemicals, such as isocyanates, will provoke an asthmatic response in sensitised cases. Similarly new onset asthma following an acute inhalation accident, of sufficient severity for the individual to have sought medical attention in its immediate aftermath, is usually regarded as sufficient evidence to be considered causal. Although causation can be identified in the individual case for many of the causes of occupational asthma, population studies have identified previously unrecognised occupations (e.g., cleaners) as having a high prevalence of asthma.

Few diseases are the outcome of a single cause, such as isocyanate exposure, which, although necessary, is usually not sufficient to cause the disease; in the great majority, cases are the outcome of multiple causes. Epidemiological investigation of occupational asthma has investigated its important risk factors (both genetic and environmental), its outcome, and the determinants of this. Important information has come from reporting schemes, as well as from formal studies, of the relative importance of the different causes of the disease, of the circumstances in which exposure to these occur, and of the frequency of occupation as a cause of asthma in adult life. The primary purpose of such study is identification of the means to reduce the incidence of the disease.

Estimated Incidence of Occupational Asthma

Reporting Schemes

The incidence of occupational asthma in different occupations and the relative importance of the different agents responsible have been estimated in the UK since 1989 by the Surveillance of Work and Occupational Respiratory Disease (SWORD) scheme. The scheme relies on reporting by specialist chest physicians in the UK of new cases of respiratory disease attributable to occupation. During the early years, the scheme achieved comprehensive coverage with reports from 385 (90%) chest physicians in the UK. From 1992, reporters were divided into a core group of 32 chest physicians with a particular interest in occupational lung disease, who continued to report monthly, and other chest physicians (some 400), grouped into 12 random samples, who reported monthly. A similar number of occupational physicians (391) also reported monthly during the early years of the scheme, with monthly sampling introduced in 1996, by which time some 800 occupational physicians were participating in a separate scheme, Occupational Physicians Reporting Activity (OPRA), reporting all types of work-related disease,

which included respiratory disease. Occupational physicians do not generally see patients after retirement and reported fewer occupational respiratory diseases of long latency (e.g., asbestos-related diseases) than chest physicians, but reported more cases of short latency conditions such as acute inhalation accidents and occupational asthma.

During the period of reporting (from 1989), the relative importance of the different agents identified as causes of occupational asthma and the estimated incidence of disease in different occupations has remained similar, with an overall decline in the estimated annual average number of cases reported from 703 in 1992 to 559 in 2001. Organic agents, such as flour, wood dust and laboratory animals, account for one third of the reported agents and chemicals, such as isocyanates and glutaraldehyde, for a further one third. Metals including welding fumes and a miscellaneous group, which includes epoxy resins, cutting oils, paints and glues, account for the majority of the remaining third. During the course of the 1990s, the number of cases reported as attributable to latex allergy rose and subsequently fell with the introduction and universal adoption of low protein non-powdered latex gloves by health care workers and others (Fig. 1).

The estimated incidence of occupational asthma by occupational group during the 1990s ranged from 1380/10^6/year in coach and other spray painters to 12/10^6/year for transport and storage workers. All occupational groups with an estimated incidence of more than 100/10^6/year, with the exception of laboratory technicians and assistants, were employed in the manufacture or processing of chemicals, metals or organic materials such as foodstuffs and wood. The estimated annual incidence for high-risk occupations between 1992 and 1997 is shown in Fig. 2. The occupations associated with the most commonly reported agents are shown in Fig. 3. The incidence in the UK was consistently higher in the Midlands (over 60/10^6/year) than in the south (less than 30/10^6/year), probably reflecting the distribution of higher risk industries and occupations [3].

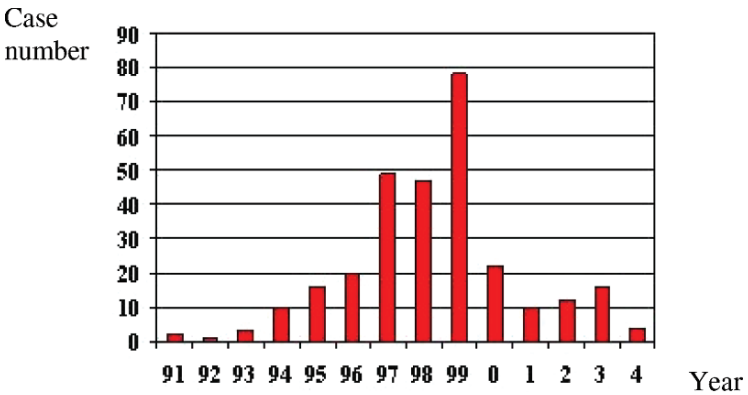

Fig. 1 Occupational asthma attributed to latex in the UK

Occupation	Annual incidence (per 10^6 pa) 1992-97	(95% CI)
Laboratory technicians and assistants	207	(150-297)
Wood workers	139	(82-221)
Food processors (exc. bakers)	280	(171-141)
Bakers	951	(618-1415)
Plastics workers	380	(220-635)
Chemical processors	573	(357-898)
Welding, soldering, electronics assembly	266	(181-389)
Metal treatment	567	(345-907)
Coach and other spray painters	1464	(968-2173)

Fig. 2 Estimated annual incidence of occupational asthma for high risk occupations 1989–1997, (From [3])

Agent	Occupation	No. of reports
Isocyanates	Spray painter	286
	Other metal or electrical processor maker or repairer	161
Flour and grain	Baker	317
Wood	Wood worker	251
Glutaraldehyde	Nurse	189
Laboratory animals	Laboratory technicians, scientists and assistants	184
Solder or colophony	Welder, solderer or electronic assembler	161

Fig. 3 Occupations associated with most commonly reported agents (>100 reports) to SWORD (1992–1997), (From [3])

Reporting schemes are dependent upon cases being seen by reporters, they recognising and attributing the occupational cause and reporting the case. In general diseases of high specificity, such as mesothelioma, or diseases with specific features, such as asbestos-related pleural disease and occupational asthma, are more likely to be reported than diseases such as lung cancer and chronic obstructive pulmonary disorder (COPD), which are not specific to occupation, have no specific features and are overwhelmingly attributable to a single non-occupational cause (cigarette smoking). The high level of participation in SWORD and OPRA by both chest and occupational physicians has continued throughout the period of the schemes. While reported cases are based on clinical opinion, several validation exercises have confirmed the occupational attribution in most cases. The relative importance of the different agents and occupations in which they occur is probably provided accurately by these reporting schemes. What remains unclear is the proportion of all cases who come to the attention of specialist chest physicians in hospitals or occupational physicians (estimated to provide a service to only 12% UK workforce [4]). The best estimate at present would suggest that about one half to one third of new cases are being reported to these schemes.

The patterns of disease reported by A SIMILAR reporting scheme in South Africa show the predominance of mining and associated disease (pneumoconiosis, tuberculosis (TB), COPD) in South Africa as compared to diseases associated with manufacturing and service industries in the UK (Fig. 4). The small proportion of mesothelioma cases seems likely to reflect low ascertainment of a rapidly fatal condition of long latency in a predominantly migrant workforce [5].

Population-based Studies

The incidence of occupational asthma has also been estimated in the population-based European Community Respiratory Health Survey (ECRHS), which initially surveyed random samples from local residents aged between 20 and 44 years in 1990–1995, in 28 centres in 13 countries. A follow-up survey of the population was undertaken in 1998–2003. This estimated the incidence of new cases of asthma in the population between these two periods and obtained information on all jobs done for at least 3 months. Acute inhalation accidents were also identified by questionnaire. Asthma was defined as either having an asthma attack or use of asthma treatment in the 12 months before interview. Airway hyperresponsiveness was also identified, by methacholine inhalation testing, in 4,438 participants. The risk of asthma, defined by asthma symptoms on questionnaire and airway hyperresponsiveness, was increased some 2.4 times in cases exposed at work to substances known to cause occupational asthma. A greater than two-fold increase in risk was found in nurses and greater than three-fold risk in cases who reported an acute symptomatic inhalation event, (e.g., chemical spills, mixing cleaning products and fire) [6]. The estimated population attributable risk for adult onset asthma for occupational exposures ranged from 10% to 25%, equivalent to an incidence of new onset occupational asthma of 250–300 cases/10^6/year, closer to the Finnish estimate of some 200 cases pa than the UK estimate of 20–30 cases per annum from SWORD. The population-attributable risk estimate in this study is consistent with that of the American Thoracic Society best estimate of 15% made from a systematic analysis of the relevant literature [7].

SWORD, % of 10477 cases reported between 1996 and 1998		SORDSA, % of 3285 cases reported between October 1996 and October 1998	
Asthma	29	Pneumoconiosis	62
Benign pleural thickening	21	Pneumoconiosis with TB	8
Mesothelioma	21	Pneumoconiosis with COPD	6.5
Pneumoconiosis	9	Asthma (with latency)	6
Inhalation accident	6	Inhalation accident	4.5
Lung cancer	3	TB (work related)	3
Infection	2	Benign pleural disease	2.5
Bronchitis/Emphysema	2	Mesothelioma	2

Fig. 4 Cases of lung disease reported to SWORD and SORDSA (From [5])

Irritant-induced Asthma

Irritant-induced asthma is chronic asthma, which persists for more than 3 months after a single inhalation, usually of short duration, of an irritant chemical in toxic concentration. Unlike hypersensitivity-induced asthma, which only develops after an interval of weeks or months from initial exposure, the manifestations of irritant-induced asthma, both symptomatic and functional (e.g., airway hyperresponsiveness), develop within hours of the inhalation accident. The majority of reports of irritant-induced asthma have been case series. The earliest report described ten patients, none of whom has pre-existing asthma [8]. All developed chronic asthma following a single exposure to a variety of respiratory irritants, which in the majority was of a few minutes, but in one case of 12 hours. These included paint containing ammonia, heated acid and smoke. At the time of follow-up, respiratory symptoms had persisted between 1 and 12 years, all 10 had increased airway responsiveness to inhaled methacholine and 7 had airflow limitation. Subsequent case reports and series have identified many other different chemical causes of the same syndrome. These include sulphur dioxide and anhydrous ammonia fumes.

In general, case reports are highly selected: symptoms are sufficiently severe and of sufficient duration to have come to medical attention. In addition, there is rarely information about lung function prior to the accident. One study, of hospital staff exposed to a spill of 100% acetic acid in a hospital laboratory, overcame several of the problems associated with case reports [9]. The study was of a random sample of the work force exposed to the spill of glacial acetic acid. An exposure–response relationship was found between the estimated intensity of the exposure and the attack rate of acute respiratory symptoms and prevalence of airway hyperresponsiveness: the risk of developing irritant-induced asthma was some 23-fold greater in those most, as compared to least, exposed to acetic acid. Finally there was partial validation of respiratory health before the inhalation accident from pre-employment questionnaires.

An investigation of the outcome of 623 acute inhalation accidents reported to SWORD, between 1990 and 1993, suggested that symptoms persisted for more than 1 month in 142 of them, which included 50 new cases of asthma [10]. A subsequent questionnaire in 1995 suggested that new asthma following an inhalation accident occurred in 34 of the original 50 cases, of whom 28 had continuing symptoms [11]. The most frequent attributable exposures were to chemical sensitizers, such as isocyanates, inhaled in toxic concentrations, sulphur dioxide, ammonia and chlorine. Failure to use respiratory protection and inappropriate procedures when mixing chemicals accounted for one third of the cases and spills leaks and faulty processes for a further one third.

The second European Community Respiratory Health Survey (ECRHS) follow-up study of 15,716 persons seen on average 9 years from the first study found an increased risk of new onset asthma in those who had reported a symptomatic acute inhalation event such as a fire, mixing cleaning products or chemical spills [6]. Among the cases of asthma in the survey, 3.8% had experienced a symptomatic acute

inhalation event, with a three-fold increased risk in those who had an asthma attack or were taking asthma treatment with evidence of airway hyperresponsiveness in the past year of having had a symptomatic inhalation event.

Hypersensitivity-induced Asthma

Hypersensitivity-induced asthma has been the subject of more study than has irritant-induced asthma, not least because of its greater frequency and impact. Indeed, recognition of the importance of occupational asthma stemmed from studies reported in the late 1960s and early 1970s of outbreaks of hypersensitivity-induced asthma worldwide among workforces engaged in the new technology of adding the *Bacillus subtilis* proteolytic enzyme to enhance the cleaning capacity of detergents, together with case reports of allergy to the protease among consumers.

Asthma caused by protease in workers employed in the detergent industry was first reported by Flindt in 1969 [12], with an accompanying report by Pepys et al. identifying specific IgE antibody and asthmatic reactions provoked by inhalation of protease [13]. Knowledge of the size of the problem was important and in 1970 two cross-sectional studies of workforces were reported in the UK [14, 15] and subsequently in the USA [18]. Cross-sectional studies are subject to survivor bias, particularly of diseases characterised by an acute reaction to an identifiable exposure. This can lead to several of those affected leaving employment and, therefore, no longer available for study. Nonetheless, the studies reported a high frequency of respiratory symptoms and skin prick test responses to the protease. One study, for instance, found a prevalence of allergic symptoms in 47% of the workforce surveyed, with an association between skin prick test responses, allergic symptoms and atopy [14].

The emphasis on the importance of atopy as a determinant of risk of developing occupational asthma reflected the contemporary belief that the disease, with the associated development of specific IgE, was a manifestation of an atopic predisposition and primarily the outcome of host susceptibility. By implication, reduction of disease incidence would primarily be achieved by the identification of the susceptible atopics, and excluding them from employment in occupations in which exposure to respiratory allergens and chemical sensitisers occurred. The enzyme detergent industry was only one, which included platinum refining, of a number of occupations in which, in the 1970s and 1980s, atopics were excluded from employment. A major advance during the 1990s was the provision of evidence that the major determinant of disease incidence in hypersensitivity-induced, as in irritant-induced asthma, was the intensity of exposure to the relevant allergen or chemical sensitiser.

The implication of this change was considerable: improved control of exposure, not the exclusion of a susceptible minority from employment, is seen as the more effective means to reduce disease incidence. Evidence to support this has come from several well-conducted cohort studies, which have investigated the relationship between disease incidence and levels of exposure and also, in a few cases,

intervention studies investigating the effect on disease incidence of reduced levels of exposure to specific agents.

The well-recognised problem of survivor bias in cross-sectional studies is a particular problem for diseases such as occupational asthma in which an acute recurrent respiratory reaction (asthma) can readily be appreciated as related to exposures at work, which individuals endeavour to avoid, either by leaving work or by reducing their level of exposure; those who accumulate exposure are those who survive to do so. Exposure measurements in cross-sectional studies can provide similar problems. The levels of exposure measured at the time of the study may differ considerably from an earlier period when asthma developed. While cross-sectional studies can provide an estimate of disease prevalence and its relationship to contemporary exposures, the potential for cases of asthma to leave work or relocate to areas of lower levels of exposure will tend to attenuate exposure–response relationships.

This effect was observed in an initial cross-sectional study of laboratory animal workers, undertaken at the start of a longitudinal cohort study, which included measurement of airborne rat urinary proteins [17]. The authors reported a gradient of increased prevalence of skin prick test responses to rat urine protein, steeper in atopics, with increasing levels of exposure to rat airborne urinary protein. However, no consistent relationship was observed between new work-related symptoms (chest, nose, eye or skin) and the level of exposure at the time of the survey. In contrast, a gradient of prevalence of new work related symptoms, particularly for contact urticaria, was reported with intensity of exposure at the time of symptom onset. This difference seems likely to reflect differential movement within and out of the workforce in relation to the development of acute symptoms. Consistent with this, the movement of employees after the onset of symptoms was more frequent and invariably to jobs with lower intensity of exposure: 24% of those with new work-related chest symptoms, 16% with new work-related eye and nose symptoms and 12% of those with new work-related skin symptoms had changed job. In contrast, only 4% of the workforce without symptoms had changed jobs, in many cases to work where the level of exposure to airborne urinary allergens was greater.

The findings of the subsequent 5-year cohort study of laboratory animal workers showed clear evidence of an exposure–response relationship [18]. Exposure intensity in different jobs was categorised into four levels of increasing exposure (1 to 4). The risk of developing any new work-related symptoms was more than five times greater in those working in category 3 than in category 1 level jobs. Exposure–response relationships were observed for new work-related chest, nose and eye and skin symptoms and for skin prick test responses to rat urine protein. The level of risk for those employed in category 3 exposure as compared to category 1 exposure categories was twice as great as the risk overall to atopics in comparison to non-atopics.

A similar exposure–response relationship was also found in companion cohort studies, one of flour mill and bakery workers, exposed to flour proteins and to fungal α-amylase, the other of acid anhydride workers. In the bakery workers, those in the high-exposure group (category 3) were 7.7 times more likely to develop chest

symptoms than those in the low-exposure group (category 1). There was no evidence of an increased risk of developing chest symptoms among atopics as compared to non-atopics. The average level of exposure to flour in the high-exposure group was 4.4 mg/m^3, suggesting the development of asthma at levels of flour in air below the contemporary exposure limit of 10 mg/m^3 [19].

A study of workforces exposed to acid anhydrides found a similar exposure–response relationship for trimellitic anhydride (TMA), used in the manufacture of cushioned flooring [20]. The risk of developing new work-related chest symptoms and of a skin prick test response to TMA increased with increasing maximum full shift exposure to TMA, a relationship not modified by atopy or smoking. Eleven of the 12 cases of new work-related chest symptoms and 6 of the 8 with an immediate skin prick test response to TMA had worked in conditions where the estimated maximum full shift exposure was less than the contemporary occupational exposure limit in UK of 40 µg/m^3.

These and other studies reported during the past 20 years have provided consistent evidence for an exposure–response relationship for occupational asthma. In recent years, measures intended to reduce the incidence of occupational asthma have focused on reducing the levels of exposure to its causes. In a few cases, the effectiveness of these interventions has been demonstrated in formal evaluative studies, the most powerful epidemiological evidence of cause and effect.

Reducing Disease Incidence: Evaluation of Intervention Studies

While the inference of exposure–response relationships is clear, the most powerful evidence of causation comes from well-designed studies, which evaluate the effectiveness in reducing disease incidence of interventions designed to reduce levels of exposure to relevant agents in the workplace.

The number of studies of intervention in occupational asthma is small and the great majority report attack rates of disease following an intervention, usually without concurrent evaluation of otherwise comparable circumstances without intervention. For two causes of occupational asthma, latex in health care workers and enzymes in detergent worker, the accumulated evidence is very convincing. For two other agents, isocyanates and laboratory animal workers, the results of the studies reported are suggestive of cause and effect.

The outbreaks of occupational asthma in the detergent industry and reports of allergy to enzymes among consumers in the late 1960s and early 1970s, which followed the introduction of powdered proteases into detergent manufacture, stimulated technological improvements and engineering controls designed to reduce airborne enzyme concentrations in the workplace and prevent exposure to consumers. Enzymes were encapsulated in granules, which would not remain airborne, and engineering controls introduced to reduce levels of airborne enzyme dust in the workplace.

Two studies the first published in 1977 [21], the second 20 years [22] later describe considerable reductions in the number of cases of enzyme-induced asthma

following the introduction of granulation and an associated reduction in the levels of airborne enzyme in the workplace. The first study reported a progressive reduction in sensitisation to enzymes and in the number of cases transferred out of the factory with respiratory symptoms in parallel with a fall in the peak levels of total dust in the packing area of the factory.

The second study published 20 years later reported a marked reduction in the number of cases of enzyme-induced asthma in the late 1960s (more than 100 between 1969 and 1974) to cases occurring on average less frequently than 1 per year from 1980 onwards to 1993. This fall paralleled a reduction in the average levels of airborne protease from about 100 µg/m^3 in the late 1960s to between 1 and 10 µg/m^3 from the mid-1970s (Fig. 5). Unfortunately, both studies reported case numbers, not disease incidence, leaving it unclear how much the reduction in the number of incident cases was due to a predominantly survivor' workforce population, for a disease of which the majority of cases occur in the first 2 years of exposure. Nonetheless the two studies provide some of the best evidence that a once important occupational health problem can be effectively controlled by reducing levels of exposure to the causative allergens.

However, the maintenance of control requires eternal vigilance. An outbreak of occupational asthma of a similar magnitude to those reported in the late 1960s occurred in a UK workforce in the 1990s in a factory, which had only used granulated enzymes [23]. More than 50 clinically diagnosed cases of enzyme-induced asthma occurred in a workforce of less than 350. Whilst not wholly explained, a plausible contributory factor to the outbreak may have been the inadvertent disruption of granules during the manufacturing process, leading to the generation of airborne enzyme dust of inhalable dimensions.

The second striking success of environmental control in virtually eliminating an international outbreak of occupational asthma is latex allergy. Several studies from

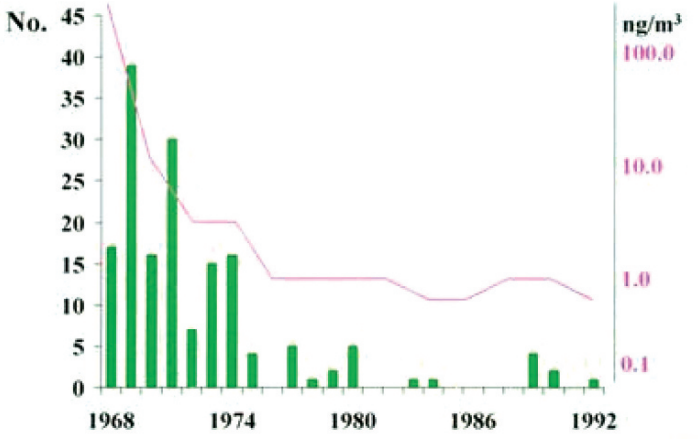

Fig. 5 Decline in number of cases of enzyme induced asthma associated with falling concentration of airborne enzyme

Europe and North America have documented the rise of latex allergy with the increasing use of powdered high-protein latex gloves in the early 1990s and the subsequent fall, following substitution by low-protein powder-free gloves. A large German study of the number of cases of latex allergy reported to an insurance company, which covered half the country's hospitals, clearly demonstrated the number of cases of occupational asthma falling with a 2-year lag following the substitution of powdered by powder-free gloves [24] (Fig. 6). Similarly the number of allowed claims for latex-induced occupational asthma fell following the increasing uptake by hospitals of powder-free low protein latex gloves. Data from the SWORD scheme also documents the rise and fall of latex allergy in Great Britain during the 1990s (Fig. 3).

Evidence for the effectiveness of improved control in reducing the incidence of occupational asthma in isocyanate and laboratory animal workers is limited to single studies. In Ontario, Canada, where isocyanates accounted for 50% of successful claims for occupational asthma, a multi-disciplinary programme to reduce isocyanate exposure to 8-hour concentrations to < 5 ppb and short-term exposure levels to < 20 ppb was introduced in 1983, together with mandatory health surveillance of isocyanate workers. No equivalent legislation was introduced for other recognised causes of occupational asthma. During the initial period of follow-up, the number of cases increased (Fig. 7) because of improved case identification. Subsequently from the late 1980s, the number of cases of isocyanate-induced asthma fell, while the number of cases caused by other agents remained essentially unchanged. Furthermore case identification occurred on average 1 year earlier (1.7 vs. 2.7 years) after the onset of symptoms, with an associated reduction in average case severity [25]. The only intervention study reported to date in laboratory animal workers is

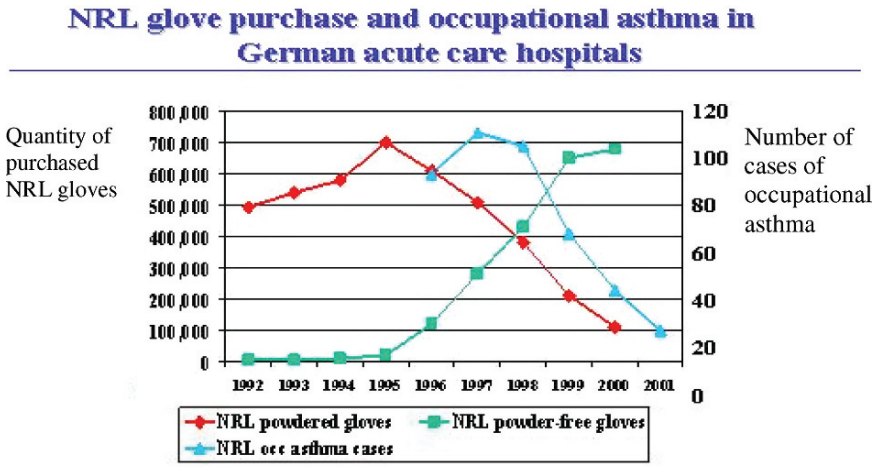

Fig. 6 The rise and fall of occupational asthma caused by allergy to latex in relation to the number of natural rubber latex (NRL) powdered and powder free gloves purchased, (From [24])

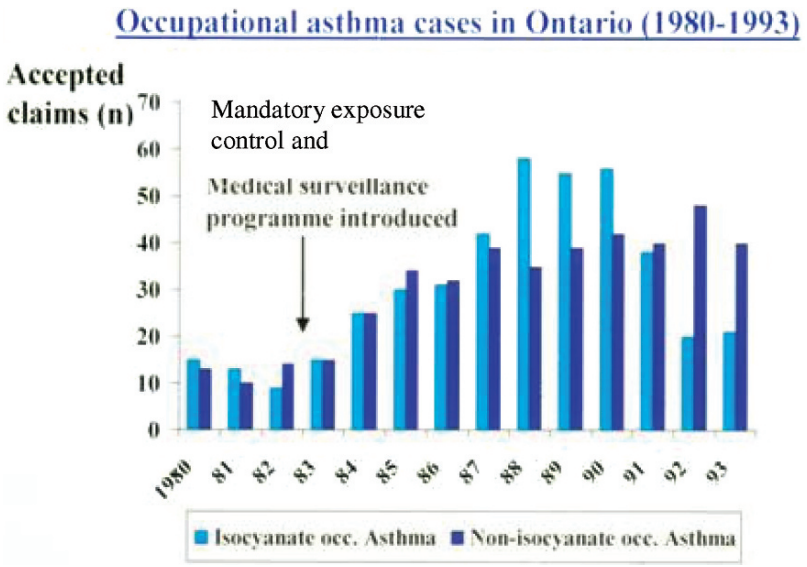

Fig. 7 Reduction in number of compensated claims for occupational asthma caused by isocyanates (but not other causes of occupational asthma) in Ontario, Canada, following mandatory control of isocyanates in workplace (1983) with medical surveillance programme, (From [25])

from a major pharmaceutical company in the UK. In 1981, a new code of practice for working with laboratory animals was introduced by the company. Workers employed each year between 1979 and 1982 were followed for 3 years subsequently, those employed in 1983 for 2 years and in 1984 for 1 year. The incidence of laboratory animal allergy fell in each of the three cohorts employed after 1981 (1982, 1983 and 1984), as compared to those employed in 1979, 1980 and 1981. No concurrent measurements of exposure were made during this period, but it seems likely the reducing incidence of laboratory animal allergy observed reflected a reduction in exposure to laboratory animal allergens [26].

Outcome of Occupational Asthma

The outcome of occupational asthma has been reported in several studies. While the majority have focussed on the long-term clinical consequences, some studies have also reported the social and financial consequences of the disease. The results of these studies need to be interpreted with some caution as the majority were based on follow-up of hospital patients whose referral was probably a reflection of more severe disease, among whom those with continuing symptoms may be overrepresented because they are more likely to maintain contact with medical follow-up.

In four studies, one of snow crab workers [27], one of tetrachlorophthalic anhydride (TCPA) workers [28], one of azodicarbonamide workers [29] and a hospital-based

survey of isocyanate workers [30], 12 from one factory and thought to represent all incident cases in the factory, the cases of asthma were identified from survey of a factory population, not hospital referral. Follow-up was complete in all the four studies. Each study found evidence of continuing asthma with persistent respiratory symptoms, reduced FEV_1 or increased airway responsiveness to inhaled histamine or methacholine in more than 50% of cases. Furthermore, in the snow crab [27] and TCPA workers [28], a progressive reduction in specific IgE during the period of follow-up was consistent with the avoidance of exposure to the specific cause.

The largest single follow-up survey of cases of occupational asthma was the attempt in 1994 to obtain information on all 1940 cases of occupational asthma reported to SWORD in 1989–1992 [31]. Although the questionnaires were returned for 1769 (91%), sufficient information for analysis was only returned by 1317 (68%). It seems likely nonetheless that the findings were reasonably representative of the cases under study. Forty-five percent of patients reported by occupational physicians had recovered as opposed to only 14% of those reported by chest physicians (even after excluding cases seen for medicolegal reasons). This marked difference probably reflects a greater average severity of the cases referred to specialist physicians. Of the cases reported by chest physicians, 48% had remained with the same employer, 16% were with another employer, 6% had retired and 30% were unemployed or had been retired on medical grounds.

None of these studies, however, have included objective evidence of normal airway function, airway calibre (FEV_1) or airway responsiveness, before the onset of symptoms. Nonetheless the findings of these studies suggest that occupational asthma, both respiratory symptoms and abnormal airway function, can persist for several years after avoidance of exposure to the cause. In addition to the findings of SWORD follow-up study, the wider social and financial consequences of occupational asthma have been reported in studies of hospital patients. Two studies found that between one half and three quarters had lost income, with one third unemployed at the time of the study and 60% reporting difficulty in finding alternative employment [32, 33].

A recent systematic review of the outcome of occupational asthma [34] found that the best estimate was that symptomatic recovery occurred 32% of cases, with the highest rate of recovery in patients with the shortest durations of exposure. Recovery rates were lower in older age groups and in clinic-based populations. On average, some three quarters of patients had evidence of continuing airway hyperresponsiveness.

Conclusion

The focus of attention of studies of occupational asthma in the past 30 years has shifted from reports of case series caused by novel agents to population-based studies estimating risk in relation to occupation and agent. The focus on individual susceptibility, a consequence of the contemporary understanding of the implications of the underlying immunological mechanisms in the early case reports, has been replaced

by a recognition of the greater importance of the intensity of exposure at the population level. In consequence, improving control of the levels of exposure is now seen as a more effective means to reduce disease incidence than pre-employment identification and exclusion of a 'susceptible' minority.

We know sufficient about the importance of the levels of exposure in determining the risk of occupational asthma to suggest that epidemiological studies of occupational asthma should now concentrate on evaluating the effectiveness of different means to reduce exposure levels and their effectiveness in reducing the incidence of the disease.

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Epidemiology of Asthma Mortality

Richard Beasley, Meme Wijesinghe, and Kyle Perrin

Introduction

In order to interpret data on long-term time trends in asthma mortality, it is necessary to firstly review the key issues of the accuracy of death certification, disease classification and diagnostic fashion. As the diagnosis of asthma as the cause of death is firmly established in the 5–34-year age group [1–6], long-term trends in asthma mortality within countries and comparisons between countries are normally confined to this age group. Although most deaths occur in the older age group, the accuracy of asthma as the cause of death declines with increasing age due to confounding with other respiratory disorders such as chronic obstructive pulmonary disease (COPD) or the presence of intercurrent medical conditions.

Changes in disease classification coding are also relevant, with the International Classification of Diseases (ICD) implementing major revisions in the coding of asthma. The ICD revisions occur about every 10 years, usually involving the manner in which deaths due to asthma and bronchitis are coded. These revisions usually have minimal effect on the coding in the 5–34-year age group [7–11].

Changes in diagnostic fashion over time are more difficult to quantify, with comparison usually made with trends in other respiratory conditions, which might be confused with asthma [7, 9]. It is likely that changes in diagnostic fashion may influence gradual changes in asthma mortality rates over long periods of time, although it is generally accepted that the method of diagnosing asthma as the cause of death in children and young adults has probably remained essentially unchanged during the past 100 years [12].

There is evidence to suggest that some of the differences in asthma mortality rates between countries may be attributed to genuine differences in nosology between countries, as well as to the quality of death certification [13, 14]. As a

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_7, © Springer 2009

result, systematic differences in the way death certificates are completed in different countries probably affect the reported national asthma mortality rates.

In this chapter, trends in country-specific asthma mortality rates and comparisons in asthma mortality rates between countries have primarily been undertaken in the 5–34-year age group.

Trends: Pre-1900

There is limited data on trends in asthma mortality prior to 1900. The only published data that appears to be available is that from England and Wales where the death rate was relatively high in the second half of the nineteenth century, particularly in males, a pattern attributed to their exposure to adverse industrial conditions [12]. These data do not support the belief that death from asthma was unknown around the turn of the century and contradicts the aphorism of William Osler that "the asthmatic pants into old age" [15].

Trends: 1900–1940

Asthma mortality data during this period is sparse, however, in a number of western countries the rates were low and stable. Indeed, the main feature of the trends in asthma mortality prior to 1940 is the relatively low rates, particularly in comparison with those later in the twentieth century [7, 10–12, 16] (Fig. 1).

Trends: 1940–1960

The intriguing feature of this time period is that there appeared to be a marked and sustained increase in asthma mortality between 1940 and 1955 in a number of western countries, which was apparently not recognised or studied at the time [7, 10–12]. As a result, there is limited data regarding the possible causes, although it does not appear to have been due to either coding artefact or changing diagnostic fashion [7, 10]. Due to the role of specific beta-agonists in the epidemics of asthma mortality in the 1960s and 1980s, it is relevant to review the introduction of new medications during this period. In this regard, it is interesting to note that isoprenaline first became available as an atomiser spray for use in asthma in the late 1940s, and may have contributed to the increase in death rates. A decline in asthma mortality in the late 1950s occurred in some, but not all, countries and has been attributed to the introduction of oral corticosteroids and their subsequent increased use.

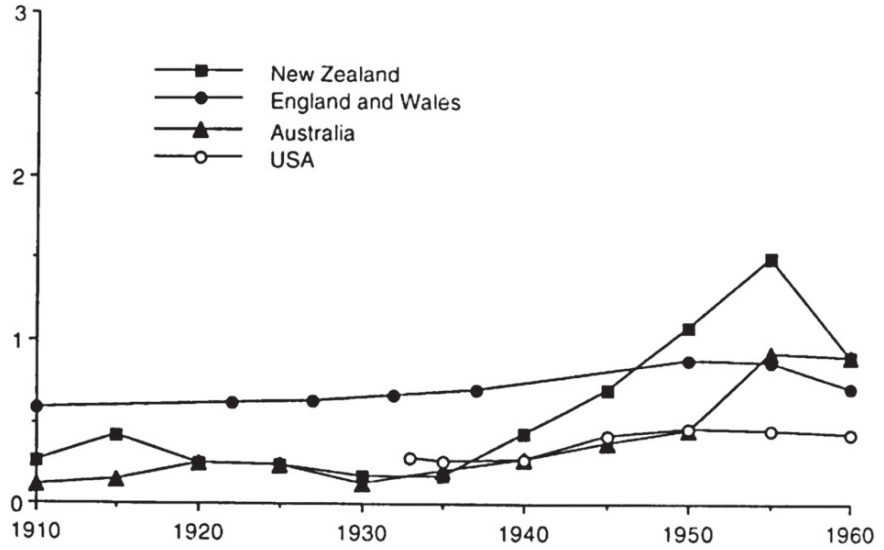

Fig. 1 Asthma mortality (per 100,000) in persons aged 5 to 34 years in New Zealand, Australia, England and Wales and the USA, 1910–1960. (Reproduced with permission from Ref. [16])

Trends: 1960–1975

The striking feature of the time trends during this decade was the dramatic increase in mortality that occurred in some, but not other western countries in the 1960s [7, 10, 17–19] (Fig. 2). These 'epidemics' occurred in England and Wales, Scotland, Ireland, New Zealand, Australia and Norway, with mortality rates increasing two- to five-fold within a 5-year period. It was apparent that the epidemics were real and could not be attributed, changes in diagnostic coding, disease classification coding, diagnostic practice or a sudden increase in asthma prevalence. Initial investigations identified that it was likely to reflect a real increase in case fatality rates due to the introduction of pressurised beta-agonist metered dose inhalers (MDIs), which were available both on prescription and direct "over the counter" [17, 19].

The deaths were often sudden and unexplained and where information on drug use was available patients had often used excessive amounts in the situation of a severe attack [20, 21]. It was proposed that this overuse could increase the risk of death by resulting in temporary relief until patients were in a state in which they did not respond to further beta-agonist therapy, which inevitably led to a delay in seeking medical help until such a life-threatening situation had occurred. Another potential mechanism was through cardiac toxicity resulting from high doses of potent non-selective beta-agonists in the situation of hypoxia. In this regard, it was shown that animals with normal blood gas tensions could tolerate large doses of

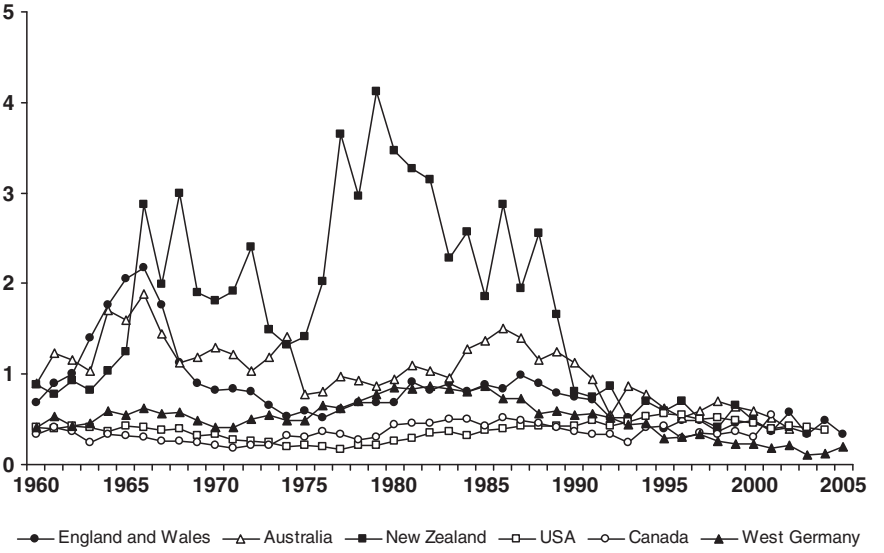

Fig. 2 International patterns of asthma mortality in persons aged 5 to 34 years, 1960–2004, showing the different trends ■___■ New Zealand Δ___Δ Australia ○___○ Canada ●___● England and Wales ▲___▲ West Germany/Germany post-1990 □___□ USA

beta-agonist, yet much smaller doses caused fatal asystolic arrest in the situation of hypoxia or a high cardiac workload [22, 23]. The other possible mechanism was that the regular use of beta-agonists could lead to more severe asthma [24–26].

The major apparent anomaly was the presence of asthma mortality epidemics in some, but not all countries, despite similar apparent use of beta-agonist MDIs. This anomaly was resolved by Stolley and Schinnar [18, 27], who noted that the asthma mortality epidemics only occurred in countries in which a high-dose preparation of isoprenaline was marketed. This preparation, which was marketed as isoprenaline forte, contained five times the dose of isoprenaline as in the standard MDI. Six of the eight countries in which isoprenaline forte was marketed had mortality epidemics, which coincided with the introduction of the drug and in the other two countries, the preparation was introduced relatively late and sales volume were low. Asthma mortality epidemics were not recognised in any country in which isoprenaline forte was not available, although in some countries such as Denmark, West Germany and Japan, modest increases in mortality were observed. In Japan, this increase in mortality, which was most marked in the 10- to 14-year age group, was closely associated with sales of bronchodilator aerosols [28].

Following recognition of the role of beta-agonists in mortality, warnings regarding their use, restriction to prescription only and reductions in their overall use, asthma mortality fell. The weight of evidence supported isoprenaline forte as being the major cause of the epidemic, and certainly there are no other credible alternative explanations proposed. A *BMJ* editorial entitled “Asthma deaths: a question

answered" concluded with the clinical recommendation that the dose of beta-agonist should not be increased in the absence of a normal response [29].

Trends: 1975 to Late 1980s

There were two distinctive trends in asthma mortality that occurred in the 1970s and 1980s. The first was a further asthma mortality epidemic, which was restricted to New Zealand, and was of greater magnitude and duration than the previous epidemic [9, 30, 31]. The other pattern was a gradual increase in asthma mortality in many other countries, which was progressive and resulted in substantial increases in the asthma death rate in some of these countries.

New Zealand Epidemic

This epidemic was essentially a repeat of that due to isporenaline forte in the 1960s, in that it was primarily due to the overuse of the high-dose forte preparation of the beta-agonist fenoterol [31, 32] (Table 1). Like isoprenaline forte, fenoterol was a poorly selective potent beta-agonist, with high intrinsic activity, marketed as a high-dose preparation with effectively four times the bronchodilator dose of the more commonly used beta-agonist, salbutamol [33–35]. The main evidence incriminating fenoterol came from a series of three case–control studies in New Zealand [36–38], each with different designs, during different periods of the epidemic. These studies identified that the only medication associated with an increased risk of mortality was fenoterol, with the risk increasing up to ten-fold in patients with the most severe asthma. This pattern was important as it effectively ruled out confounding by severity as an explanation of the findings, and was consistent with data that fenoterol was not preferentially prescribed to patients with more severe asthma [39].

Table 1 The epidemiological evidence supporting the association between the epidemic of asthma deaths in New Zealand and fenoterol

Type of studies	Cohort, case–control, clinical and ecological studies; no randomised controlled trials
Strength of association	Relative risk 1.5 to 13 (higher in severe subgroups)
Consistency	Studies from NZ, Canada, Germany, Japan and South Africa
Biologically appropriate temporal relationships	Yes
Dose-response	Possible
Biological plausibility	Acute and/or chronic pharmacological effects greater than other commonly used beta-agonist drugs
Analogy	1960s epidemic – isoprenaline forte
Ecological evidence	NZ sales of fenoterol versus onset and end of the epidemic of deaths
Alternative explanation	None

The findings of the New Zealand case–control studies were subsequently confirmed by epidemiological studies from Canada [40, 41], and Japan [42, 43], which reported a similar increased risk of death with fenoterol compared with salbutamol. In addition, a cohort study based in Germany reported that in COPD, there was a ten-fold increased risk of death with fenoterol compared with salbutamol [44] and a case–control study in South Africa reported a six-fold risk of a life-threatening attack of asthma with fenoterol use [45].

The epidemics of asthma deaths was limited to New Zealand due to the very high sales of fenoterol in New Zealand, with by far the highest per capita use internationally [32]. In most other countries, fenoterol had a small market share and was not approved for use in the USA due to safety concerns. In 1989, the New Zealand Ministry of Health withdrew fenoterol from the market in New Zealand, which resulted in an end to the epidemic, with an immediate two-thirds reduction in asthma death rates [46].

A Gradual Increase During This Period

The other international trend in asthma mortality has been the progressive increase in rates in many countries worldwide [47] (Fig. 2) (Table 2). This pattern has been observed in countries in many different regions throughout the world, and although not of epidemic proportions, the magnitude of the increases has in some countries been substantial with mortality increasing at least two-fold. The causes of these trends have been difficult to determine, as death from asthma is a complex phenomenon and many potential causative factors have changed to differing degrees in different countries during this period.

Table 2 Asthma mortality (per 100,000) in persons aged 5 to 34 years in 16 countries between the mid-1970s and mid-1980s

Country	1975–1977	1985–1987	% change
Australia	0.86	1.42	65
Canada	0.33	0.47	42
Denmark	0.14	0.36	157
England and Wales	0.57	0.90	58
Finland	0.29	0.21	–28
France	0.24	0.51	113
Hong Kong	0.24	0.42	75
Israel	0.27	0.42	56
Italy	0.05	0.17	240
Japan	0.44	0.59	34
Netherlands	0.20	0.22	10
Singapore	0.75	0.88	17
Sweden	0.37	0.54	46
Switzerland	0.31	0.45	45
USA	0.19	0.40	111
West Germany	0.59	0.78	32

The most important consideration is the potential role of a class effect of beta-agonist drug therapy, the use of which increased markedly throughout this period. This issue is difficult to investigate through epidemiological studies, because almost all asthmatics use this class of drug, a situation which is analogous to a clinical trial with no placebo group [31, 48]. The previous studies of fenoterol and asthma mortality essentially involved a comparison of fenoterol with other drugs within the same class and could not accurately address the more difficult issue of a class effect of beta-agonists. It is debatable whether this question can ever be resolved by epidemiological studies and the one study which did attempt to do so [40] had major bias due to confounding by severity, particularly in the dose–response analyses [31, 48, 49]. As a result, the association between increased beta-agonist use and asthma mortality in this study was predominantly due to beta-agonist use being a marker of risk. However, it is likely that there is some degree of risk with other beta-agonists such as salbutamol or terbutaline, not least because the mechanisms associated with an increased risk of mortality associated with isoprenaline and fenoterol should also apply to other beta-agonists, although to a lesser extent. As a result, the balance of evidence would suggest that the progressive increase in beta-agonist use in many countries throughout this period may have contributed to some extent to the gradual increase in asthma mortality observed. In some countries such as Australia, the marked increase in beta-agonist use related in part to their availability without prescription "over the counter" [50]. In some countries, such as Germany [32] and Japan [43, 51], a significant proportion of the increase in mortality is likely to be due to fenoterol use, however, in other countries such as the USA, fenoterol had no role whatsoever as it was never approved for use.

Another consideration is whether the gradual increase in mortality may have been due to increasing baseline asthma prevalence. Asthma prevalence studies, which have been repeated during this period using standardised methods in the same population group, have demonstrated a consistent increase in the prevalence of asthma [52]. These increases have been observed in a wide range of countries with differing lifestyles and in some countries, the prevalence has been of considerable magnitude. As a result, it is likely that in many countries, this increase in the prevalence of asthma may have contributed to some extent to the mortality trends observed. Any increase in asthma prevalence will inevitably result in an increase in asthma mortality rate if the case fatality rate remains unchanged.

Trends: Late 1980s to 2000 and Beyond

Since the late 1980s, the predominant trend in asthma mortality in many countries in different regions worldwide has been that of a progressive reduction [10, 53–60] (Table 3). However, in some countries, this trend of decreasing asthma mortality has not been observed [61–63]. In the USA, the asthma mortality rate increased progressively until 1997 when the trend reversed, with rates decreasing since that time [64].

Table 3 Asthma mortality (per 100,000) in persons aged 5 to 34 years in 20 countries between the mid-1980s and mid-1990s

Country	1985–1987	1995–1997	% change
Argentina	0.85	0.25	–71
Australia	1.42	0.58	–59
Canada	0.47	0.38	–19
Denmark	0.36	0.22	–39
England and Wales	0.90	0.45	–50
Finland	0.21	0.10	–52
France	0.51	0.38	–25
Germany	0.78	0.31	–60
Hong Kong	0.42	0.57	36
Israel	0.42	0.23	–45
Italy	0.17	0.16	–6
Japan	0.59	0.57	–3
Netherlands	0.22	0.11	–50
New Zealand	2.22	0.60	–73
Singapore	0.88	0.62	–30
Sweden	0.54	0.16	–70
Switzerland	0.45	0.14	–69
Taiwan	0.34	0.31	–9
USA	0.40	0.54	35
Uruguay	0.50	0.29	–42

Taiwan and Korea mid-1980 rate based on 1986 and 1987 rate only. The mid-1980s rate for Germany is restricted to West Germany

The most likely explanation for this widespread reduction in asthma mortality is that it is due to the international trends of increasing use of inhaled corticosteroid therapy, together with other improvements in asthma management. Inhaled corticosteroid therapy represents the only treatment associated with both a reduction in the risk of a life-threatening attack of asthma leading to hospital admission, and risk of death [65–69]. A dose–response relationship has been determined between inhaled corticosteroid use and asthma mortality, with most of the benefit achieved with low doses [65]. This is consistent with the clinical studies that have demonstrated that most of the maximum benefit of inhaled corticosteroid therapy is achieved with daily doses of around 200 µg fluticasone or equivalent [70, 71].

The increase in inhaled corticosteroid use over recent decades has been substantial in many countries, and is likely to have contributed to the reduction in hospital admission rates for asthma [72, 73], as well as mortality [56, 59, 60, 74–76]. For many countries, the increased use of inhaled corticosteroids represented part of a comprehensive public health programme to reduce the burden of asthma.

International Comparisons in Asthma Mortality

The traditional approach to the comparison of asthma mortality rates between countries has been to examine rates expressed as the number of deaths per 100,000

population in the 5–34-year age group [7, 9, 11, 47]. This approach provides asthma mortality rates, which are determined to a large extent by the prevalence of asthma in the populations studied. It is evident that there is a wide variation in the reported asthma mortality rates globally [77] (Fig. 3).

An alternative approach is to examine case fatality rates, expressed as the number of deaths per 100,000 asthmatics in the 5–34-year age group [77]. This provides an estimate of the risk of a person with asthma dying, thereby controlling for the prevalence of asthma in each country, determined from the standardised international prevalence studies in adults and children [78, 79]. Utilising this method to determine case fatality rates, a different perspective of international differences in asthma mortality rates is obtained [77] (Fig. 4). Wide variations in asthma case fatality rates are observed worldwide, which suggests that in addition to the prevalence of asthma, other factors also play a role. As management is a major determinant of case fatality rates, these comparative data provide a crude measure of the provision of, and standard of, asthma management in different countries. It is notable that a number of low and middle income countries have relatively high case-fatality rates. This may be due to limited access to medications required for the treatment of asthma, resulting in a barrier to effective management [80, 81].

In considering the trends in asthma mortality rates between countries, mention should also be made of the differences within countries, particularly in relation to specific disadvantaged population groups. This is illustrated by studies from the USA, in which asthma mortality rates are greater in disadvantaged populations such as African-Americans and Hispanics, those that are poorly educated, live in large cities or are poor [82–84]. In China, the asthma mortality rate in rural areas is about twice that recorded in urban areas, despite a higher prevalence of asthma in urban communities [77]. This difference is likely to be due to socioeconomic factors, including provision of medical care and access to essential medications. In Singapore, the death rate was five times higher in Malays than Chinese, a difference, which has been attributed to medical care factors in addition to genetic factors and environmental exposures [85].

One feature which is not evident from national mortality data is the occurrence of epidemics in discrete locations, associated with environmental exposures. Probably the best-studied example is that of the epidemics of fatal asthma in Barcelona in the 1980s, associated with environmental exposure to airborne soybean dust [86]. Other examples include the Asian dust storms blown from the deserts in Mongolia and China, which result in increased respiratory mortality in South Korea [87], the Bhopal disaster in India [88, 89] and the effects of air pollution, which may result in regional differences in asthma mortality [90, 91]. These experiences suggest that environmental exposures can lead to recurrent episodes of life-threatening attacks of asthma in a community whenever exposure reaches a sufficient level.

Age-related seasonal trends in asthma mortality have been observed in a number of countries [76, 92–95]. In each of these countries, asthma mortality in the 5–34-year age group is highest in the summer months, in contrast to older age groups, in which the peak occurs in the winter. This pattern in the younger age group is likely

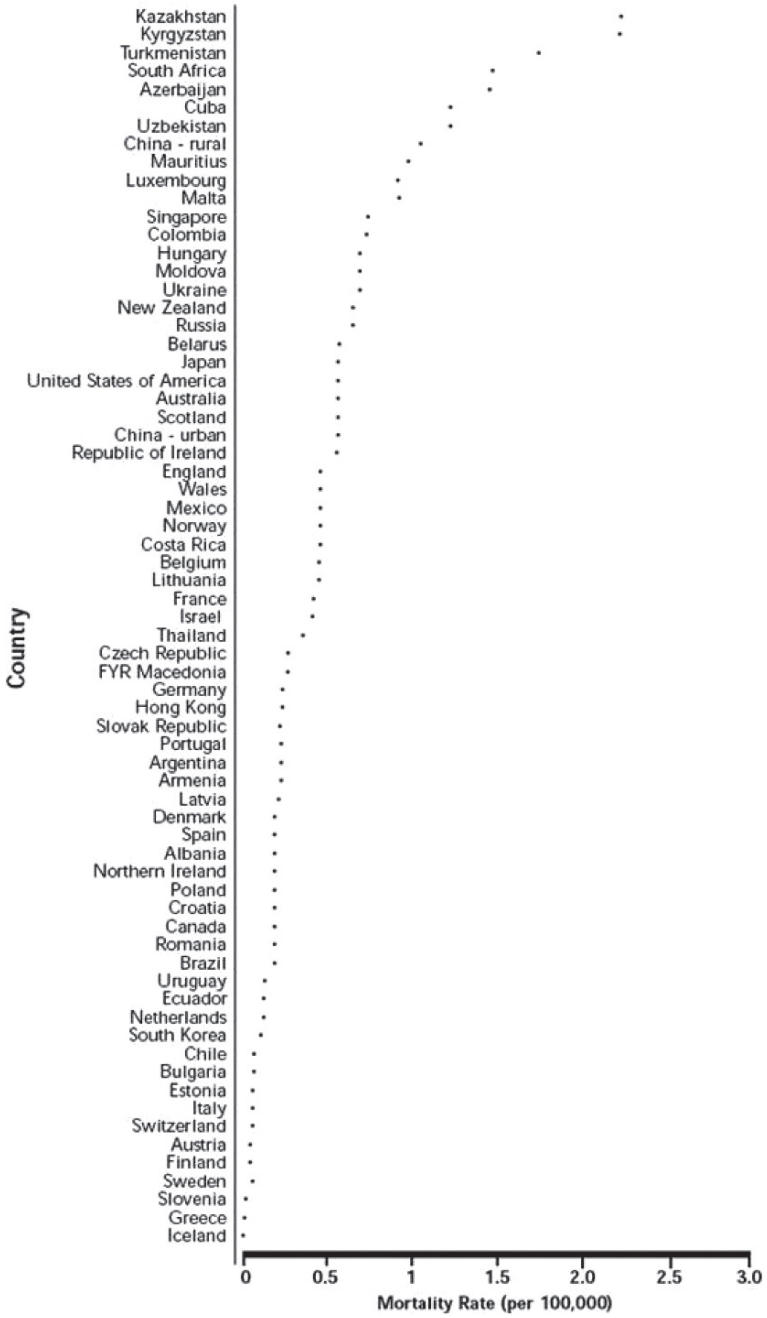

Fig. 3 The ranking of asthma mortality per 100,000 persons aged 5 to 34 years. (Reproduced with permission from Ref. [77])

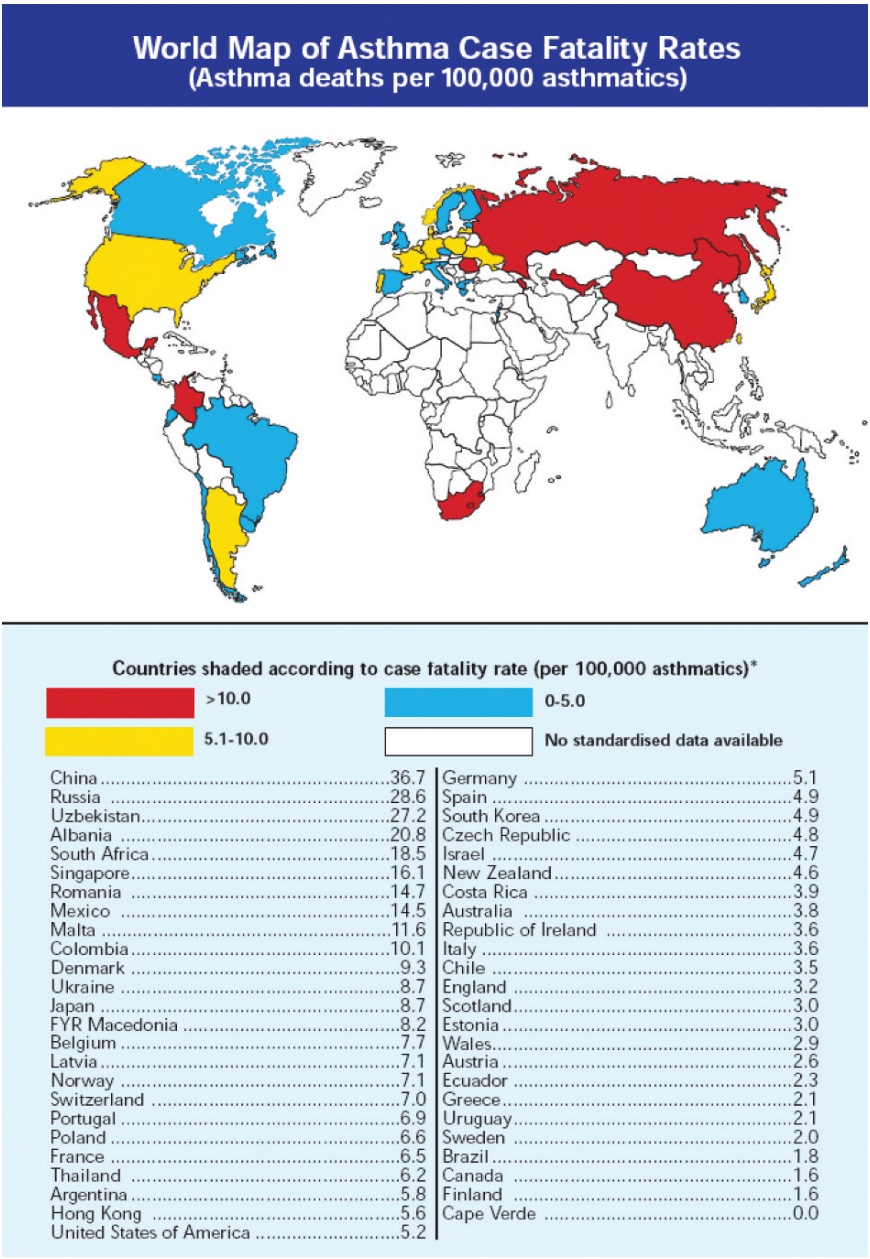

Fig. 4 World map of asthma case fatality rates expressed as asthma deaths per 100,000 persons with asthma aged 5 to 34 years. (Reproduced with permission from Ref. [77])

to be due to a reduced access to or availability of medical care during summer holidays, as reflected by the associated reduction in hospital admissions during this period. This pattern contrasts with the older age groups in which the increase in asthma mortality in winter is associated with a similar peak in hospitalisation rates. An alternative explanation is that exposure to outdoor aero-allergens may account for these trends in the younger age group [96].

Over recent decades, different patterns in the risk of asthma mortality in males and females have been observed in different countries. For example, in the UK, the death rate from asthma in the 5–34-year age group has been consistently higher in females than in males [12], whereas in Japan mortality rates have been higher in males [97], whereas in Australia, no gender differences have been observed [76]. In countries where higher female mortality rates have been observed, this is thought to relate to the higher prevalence of asthma in women after adolescence and the lack of access to or utilisation of medical care, whereas higher death rates in males have been attributed to occupational exposures.

Most asthma deaths occur in older adults with the risk of death increasing progressively with increasing age. In many countries the asthma death rate is over 10 times higher in adults older than 65 years of age compared with the 5–34-year age group [76, 92, 94]. While there may be some misclassification due to deaths from concomitant chronic bronchitis and emphysema, it is unlikely to account for the marked differences observed.

In contrast to mortality rates, the hospital admission rates for asthma decrease progressively with increasing age. For example in New Zealand, the number of hospitalisations due to asthma per asthma death is 30 times higher in the 5–14-year age group than in the 45+ age group [94]. This suggests that there may be a lack of awareness of the risk of mortality in older subjects with asthma, with a reduced likelihood of referral to hospital in the situation of a life-threatening attack, a factor which may by itself increase the risk of mortality.

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Epidemiology of Anaphylaxis

David J. Chinn and Aziz Sheikh

Introduction

Epidemiology is the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to the control of health problems [1]. Epidemiological measures of interest for anaphylaxis include the incidence, incidence rate, lifetime prevalence of its occurrence and case fatality rate (Box 1). Other aspects of interest concern features of persons who experience it, temporal relationships, and the factors that lead to its development and recurrence. Anaphylaxis is a potentially life-threatening hypersensitivity reaction to a substance or set of factors to which the affected person is sensitive and people who experience an anaphylactic reaction remain at risk of further reactions. Accordingly, a description of its epidemiology is important to inform the development and evaluation of strategies to reduce its frequency of occurrence.

Anaphylaxis affects children and adults alike, but estimates of its incidence and lifetime prevalence vary across populations, with time in the same population, and with the data sources used to estimate them. One important reason for this imprecision relates to the great variability in clinical symptoms experienced [2]. An anaphylactic reaction can present with cutaneous, respiratory, cardiovascular or gastrointestinal symptoms that can be misinterpreted for other disorders [3]. The variety of physiological responses experienced by patients and the failure to identify specific biomarkers present during all attacks contributes to the uncertainty of diagnosis [4]. Accordingly, agreement on a case definition has proved elusive and this has contributed to difficulties of conducting research into its epidemiology [5, 6].

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_8, © Springer 2009

Box 1. Epidemiological Definitions Related to Anaphylaxis

Incidence: The number of incident events of anaphylaxis that occur during a given period in a defined population.
Incidence rate: The rate at which new events of anaphylaxis occur in a population where the numerator is the number of new events that occur in a defined period and the denominator is the population at risk of experiencing the event during this period (sometimes expressed as person-time).
Lifetime prevalence: The proportion of a defined population known to have experienced anaphylaxis during their lifetime. Care is required in defining the appropriate denominator.
Occurrence: The frequency of anaphylaxis attacks in a defined population, without distinguishing between first-ever or recurrent events.
Case fatality rate: The proportion of cases of anaphylaxis that prove fatal (usually defined within a time period). This is also sometimes known as the case fatality ratio.
Source: Adapted from Ref. [1].

Box 2. Examples of Definitions of Anaphylaxis

American Academy of Pediatrics, 1990 [Source: Ref. 7]
"Anaphylaxis is a rapidly evolving generalised allergic reaction resulting in multi-system involvement with symptoms of airway tract obstruction (wheezing, stridor), skin rash (urticaria, angioedema), gastrointestinal involvement (nausea, vomiting, abdominal pain, diarrhoea), and cardiovascular involvement (loss of consciousness)."

International Collaborative Study of Severe Anaphylaxis, 1998 [Source: Ref. 8]
The authors adopted a two-stage approach to defining anaphylaxis occurring in hospital as a result of adverse medication reactions:

Stage 1: "An acute episode (usually evolving within one hour) of unexpected and substantial decrease in arterial blood pressure (defined as a systolic blood pressure < 90 mm Hg, or < 100 mm Hg *and* a decrease of ≥30 mm Hg, or a decrease of ≥40 mm Hg) requiring treatment with sympathomimetic amines, parenterally administered corticosteroids, or volume replacement, or resulting in death, and excluding other clinical causes of shock (for example myocardial infarction, pulmonary embolism, massive trauma, acute major haemorrhage, septicaemia, terminal uraemia, hepatic coma, and acute intravascular coagulation)."

(continued)

Box 2 (continued)

Stage 2: "An acute episode of unexpected laryngospasm, laryngeal oedema, or bronchospasm requiring treatment with sympathomimetic amines or parenterally administered corticosteroids, or resulting in death, and excluding those with chronic obstructive pulmonary disease or known active asthma defined as having had an asthmatic attack within two years, or being on current treatment with anti-asthmatic drugs."

Additional exclusion criteria were:

- Episode occurred during surgery or as a direct result of a surgical procedure.
- Episode has an obvious mechanical cause.
- Patient is an intravenous drug abuser.
- Patient has grade IV heart failure.
- Patient has received an organ transplant in previous 12 months.

Australasian Society of Clinical Immunology and Allergy Inc. (ASCIA), 2004 [Source: Ref. 9]
"Anaphylaxis is a rapidly evolving generalised multi-system allergic reaction characterised by one or more symptoms or signs of respiratory and/or cardiovascular involvement, and involvement of other systems such as the skin and/or gastrointestinal tract."

Joint Task Force on Practice Parameters; American Academy of Allergy, Asthma and Immunology; American College of Allergy, Asthma and Immunology; and Joint Council of Allergy, Asthma, and Immunology, 2005. [Source: Ref. 10]
"Anaphylaxis is an acute systemic reaction caused by IgE-mediated immunological release of mediators from mast cells and basophils to allergenic triggers, such as food, insect venoms, latex and medications."

National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network Symposium, 2006 [Source: Ref. 4]
"Anaphylaxis is a severe, potentially fatal, systemic allergic reaction that occurs suddenly after contact with an allergy-causing substance."
The panel also proposed a simplified definition for the medical and lay community: "Anaphylaxis is a serious allergic reaction that is rapid in onset and may cause death."

Defining Anaphylaxis

Several working definitions of anaphylaxis are in use for clinical applications (Box 2), but, with the exception of that adopted by the International Collaborative Study of Severe Anaphylaxis [8], these are of limited utility for epidemiological

studies where the presumption of exposure to a known trigger can substantially increase the likelihood of making the diagnosis. Hence, current estimates of incidence and lifetime prevalence will be subject to uncertainty arising from use of different definitions of the event and its severity. For example, mild systemic allergic reactions that do not involve the respiratory or cardiovascular system may not be considered anaphylaxis by some authorities as they are unlikely to be life-threatening [2].

Data Sources

Data sources include population surveys, health care records—primary care records, hospital activity statistics, community allergy service records, adrenaline (epinephrine) prescribing records—and mortality statistics. Considerations in use of different sources of data concern case ascertainment and diagnostic uncertainty whereby different definitions are used worldwide and in different health care settings where the availability of confirmatory tests will vary. Furthermore, the distribution of severity amongst cases recorded is likely to vary according to the source of data. For example, the most severe reactions are more likely to be dealt with in hospital emergency departments (EDs) where some may lead to an admission; milder cases are more likely to be dealt with only in primary care. Some cases, irrespective of severity, may not get to the attention of the health services if they resolve spontaneously or following appropriate patient-initiated treatment.

All data sources have their own strengths and limitations that should be considered when critically reviewing the results of epidemiological studies. Patient surveys using self-completed questionnaires are subject to response bias and to reporting and recall biases. Primary care data are subject to incomplete data capture and are likely to exclude those anaphylaxis cases that occur in hospital due, for example, to a reaction to an anaesthetic. Hospital in-patient data will be influenced by differences between hospitals in thresholds for admission and in health insurance coverage. Hospital ED statistics will not capture reactions managed solely in the community. Mortality data can be incomplete whereby cause of some deaths due to anaphylaxis may be assigned to, for example, asthma [11]. Although all sources will have some utility the population-based studies are likely to be the most useful, provided they meet quality standards of research design and data capture.

One important issue affecting all epidemiological estimates is that of under-reporting for which evidence of its presence is available for primary care data [12, 13], hospital-based data [14–16] and mortality statistics [17]. Hence, deficiencies in capture of cases (numerators) and in estimates of the population at risk (denominators) contribute to the imprecision in epidemiological estimates.

Epidemiology of Anaphylaxis

There have been a number of studies aiming to describe the epidemiology of anaphylaxis. A comprehensive review up to 2001 noted a large variation in estimates, with a significant risk of under-recording, and concluded that, "no exact incidence can be established based on available data" [18]. A more recent review by a Working Group of the American College of Allergy, Asthma and Immunology summarised the findings from some principal studies published in English. They concluded that the overall frequency of episodes of anaphylaxis using current data lies between 30 and 60 cases per 100,000 persons at the lower end and 950 cases per 100,000 persons at the higher end, with a lifetime prevalence between 50 and 2000 episodes/100,000 persons or 0.05–2.0% [6]. However, the Working Group also considered that even the higher figure could be an underestimate due to under-diagnosis and under-reporting.

Population-based Studies

An early population-based study of Danish hospital records between 1973 and 1985 identified 20 cases with 'anaphylactic shock', for which the incidence, and its 95% confidence interval (CI) was estimated as 3.2 (1.9 to 4.9)/100,000 inhabitants per year [16]. The patients were aged 29 to 77 years and the triggers were medications (10 cases), insect stings (8 cases) and foods (2 cases). All events were triggered outside hospital and would not have included those arising from medication-induced responses amongst hospital in-patients. The authors noted that none of the 10 drug-induced reactions had been reported by hospital staff to the National Adverse Drug Reaction Board and that, for many cases, the hospital discharge diagnosis was incorrect drawing attention to the risk of under-reporting of incident cases.

The lifetime prevalence of anaphylaxis in populations of school-aged children was determined in two surveys of school health records in Australia [19] and the UK [20]. Prevalence estimates were 600/100,000 children (95% CI 360 to 820/100,000) and 430/100,000 children (95% CI 380 to 480/100,000), respectively (Table 1). In both studies, the authors considered their lifetime prevalence estimates to be a minimum.

Large-scale population-based studies of primary care medical records and hospital episode statistics in the USA and UK have generated estimates of incidence rates in the range 6–21 cases per 100,000 person-years at risk and a lifetime prevalence of around 50–75 per 100,000 population [12, 15, 21–25] (Table 1). These may also be considered underestimates as some studies may not capture those cases

Table 1 Incidence and lifetime prevalence of anaphylaxis from selected population-based studies

Author, year, reference	Country	Data source, population, time period	Findings	Summary statistic
Sørensen et al., 1989 [16]	Denmark	Review of hospital records of patients with 'anaphylactic shock', population base 48,000, 1973–1985.	20 adult patients noted (one fatality)	Incidence = 3.2 (95% CI 1.9–4.9)/100,000 inhabitants per year.
Yocum et al., 1999 [21]	USA	Medical records (Rochester Epidemiology Study). Residents of Olmsted County, Minnesota, 1983–1987.	133 residents (all ages) had 154 episodes. 116 single episodes, 13 had 2 episodes and 4 had 3 episodes. 110 new cases (23 had prior H/O anaphylaxis). 1 death.	Incidence = 21 (95%CI 17–25)/100,000 person-years Occurrence = 30 (95% CI 25–35)/100,000 person-years
Boros et al., 2000 [19]	Australia	Parent reports from school records of 4,173 children aged 3–17 years, 1996.	25 cases of anaphylaxis (later confirmed by telephone interview with the parent).	Overall prevalence = 600 (95%CI 360–820)/100,000 children. Prevalence in children aged 3–5 years = 680 (95%CI 340–1,020)/100,000 Prevalence in children aged 5–17 years = 510 (95%CI 200–820)/100,000.
Peng and Jick, 2004 [12]	UK	General practice records for approximately 4 million patients yielding about 8 million person-years of data, 1994–1999.	897 records identified with entry for anaphylaxis. Detailed review of random selection of 120 records, of which 87 considered to have anaphylaxis confirmed.	Crude incidence rate = 8.4/100,000 person-years
Gupta et al., 2004 [22]	England	Hospital admissions, 2000–2001 for 'anaphylactic shock'. Denominator population estimate.	1,964 admissions for 'anaphylactic shock' (all ages).	Annual prevalence 3.8/100,000 persons
Bohlke et al., 2004 [15]	USA	Case note review of hospital records, ED visits and outpatient appointments of 229,422 children and adolescents aged <18 years enrolled in a HMO, 1991–1997 (640,324 person-years of follow-up).	85 confirmed or probable episodes noted in 80 individuals, but statistics based on 67 cases with 'provider diagnosed anaphylaxis'. No deaths.	Incidence rate = 10.5 (95%CI 8.1–13.3)/100,000 person-years.

(continued)

Table 1 (continued)

Author, year, reference	Country	Data source, population, time period	Findings	Summary statistic
Helbling et al., 2004 [23]	Switzerland	Medical records from allergy clinics and 17 hospital EDs. Canton Bern (population about 940,000), 1996–1998.	Only noted 'severe anaphylaxis with circulatory involvement'. 226 patients identified who had 246 episodes. 214 single episodes, 9 had 2 episodes and 3 had 3 or more episodes. 3 deaths.	Incidence rate = 8 – 10 /100,000 person-years
Rankin and Sheikh, 2006 [20]	Scotland	Survey of head teachers from 148 schools representative of schools across Scotland, 2005. Requested details of the number of children with a history of anaphylaxis.	282 children identified from a school population of 65,185 registered pupils aged 4 to 17 years.	Prevalence = 430 (95%CI 380–480)/ 100,000 children.
Sheikh et al. [25]	England	General practice records for approximately 3 million patients (all ages), 2001–2005. QRESEARCH health database. http://www.qresearch.org	Annual estimates calculated for incidence and prevalence 2001 – 2005 adjusted for age and sex using the mid-year population of England.	Age-sex standardised incidence: (/100,000 person-years with 95%CI) 2001 – 6.7 (5.7–7.7) 2002 – 6.6 (5.7–7.6) 2003 – 6.8 (5.9–7.9) 2004 – 8.5 (7.5–9.6) 2005 – 7.9 (7.0–9.0) Age-sex standardised lifetime prevalence (/100,000 population): 2001 – 50.0 (47.5–52.7) 2002 – 55.9 (53.3–58.7) 2003 – 61.8 (59.0–64.7) 2004 – 68.5 (65.6–71.6) 2005 – 75.5 (72.4–78.7)
Mulla and Simon, 2007 [25]	USA	Hospital admissions for anaphylaxis (all ages) from 153 non-Federal hospitals in Florida State, population 16.4 million, 2001	464 cases, 4 deaths.	Annual prevalence 2.8/100,000 population and 19.8/100,000 admissions.

HMO Health Maintenance Organisation, *CI* confidence interval, *ED* emergency department, *H/O* history of

of anaphylaxis that occur in hospital [12] or those that occur amongst segments of the population with reduced or no health insurance as is the case in, for example, the USA [21, 25]. Hospital admission rates for anaphylaxis were estimated as 3.8/100,000 persons in England in 2000/01 [22] and 2.8/100,000 persons in Florida, USA in 2001 [25] (Table 1).

Community Allergy Services

A study of patients referred to a community-based allergy service in Australia between 1995 and 2000 identified 179 incident cases yielding an incidence rate of 9.9/100,000 person-years [13]. The overall occurrence rate of anaphylaxis based upon 259 residents registered at the clinic was 12.9 episodes/100,000 person-years.

Hospital Activity Statistics

During the 1990s, the number of anaphylaxis cases admitted to hospital expressed as a proportion of all hospital admissions was reported as 0.04% in Germany [26] and England [27], and 0.09% in USA [14]. A recent study using a state-wide hospital database in Florida reported an admission rate of 0.02% (19.8/100,000 admissions) [25]. The number of cases admitted as a proportion of emergency admissions in England was 0.02% (17.2/100,000) [28] (Table 2).

More recently, reviews of visits to hospital EDs have recorded consultation rates for anaphylaxis of 0.36% in Italy [29], 0.22% in Thailand [33], 0.23% in Australia [30] and 0.10% in an Australian paediatric unit [32] (Table 2).

Adrenaline Dispensing

Adrenaline prescribing may possibly be considered a reliable measure of demand for patients considered at risk of anaphylaxis as a history of anaphylaxis is the only approved indication for prescribing self-injectable adrenaline [35]. However, the decision to prescribe adrenaline raises many challenges for physicians [36]. Summary statistics are subject to a number of influences due, for example, to prescribing behaviour, patient costs and limitations of the source data. Physicians may prescribe prophylactic adrenaline injectors to patients with newly diagnosed allergies that may be considered to put them at risk of a severe adverse reaction, for example, food allergy. In some countries (for example, Canada), the patient is required to pay for the prescription whereas in other parts of the world (for example, UK), the prescription is free to many patients. The information contained in the pharmaceutical databases cannot distinguish between a repeat prescription issued

Table 2 Hospital activity statistics

Author, year, reference	Country	Data source, time period.	Findings	Summary statistic
Sheikh and Alves, 2001 [28]	England	Hospital admissions, 1991–1995 for 'anaphylactic shock' or 'anaphylactic shock due to serum'. Denominator all emergency admissions	2,323 admissions due to anaphylaxis from amongst 13.5 million emergency admissions (12 deaths)	0.017% of emergency admissions
Pastorello et al., 2001 [29]	Italy	Hospital ED, retrospective case note review, 1997–1998	140 patients with anaphylactic symptoms (13 severe with loss of consciousness) from 38,685 attendances	0.36% ED attendances
Brown et al., 2001 [30]	Australia	Hospital ED, retrospective case note review over 1 year, 1998/99	142 adult patients aged 13 years and older with anaphylaxis (60 severe) from 62,361 attendances (1 death)	0.23% of ED attendances
Smit et al., 2005 [31]	Hong Kong	Hospital ED, retrospective case note review, 1999–2003	282 cases (number of attendances not specified). No deaths.	No summary stats
Braganza et al., 2006 [32]	Australia	Pediatric hospital ED, age < 16 years, retrospective case note review, 1998–2001	57 children with anaphylaxis (28 severe) from amongst 56,655 attendances. No deaths	0.1% of ED attendances
Poachanukoon and Paopairochanakorn, 2006 [33]	Thailand	Hospital ED, retrospective case note review, 2003–2004	64 patients with 65 episodes	0.22% of ED attendances
Gupta et al., 2007 [34]	England	Hospital admissions, 1990–2004 for anaphylaxis. Denominator all admissions	Increase from 0.5 to 3.6/100,000 admissions 1990–2004	0.0036% of all admissions (2003/04)

ED emergency department.

to replace out-of-date stock or as a replacement for one used to treat an attack. Hence, the statistics should be viewed with caution.

The University of Manitoba Health Research Database, incorporating the Drug Programs Information Network (an administration claims pharmaceutical database for out-of-hospital prescriptions dispensed) in Canada, has been used to provide estimates of the population prevalence of anaphylaxis in children [37] and adults [35]. Between 1995 and 1999, self-injectable adrenaline pens were dispensed to 3,340 children (59.5% boys) aged up to 17 years, or 1.2% of the paediatric population [37]. These infants and children were considered to be at risk of an anaphylactic reaction, and therefore the number of prescriptions issued did not reflect the

number of attacks. One disturbing aspect of the review was that up to 7.3% of dispensed pens may have been inappropriate for the age and weight of the child, leading to potential over-dosing (6.7%), or under-dosing (0.6%) if used.

A subsequent review of the database covering the period 1995–2000 was undertaken for all 1.15 million residents of Manitoba [35]. Patients were counted if they had ever had a prescription for self-injectable adrenaline dispensed. Population estimates on 31 December 1999 were used as a denominator. Over the five years, 10,949 persons had an adrenaline prescription issued (0.95% of the population). The proportions were 1.4% for those aged < 17 years, 0.9% for those aged 17–64 years and 0.3% for those aged 65 years or older. The rate in those aged < 17 years was greater in boys than girls but the trend reversed in those aged 17–64 years and did not differ between the sexes in those aged 65 years or older [35].

In England, between 1991 and 2004, the number of prescriptions issued in primary care for self-injectable adrenaline ('allergic emergencies', British National Formulary 3.4.3) increased 12-fold to 124,000 [34].

Mortality Statistics and Death Registers

Registers of anaphylaxis deaths have been established in a number of countries, for example, the UK [11], the USA (American Academy of Allergy, Asthma and Immunology and The Food Allergy and Anaphylaxis Network) [38, 39] and France (French Allergy Vigilance Network) [40]. These important registers provide useful summary statistics and insights into the circumstances surrounding fatal episodes. For example, reviews of the deaths consistently show that many may have been avoided by timely and proper use of adrenaline [11, 40].

In the UK, with a population of 60.2 million [41] the death register suggests that there are about 20 deaths per year, but this is likely to be an underestimate as some anaphylaxis deaths are recorded as deaths from asthma [11]. Although rare, fatalities from anaphylaxis remain important because they are mostly avoidable and many occur in children and young adults so the potential years of life lost can be large. In the UK, about 25% of deaths are related to foods, 25% to venoms and 50% to adverse drug reactions (iatrogenic) [11]. Between January 2005 and June 2006, there were five out of 92 incidents of severe harm or death reported to the English National Patient Safety Agency in which a medicine had been administered to a patient known to be allergic to it [42]. Between 1992 and 2001, the peak age for deaths from anaphylaxis triggered by foods was 17–27 years, for stings 45–70 years and for medications 60–75 years [43].

In the USA, the estimated number of annual deaths from anaphylaxis is 1,500, of which 1,300 will be iatrogenic, 100 will be due to foods and up to 100 due to stings [5]. However, a study using data from a register of anaphylaxis deaths due to foods extrapolated the number of fatalities could be as high as 150 per year, most of which will be in children and young adults [38].

Fatalities due to Anaphylaxis

The case fatality rate was 5% in the study by Sørensen et al. [16], but this was based on only 20 cases giving a 95% confidence interval of 0.1% to 24.9%. More commonly, case fatality rates based on larger numbers of patients vary from 0% [15, 31, 32] to less than 1% [21, 28, 30]. The case fatality rate was 1.3% amongst 226 Swiss patients with 'severe' anaphylaxis [23] and 1.7% amongst 229 French patients with severe food-mediated anaphylaxis [40]. In this latter series, two of 89 children died (case fatality rate 2.2%) and two of 140 adults died (case fatality rate 1.4%). The International Collaborative Study of Severe Anaphylaxis collected in-hospital data on medication-induced anaphylactic reactions from hospitals in Hungary, Spain and India and reported a case fatality rate of 1.6% [10]. Overall, the estimated number of deaths expected from anaphylaxis is 1–5.5 per million population per year [5, 40].

Risk Factors for Fatal Anaphylaxis

The majority of fatal anaphylactic episodes are unpredictable, particularly for those initiated by medications or insect venoms [44]. In comparison, for those whose fatal attack was initiated by a food, there was usually a history of previous allergic reaction and a history of asthma [11, 38, 44, 45]. Poorly controlled asthma has been described as a risk factor for death from anaphylaxis due to allergen immunotherapy, skin prick testing [46] and to foods [45].

Variation in Incidence of Anaphylaxis by Time, Place and Person

Time

Time trend studies may be confounded by change in coding conventions, for example following the change in the mid-1990s from Version 9 to Version 10 of the International Classifications of Diseases (ICD9 to ICD10). However, the impact of such change was judged minimal in a study of hospital admissions from England [47]. In the USA, Bohlke and colleagues considered the incidence of anaphylaxis in children and adolescents to be stable over the period 1991 to 1997 [15]. However, in other studies, the incidence of anaphylaxis appears to be increasing over recent decades, including amongst children [34]. Gupta and colleagues studied hospital admission rates for anaphylaxis in England and noted an increase from 0.5 to 3.6 admissions per 100,000 between 1990 and 2004 (Fig. 1), an increase of 700% [34]. This may have been due partly to better awareness and

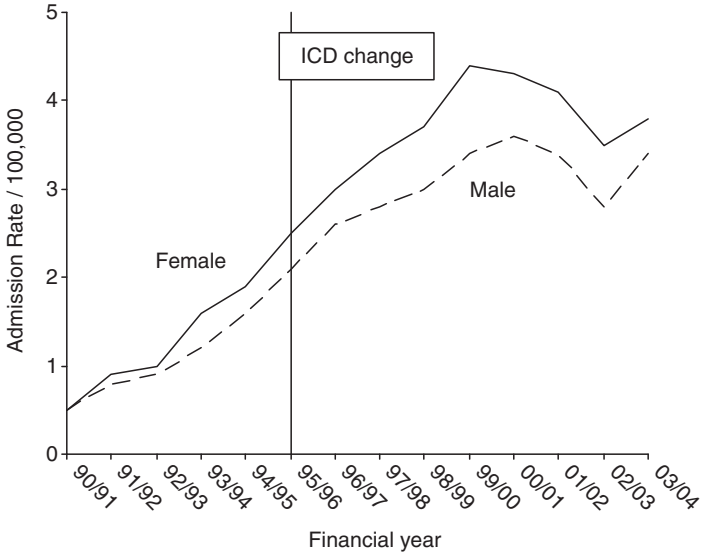

Fig. 1 Hospital admission rates for anaphylaxis, 1990 to 2004, England (modified from 47 with permission from the Lung and Asthma Information Agency, St Georges Medical School, London)

better recording though the figures are likely to at least in part reflect a true increase in incidence for which possible explanations are increases in allergies to foods in children [34] and drugs in adults [48].

The frequency of anaphylaxis attacks does not vary by season except for those caused by hymenoptera (insect venom from stings/bites), which exhibit a peak incidence in summer months [12, 13, 21]. The attack rate does appear to vary according to the time of day, an influence possibly of exposure. Of 282 patients treated for anaphylaxis at a hospital ED in Hong Kong, 78% presented between 4 pm and 8 am (66% of the day) [31]. The authors argued this has important implications for staffing levels of EDs where, traditionally, less senior staff cover these hours.

Place

Until recently, geographic variations were considered unlikely, the variation in incidence of anaphylaxis being explained by deficiencies in the data sources used to estimate it and biological factors related to the population prevalence of allergic sensitisation. However, a study of hospital admission data from England revealed clear evidence of differences in incidence of anaphylaxis between rural (higher incidence) and urban areas, and in geographic location (greater incidence in the

South and West compared with the North and East) [28]. The authors speculated that environmental exposures such as differences in diet, hygiene, vaccination coverage and childhood infections could be explanatory features, along with regional variations in thresholds for admission.

Person

Age

Emergency hospital admission rates for anaphylaxis in the UK average about 17/100,000; they rise during childhood, plateau at about 25–35/100,000 in those aged 15–54 years and decline thereafter with increasing age [28]. In Canada, the prescription of self-injectable adrenaline pens for out-of-hospital use is proportionately higher in young, compared with older people [35] (see above). Susceptibility to triggers also varies by age group with children affected mostly by foods and adults affected predominantly by medications [25, 48].

Gender

Gender and age interact in the occurrence of anaphylaxis. Amongst children, the incidence of anaphylactic reactions is greater in boys than girls, but in most adult studies women are affected more than men [30, 32] (see Fig. 1). This relationship between age, gender and occurrence of anaphylaxis has been confirmed by comparing adrenaline prescribing rates (see above).

Amongst female patients, the rate ratio for those admitted to hospital with anaphylaxis in England was 1.19 [28] and for those attending a hospital ED, it was 1.5 in Australia [30] and 2.4 in Italy [29]. However, this finding of adult female preponderance is not universal; in a study of 282 patients with anaphylaxis seen in a hospital ED in Hong Kong, only 41% were females (rate ratio 0.7) [31].

Socio-economic Position

Hospital admission rates across England for anaphylaxis were higher for persons resident in more affluent postcode areas (adjusted rate ratio for affluent residence 1.32 (95%CI 1.19–1.46) [28]. Similarly, a study of UK general practitioner records revealed a higher prevalence rate of anaphylaxis in the most affluent quintile (1 in 1,200 patients) compared with that in the most deprived quintile (1 in 1,640 patients) [24]. Black et al. noted in an urban population (Manitoba) that self-injectable adrenaline pens were dispensed more frequently in higher income (1.3%) than in lower income (0.6%) quintiles [49].

Ethnicity

There are only very limited data on anaphylaxis risk by ethnic group. In their study of 464 hospitalisations for anaphylaxis across Florida state, Mulla and Simon [25] noted that White non-Hispanic patients were twice as likely to be admitted for anaphylaxis due to insect venom than other ethnic groups (relative risk 2.2, 95% CI 1.4–3.5 after adjustment for age, sex and health insurance provider).

Biological Susceptibility

Atopy appears to be an important risk factor for anaphylaxis attributed to food allergies [13, 50] and latex [51], but not apparently to medications or hymenoptera [13, 18, 50] though the evidence is not robust for either medications or venom [52]. However, atopy is common in the general population and only a minority develop anaphylaxis in response to an allergen to which they are sensitive [18].

Genetic factors associated with risk of developing anaphylaxis are emerging in, for example, studies of patients who have experienced severe reactions to non-steroidal anti-inflammatory drugs [53] and latex [54].

Recurrence Rates and Risk Factors

A recurrence rate of 15% was noted in a prospective study of 567 patients (70% aged < 16 years) with nut allergy referred to a specialist allergy service and followed up for a median of 21 months [55]. Seventy percent of the reactions were mild and these occurred mostly in children (median age 9 years). In comparison, those who experienced more severe reactions were significantly older (median age 18 years).

In another prospective study of 304 patients referred to a specialist service, Mullins [13] noted a recurrence rate of 43% (674 patient-years, maximum follow-up 5.5 years). Of 386 episodes experienced by these patients, 59% were mild and only 18% serious. Risk factors for recurrence included gender (recurrence rates 49% in females, 36% in males), exercise or unknown trigger (idiopathic), but not atopy. Of 45 patients who had a serious recurrence, all but one had had a previous serious reaction. Overall, the annual risk of a recurrence was estimated as approximately 1 in 12, of which a quarter of reactions are likely to be serious and the rest less severe than the original episode.

Although Mullins did not find atopy to be a significant risk factor for recurrence of anaphylaxis, this was not the case in a 7-year follow-up of 46 children in which 14 children (30%) experienced a recurrence and the risk of recurrence was greater in those with atopic dermatitis at initial presentation (64% vs. 34%, $P = 0.04$) [56].

Table 3 Causes of anaphylaxis from selected studies

Source	Country	*N* (no. of patients)	Age (years)		Trigger (%)				
			Range	Median (m) or mean ($\bar{x}$)	Foods	Medications	Insect stings/ bites	Other	Unknown (or unrecorded)
Yocum and Kahn, 1994 [57]	USA	179	N/A	36 ($\bar{x}$)	33	13	14	21	19
Yocum et al., 1999 [21]	USA	133	0.5 – 89	29 ($\bar{x}$)	36	17	15	-	32
Bohlke et al., 2004 [15]	USA	85	0 – 18	N/A	42	12	22	12	12
Mulla and Simon, 2007 [25]	USA	464	0 – 94	53 (m)	16	12	34	36	N/A
Peng and Jick, 2004 [12]	UK	87	0 – 80	N/A	22	30	32	16	-
Pumphrey and Stanworth, 1996 [50]	England	172	0.5 – 69	N/A	60	9	16	3	12
Sheikh and Alves, 2000 [58]	England	2,424	N/A	N/A	8	32	6	6	49
Helbling et al., 2004 [23]	Switzerland	226	5 – 74	41 ($\bar{x}$)	10	18	59	8	5
Pastorello et al., 2001 [29]	Italy	140	14 – 91	38 (m)	39	36	1	3	21
Brown et al., 2001 [30]	Australia	142	14 – 86	37 (m)	17	28	18	11	27
Mullins, 2003 [13]	Australia	432	1 – 82	26 (m)	61	8	20	2	8
Brown, 2004 [59]	Australia	1,149	0 – 96	29 (m)	18	22	30	5	25
Braganza et al., 2006 [32]	Australia	57	0.2 – 14	4 (m)	56	5	5	2	32
Smit et al., 2005 [31]	Hong Kong	282	1 – 91	28 (m)	44	36	6	2	11

N/A not available

Triggers

The commonest triggers for anaphylaxis are foods, medications and diagnostic agents, and hymenoptera venom, though the relative proportions cited as causative agents can vary markedly between studies (Table 3). Some of the variation is accounted for by differences in the age distribution of the subjects studied as the susceptibility to triggers varies by age [48]. For example, anaphylaxis in children is mostly triggered by foods and that in adults mostly by medications or venom. Less common causes of anaphylaxis include exposures to biological and other material in occupational settings (e.g., latex).

Foods

Self-reported food allergies are common in Western societies with 1.1% (95%CI 1.0 to 1.4) of the USA population reporting an allergy to nuts [60]. However, the general perception of food allergy amongst the general population is considerably greater than that confirmed by formal challenge testing, both in the USA [61] and in the UK amongst both teenagers [62] and parents of infants [63]. For example, amongst 1,532 British teenagers, the prevalence of food sensitivity was 12% by self-report, but only 2.3% using an objective test with an open food challenge [62].

The commonest food triggers are seafood (particularly shellfish), peanuts and tree nuts (particularly so for children), milk, eggs, wheat, soya, vegetables/fruits and food additives. Exercise can be an important co-factor for anaphylaxis episodes triggered by foods and non-steroidal anti-inflammatory drugs in some individuals [13].

Medications

Antibiotics and non-steroidal anti-inflammatory drugs are responsible for the majority of drug-related anaphylactic reactions; additional agents include dextrans and radio-contrast media [18]. The International Collaborative Study of Severe Anaphylaxis has estimated the risk of anaphylaxis due to medications per million hospital admissions as 149 in Hungary, 150 in Spain and 200 in India (overall risk 196) [10]. These figures included cases judged ‘definite’ or ‘probable’ using a definition of anaphylaxis that was independent of exposure in that the two physicians who reviewed each patient’s notes were unaware of the potential trigger.

Insect Venom (Hymenoptera)

The proportion of all anaphylactic reactions caused by insect venom was particularly high in a report from Switzerland (Table 3). Although many people get stung each year, only a minority develop anaphylaxis. The number of hymenoptera-induced

anaphylaxis episodes may be markedly under-reported as less severe reactions are unlikely to get reported to any health care workers. Also, some patients with known insect venom allergy may manage an anaphylaxis attack successfully with self-injectable adrenaline and therefore only the most severe, untreated reactions are likely to be seen by hospital staff.

Latex

Increased use of protective gloves by health care workers, and occupational groups generally, has exposed a large number to potential sensitisation against latex. The prevalence of latex allergy in health care workers is estimated as 8–17% and in the general population as 1–6% [5].

Implications for Health Care Policy and Delivery of Care

The epidemiological estimates of anaphylaxis derived from population sources depend on the quality of recording and coding conventions used in computerised medical records. There are deficiencies in current systems that limit the refinement of secondary analyses of hospital and primary care records of anaphylaxis episodes [58]. The College of American Pathologists' Systematised Nomenclature of Medicine (SNOMED) improves the level of detail collected and should help with future epidemiological studies once codes have been agreed and the system is in widespread use [64]. In the meantime, when planning services, the current estimates of the epidemiology of anaphylaxis should be accepted as likely minimal estimates acknowledging the major issue of under-recording [13–15]. Given the relatively high number of children now deemed to be at risk, schools need to develop coherent policies for the prevention and management of anaphylaxis [19, 20]. Also, given the known relatively high risk of recurrence, there is a need to develop and evaluate policies for reducing the risk of further episodes in those with a previous history of anaphylaxis [65]. These interventions could, for example, focus on approaches to reducing the risk of iatrogenic harm from prescribing medications to those with a known allergy to medicines and a history of anaphylaxis [42, 66]. These data can also be useful when planning service provision.

Future Research

Anaphylaxis is a relatively uncommon event, but its occurrence can have a profound effect on the quality of life of the sufferer and their family [67]. The risk of recurrence may be high and some attacks prove fatal, sometimes despite immediate, on-site treatment with adrenaline. Successfully identifying those at greatest risk of an initial attack, and a recurrence, could reduce morbidity, but this has proved difficult in practice using demographic and clinical markers. Genetic epidemiology

Table 4 Summary of the epidemiology of anaphylaxis from selected population-based studies

Sector	Incidence rate (population-based estimates) (per million population per year)	Sources
Mortality	1–5.5	[5, 40]
Hospital admissions	28–100	[15, 16, 22, 25]
Primary care records	70–84	[12, 24]
General population	80–210	[21, 23]

may have the potential to help fill this gap by identifying those at particularly high risk of severe reactions.

Secondary analyses of routine sources of data have proved invaluable in describing the epidemiology of anaphylaxis though the estimates generated would be considered more reliable if the data could be validated and linked across primary and secondary care sectors [68]. Such validation work needs to be prioritised.

Vigilance is needed as new drugs are introduced into our pharmaceutical armamentarium. National reporting systems of adverse drug reactions associated with anaphylaxis may need reinforcing, perhaps through the use of prompts during patient consultations [42, 66]. Methods of improving alerts for potential adverse drug reactions are legitimate areas for the research agenda.

Conclusions

Population estimates of the incidence of anaphylaxis are unreliable being subject to biases from case ascertainment and other sources. However, though imprecise, any information about the burden of anaphylaxis is better than none and current estimates can be useful for health planning, for comparing populations and determining trends with time. Some summary statistics can be proposed though most are likely to be underestimates (Table 4).

From data available over recent decades, incidence appears to be increasing, partly reflected in greater awareness by medical and lay groups, and possibly due to increases in allergies to foods and adverse drug reactions. Evidence is emerging that incidence of anaphylaxis varies by age, gender, geography and socio-economic position. The usual, inverse relationship between prevalence of illness and income seems to be reversed with anaphylaxis. However, the evidence comes partly from Canada where it may be related to ability to pay for adrenaline prescriptions. Common triggers are foods in childhood and drugs and insect venom in adulthood. Annual recurrence rates may be as high as 8% despite vigilance on the part of sufferers. Death is infrequent, but should be mostly avoidable. Improved data capture in and across routine health databases is required if we are to obtain more accurate estimates of the burden of anaphylaxis. This may be obtained through agreement on an acceptable definition of

anaphylaxis [69] and use of standard coding conventions (e.g., ICD10, SNOMED). At present, the best epidemiological estimates appear to come from the developed world, but more information is needed from developing countries.

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Epidemiology and Food Hypersensitivity

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Introduction and Definitions

Food hypersensitivity (FHS) has attracted much awareness over the last three decades and the general public perceives FHS as a major health problem. A revised nomenclature for allergy has recently been published as a position paper by the European Academy of Allergology and Clinical Immunology (EAACI) [1]. Generally, hypersensitivity causes objectively reproducible symptoms or signs, initiated by exposure to a defined stimulus at a dose tolerated by normal subjects [1]. Allergy is a hypersensitivity reaction initiated by immunologic mechanisms, whereas sensitization just reflects presence of specific antibodies to an allergen. Allergens are antigens with the capacity to bind IgE (and IgG) antibodies [1].

FHS is subdivided into toxic reactions and non-toxic reactions [2]. Toxic reactions typically reflect contamination (e.g., bacterial), whereas non-toxic reactions are subdivided into immune mediated and non-immune-mediated reactions [1, 2].

Immune-mediated reactions comprise IgE-mediated and non-IgE-mediated reactions. IgE-mediated (classical type I response) symptoms (e.g., acute urticaria) are mostly immediate reactions (≤ 2 hours after intake of culprit food), whereas non-IgE-mediated (classical type IV response) symptoms (e.g. eczema) are delayed reactions (>2 h after intake of culprit food) [2]. Non-immune-mediated reactions are pharmacological (e.g., tyramine in red wine), enzymatic (e.g. lactose deficiency) or undefined reactions (e.g., additives) [2].

Primary FHS is defined as hypersensitivity to foods independent of pollen sensitization, whereas secondary FHS is defined as reactions to pollen-related fruits and vegetables in pollen-sensitized individuals.

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_9, © Springer 2009

Epidemiologic Population Studies of Primary Food Hypersensitivity in Adults

Epidemiologic studies have been performed all over the world with Europe and USA representing the major part. In general, there is a discrepancy between the prevalence of self-reported and confirmed FHS by oral challenge. The prevalence of FHS to specific food items all over the world is linked to different food cultures such as more consumption of rice in Asia and peanut in USA. The self-reported prevalence of FHS has been reported between 3.5% and 38.4% and the self-reported prevalence of FHS is high both in children and adults.

Table 1 lists the most important epidemiologic population studies about FHS in children and adults. One of the first population studies including ordinary unselected families was conducted by Young et al. in the UK in 1994 and comprised about 7,500 households from the Wycombe Health Authority area and the same number of randomly selected households nationwide [3]. A questionnaire was handed out and returned by 10,552 individuals (52.7%) from the Wycombe Health Authority area and 8,328 (41.8%) nationwide. In the Wycombe Health Authority area, 19.9% suspected FHS and 20.4% of the nationwide sample complained FHS [3]. The prevalence of FHS was estimated between 1.4% and 1.8% in the participants [3]. The study by Young et al. [3] was one of the first studies focusing that the general public perceived FHS as a major health problem, and thereby limiting the daily living in thousands of ordinary families with different self-appointed eliminations diets [3]. However, the estimated prevalence of FHS in the study by Young et al. [3] was correlated with bias. The true prevalence of FHS in the study by Young et al. [3] might be even higher as only eight different foods were selected for oral challenge representing only 50% of the reported reactions. Further, only about 50% returned the questionnaire in both groups, making the estimated prevalence of FHS uncertain. In the study by Young et al. [3], a major part of symptoms in oral challenges were subjective such as headache, behavioural symptoms and joint symptoms. The subjective symptoms, in the study by Young et al. [3], are previously reported with a doubtful correlation to FHS [2]. A recent paper by Briggs et al. statistically demonstrated that using subjective symptom as the only verifying symptom in positive oral food challenges should be followed up with three active and three placebo oral food challenges eliminating statistical uncertainty [4].

Few prevalence studies have been performed in adults. Jansen et al. investigated the prevalence of FHS in the Netherlands including adults by a door to door interview [5]. The response rate was 86% and comprised 1,483 adults [5]. The self-reported prevalence was 12.4% and the estimated prevalence of FHS was calculated to 2.4% [5]. The study by Jansen et al. also clearly demonstrated a gap between the self-reported prevalence and confirmed by oral challenge [5]. In the study by Jansen et al. [5], a very surprising list of confirmed food items causing FHS was found such as pork, glucose, menthol, white wine and no one was allergic to peanut, hen's egg or cow's milk. The study by Jansen et al. could suggest that FHS in adults differ significantly from children [5].

Table 1 Epidemiological population studies of primary FHS including a broad range of food items

Author	Year	Country	Study[a]	*N*[b]	Group	Self-reported (%)	Confirmed (%)
Bock [20]	1987	USA	Selected	480	Children	28	8[c]
Young et al. [3]	1994	UK	Unselected	18880	Adults	20	1.4–1.8[c]
Jansen et al. [5]	1994	Netherlands	Unselected	1483	Adults	12.4	2.4[c]
Altman et al. [62]	1996	USA	Unselected	3750	Households	16.6	NC[d]
Brugman et al. [63]	1998	Netherlands	Unselected	4375	Children	7.2	NC[d]
Eggesbo et al. [64]	1999	Norway	Unselected	2803	Children	33	NC[d]
Schaefer et al. [11]	2001	Germany	Unselected	1537	Adults	20.8	NC[d]
Kanny G et al. [65]	2001	France	Unselected	16174	Households	3.5	NC[d]
De Vries et al. [66]	2001	Netherlands	Selected	907	Children	13.7	NC[d]
Woods et al. [67]	2002	Australia	Unselected	457	Adults	22	NC[d]
Zuberbier et al. [10]	2003	Germany	Unselected	4093	Adults	34.9	3.7
Roehr et al. [12]	2004	Germany	Unselected	2354	Children and adolescents	38.4	4.2
Osterballe et al. [31]	2005	Denmark	Unselected	936	Adults	9.7	3.2
Osterballe et al. [31]	2005	Denmark	Unselected	486	Children	10.3	2.3
Rance et al. [68]	2005	France	Unselected	2716	Children	6.7	NC[d]
Pereira et al. [16]	2005	UK	Unselected	757	Children 11 years	11.6	0.1
Pereira et al. [16]	2005	UK	Unselected	775	Children 15 years	12.4	0.5
Venter et al. [17]	2006	UK	Unselected	969	Infants	5.5 to 14.2	4
Venter et al. [13]	2006	UK	Unselected	798	Children 6 years old	11.8	1.6

[a]Selected or unselected study population
[b]Number of participants
[c]Confirmed by oral challenge
[d]Not challenged

Although data are limited, it is well-known that FHS not always is a permanent affliction. A convincing study by Høst et al. [6] demonstrated development of tolerance to cow's milk before 3 years of age in 87% of children with previously diagnosed cow's milk allergy. Recent studies estimate that approximately 20% of young children with peanut allergy become tolerant to peanut over time [7, 8]. Ford and Taylor [9] reported that 44% of 25 egg allergic children became tolerant over time.

Zuberbier et al. examined the prevalence of FHS in a random sample of 13,300 residents of Berlin by questionnaire followed by oral challenge if suspecting FHS [10]. The questionnaire was returned by 4,093 persons with a mean age of 41 years (range 18 to 79 years). The self-reported lifetime prevalence of FHS was 34.9% compared to point prevalence on 3.7% confirmed by oral food challenge [10]. The highest prevalence of IgE-mediated FHS was found in the age group between 20 and 39 years with pollen-related food such as hazelnut and apple as the most common allergenic food [10]. The authors calculated with a response rate between 30% and 40% from a random sample on 15,000 with an expected prevalence rate of FHS on 2% and confidence interval from 1.5% to 2.5% [10]. However, the prevalence of FHS was 3.7%, making the response rate in study as a possible bias of the true prevalence of FHS. Further, although data is analysed weighting age, it is not clear how many participants included in specific age groups, thus some age groups could be overrepresented, making this a possible bias. The study by Zuberbier et al. [10] also demonstrates a discrepancy between the prevalence of self-reported FHS and confirmed FHS by oral challenge as previously reported by Jansen et al. [5] in a similar adult population. However, the culprit food in the study by Jansen et al. [5] differ significant from the culprit food in the study by Zuberbier et al. [10]

More studies have described the prevalence of FHS in adults, but without diagnosing FHS with oral food challenge. Schäfer et al. found the prevalence of self-reported FHS in adults (mean age 50 years and range 25–74 years) at 20.8% based on questionnaires and interviews [11].

Epidemiologic Population Studies of Primary Food Hypersensitivity in Children and Adolescents

Roehr et al. investigated the prevalence of FHS in children and adolescents in Germany, Berlin, handing out questionnaires to a random sample including 2,354 participants [12]. The response rate was 31.4% and confirmed FHS by oral food challenge was 4.2% with 3.5% representing an IgE-mediated reaction [12]. The most common allergenic foods were pollen-related such as apple, hazelnut, kiwi and carrot [12]. Roehr et al. subdivided the study group into two age groups, one between 0 to 14 years and the other one between 15 and 17 years of age [12]. There is no clear-cut explanation for this subdivision, and no clear-cut information about an equally age distribution of children, e.g., 0–1 years, 1–2 years, etc. The study by Roehr et al. [12] shows that pollen-related food is a major source for allergic reactions

in children younger than 18 years, however, it is important to have in mind that allergic food ingredients differ in different age groups, i.e., cow's milk allergy in small children and pollen-related food in adolescents following the natural allergic march with pollen allergy.

Venter et al. established a study population comprising 1,440 six-year-olds resident on the Isle of Wight in UK, and the final response rate was 798 children [13]. The self-reported prevalence of FHS was 11.8% and confirmed FHS was 1.6% confirmed by oral challenge [13]. The study by Venter et al. [13] clearly emphasize that a detailed case history is of vital importance as totally 94 children reported FHS, but only 28 were regarded as possible allergic. Sixty-six children were excluded from oral challenge mostly because of an inconsistent history eating the food frequently in a variety of forms without any reactions [13]. However, 12 children refused oral food challenge because of variety of reasons [13]. Five children were previously diagnosed with FHS and 4 of 10 children with a positive open-controlled oral food challenge (OCFC) declined double-blind, placebo-controlled food challenge (DBPCFC) [13]. Venter et al. included all five children with previously diagnosed FHS in the estimated prevalence of FHS, a possible bias as we have no information about sort of FHS and time of diagnosis. It is well-known that a significant number of cow's milk allergic children become tolerant over time, whereas in peanut allergic children only 50% become tolerant over time. Cross-reactivity between grass and wheat is well-known [14, 15], Venter et al. [13] reported 3.1% of the children sensitized to grass and wheat despite regular intake of wheat without any symptoms, thus asymptomatic cross-reactivity.

Pereira et al. investigated the prevalence of FHS among teenagers by establishing two cohorts comprising 1,636 eleven-year olds and 1,508 fifteen-year-olds in the UK with a final response rate on 47.4% (n = 757) and 50.2% (775), respectively [16]. Lack of interest was the main reason for not participating in a sample of non-responders [16]. The prevalence of self-reported FHS among these cohorts was 11.6% and 12.4% [16]. Cow's milk and additives were the most common food ingredients reported in the 11-year-old children, whereas cow's milk, hen's egg and peanuts were the most frequently reported food ingredients in the 15-year-old cohort [16]. All children with self-reported FHS and all children with a positive skin prick test (SPT) never previously knowingly eaten a large amount of the food were included for oral challenge [16]. Of the 90 eleven-year-olds and 94 fifteen-year-olds reporting FHS 21 eleven-year-olds and 14 fifteen-year-olds underwent a total of 25 and 17 open oral challenges, respectively [16]. A significant part of children was excluded for a variety of reasons, mainly because of an inconsistent history [16]. With DBPCFC as the end point, the calculated prevalence of FHS was 1.4% in 11-year-olds and 2.1% in 15-year-olds, but altogether 18 children previously diagnosed as food allergic were also included in calculations. This may bias the estimated prevalence, as there is no clear-cut information about time or type of previously diagnosed FHS. Further, it is not clear what sort of additives included in oral challenge.

Venter et al. investigated the incidence of parentally reported and clinically diagnosed FHS in the first year of life by establishing a birth cohort with 969 pregnant

women with a very high follow-up rate as 900 questionnaires were completed at 12 months [17]. Adverse reactions to foods were reported by 14.2% at 3 months, 9.1% at 6 months, 5.5% at 9 months and 7.2% at 12 months [17]. Cumulative incidence of FHS by 12 months was 4% based on OCFC and 3.2% based on DBPCFC [17]. More than 90% of the parents consented to OCFC and 60% consented to DBPCFC at 12 months [17]. However, a major part of the children were not undergoing DBPCFC because of severity of reaction on OCFC, thus making the estimate of the cumulative incidence of FHS using OCFC more valid. Further, a recent position paper [18] from EAACI recommends OCFC as a standard procedure in children less than 3 years of age in case of objective symptoms during oral challenge. Venter et al. did not include children with possible allergy to peanut and sesame of ethical reasons, resulting in a possible underestimate of the cumulative incidence of FHS. The cumulative prevalence of cow's milk allergy was 2.3% in OCFC and 1.0% in DBPCFC, and hen's egg allergy 1.3% in OCFC and 0.8% in DBPCFC. Only one of ten infants with a positive DBPCFC to cow's milk was positive in SPT to cow's milk, whereas five of eight hen's egg hypersensitive infants were positive in SPT to hen's egg. The study by Venter et al. [17] shows that a major part of cow's milk hypersensitivity in infants seems to be non-IgE-mediated compared to IgE-mediated hen's egg allergy in the major part of the infants. However, SPT was performed with commercial extracts of standard food, which could bias the result as the prick-prick technique using fresh food gives a higher sensitivity [19].

A major part of the clinical reactions were delayed symptoms over days (1–7 days) with cow's milk as the most common allergenic food. Except two infants with delayed reactions, all other infants with delayed reactions were negative in SPT to the culprit food, whereas about 50% of infants with immediate reactions were positive in SPT and mostly to hen's egg.

Bock enrolled 501 children and 480 (96%) children were finally included and followed prospectively from birth to their third birthdays [20]. In total, 28% thought to have symptoms produced during food ingestion, and in 8% were these reactions reproduced with cow's milk as the most common allergenic food item [20]. Bock demonstrated that the cumulative prevalence of FHS the first 3 years of life was 8%, thus including previously diagnosed food-allergic children now tolerant to the culprit food during this period of life [20].

Epidemiologic Studies of Primary Food Hypersensitivity Including Specific Food Items

Table 2 lists prevalence studies of FHS to specific food items. In the last decades, an increased prevalence of peanut allergy has been reported. A study by Mortz et al. estimated the prevalence of peanut allergy to 0.5% in a cohort of unselected adolescents [21]. OCFC was performed in 27 of 61 adolescents with a positive SPT or specific IgE (CAP technique) combined with a positive case history [21]. Further, OCFC was negative in 22 cases with negative case history but positive SPT

Table 2 Epidemiological studies of FHS to specific foods

Author	Year	Country	Study[a]	n[b]	Group	Food	Confirmed (%)
Gerrad et al. [69]	1973	Canada	Selected	787	Children	Cow's milk	7.5
Halpern et al. [70]	1973	USA	Selected	1084	Children	Cow's milk	1.8
Jakobsson et al. [71]	1979	Sweden	Unselected	1079	Children	Cow's milk	1.9
Hide et al. [72]	1983	England	Unselected	609	Children	Cow's milk	2.5[c]
Bock [20]	1987	USA	Selected	480	Children	Cow's milk	2.3
Young et al. [3]	1987	England	Unselected	18582	Households	Additives	0.01–0.23
Høst et al. [6]	1988	Denmark	Unselected	1749	Children	Cow's milk	2.2
Fuglsang et al. [51]	1993	Denmark	Unselected	4274	Children	Additives	1–2
Schrander et al. [73]	1993	Netherlands	Unselected	1158	Children	Cow's milk	2.3
Tariq et al. [74]	1996	England	Unselected	1218	Children	Peanut	1.1[c]
Sicherer et al. [75]	1999	US	Unselected	2998	Children	Peanut	0.4[c]
Sicherer et al. [75]	1999	US	Unselected	8049	Adults	Peanut	0.7[c]
Saarinen et al. [28]	1999	Finland	Unselected	6209	Children	Cow's milk	1.9
Emmett et al. [76]	1999	England	Unselected	16420	Adults	Peanut	0.48[c]
Eggesbo et al. [77]	2001	Norway	Unselected	2721	Children	Cow's milk	1.1
Eggesbo et al. [30]	2001	Norway	Unselected	2721	Children	Hen's egg	1.6
Grundy et al. [23]	2002	England	Unselected	1246	Children	Peanut	1.5
Kagan et al. [24]	2003	Canada	Unselected	7768	Children	Peanut	1.5
Sicherer et al. [78]	2004	US	Unselected	5529	Households	Seafood	2.3[c]
Mortz et al. [21]	2005	Denmark	Unselected	979	Adolescents	Peanut	0.5

[a]Selected or unselected study population
[b]Number of participants
[c]Not challenged

or specific IgE to peanuts [21]. Mortz et al. estimated the prevalence of peanut allergy to 0.5% in adolescents [21]. The study by Mortz et al. also demonstrated a correlation between peanut and grass sensitization, emphasizing the importance of obtaining a detailed case history followed by oral challenge if still suspecting peanut allergy [22, 15].

Grundy et al. reported a prevalence of peanut allergy to 1.5% in small children. Unfortunately only 43% of the target population participated in the study, thus making the estimated prevalence of peanut allergy as a possible overestimate [23].

Kagan et al. estimated the prevalence of peanut allergy in Montreal, Canada, by administrating questionnaires to 7,768 children and 4,339 children responded with a mean age of 7.4 years [24]. SPT with peanut was performed in children reporting 'never-rarely ingest peanut' or 'uncertain history of peanut allergy' and if a positive SPT measurement of peanut-specific IgE was undertaken and by levels greater than 15 kU/l children were considered peanut allergic [24]. DBPCFC was performed in children with peanut specific IgE levels less than 15 kU/l [24]. Children with a convincing history of peanut allergy were considered peanut allergic without further testing if a positive SPT to peanut [24]. The prevalence of peanut allergy was estimated to be 1.34%, a result that seems to be an overestimate as the authors criteria for peanut allergy included 1,737 children, all of them with a convincing history of peanut allergy combined with positive SPT without performing oral challenge [24]. Further, children with peanut-specific IgE level exceeding 15 kU/l were diagnosed peanut allergic without performing oral challenge [24]. Recent studies have demonstrated that the positive predictive value of a positive SPT is about 50% and diagnostic levels of specific IgE seems to vary between different centres [25–27].

Saarinen et al. demonstrated the prevalence of cow's milk in unselected healthy full-term infants by initial including 15,400 mothers after delivery and 6,267 (41%) agreed to participate [28]. The infants were subdivided into three groups, group 1 (n = 1,789) receiving CM formula, group 2 (n = 1,859) receiving pasteurized human milk and group 3 (n = 1,737) receiving whey hydrolysate formula [28]. Further, a comparison group (n = 824) exclusively breast-fed was also established [28]. The cumulative incidence of CMA in the infants fed with CM was 2.4% compared with 1.7% in the pasteurized group and 1.5% in the whey hydrolysate group [28]. In the exclusively breast-fed group, CMA developed in 2.1% infants [28]. Saarinen et al. clearly showed that feeding of CM increases the risk of CMA, however, infants exclusive breast-fed CMA is still a health problem with a prevalence of 2.1% [28].

In a prospective study by Høst et al. investigating the prevalence of CMA in unselected Danish infants (n = 1,749) during the first 3 years of life, Høst et al. diagnosed 39 infants with CMA [29]. Høst reported the prevalence of CMA to be 2.2% with a peak in the first year of life [29]. Further, Høst clearly demonstrated that prognosis was good in CMA children as about 90% were tolerant to cow's milk before 3 years of age [29].

Hen's egg allergy seems to be a major allergic problem in small children. Eggesbø et al. estimated the prevalence of hen's egg allergy to 1.6% in children aged 2½ years by investigating 3,289 children with a response rate on 83% [30].

A Danish study investigated the prevalence of FHS in an unselected population of children and adults using a questionnaire, skin prick tests, determination of specific IgE and histamine release followed by oral challenge if suspecting FHS [31]. The study population comprised 486 children (probands) 3 years old, their siblings with 111 less than 3 years of age and 301 older than 3 years of age, and their parents ($n = 936$) with a mean age of 33.7 years [31]. In total, 698 cases of possible FHS were recorded in 304 (16.6%) participants [31]. The prevalence of FHS confirmed by oral challenge was 2.3% in the children 3 years of age, 1% in children older than 3 years of age and 3.2% in adults [31]. Although an unselected study population, there may be bias in the estimate of 'the true prevalence' of FHS such as participants with a history of FHS are likely to be overrepresented and this could overestimate the prevalence of FHS. The most common allergenic foods were hen's egg affecting 1.6% of the children 3 years of age and peanut in 0.4% of the adults. In the adults, 0.2% were allergic to codfish and 0.3% to shrimp, whereas no challenges with codfish and shrimp were positive in the children. Surprising, the prevalence of primary FHS to fruits and vegetables in adults was 2.7%, thus without a positive SPT to pollen and without any allergic symptoms in pollen season. A relatively high proportion of clinical reactivity to fruits or vegetables in absence of pollen allergy is a common phenomenon in the Mediterranean area but has not been reported in the Scandinavian area. Previous studies from the Mediterranean area reported between 15% and 21% of subjects allergic to fruits and vegetables without pollen sensitization [32–34]. In the Mediterranean area, allergic reactions to a wide range of pathogenesis-related (PR) protein are reported. Most of the PR proteins are not found in pollen such as seed storage proteins, and this may be an explanation of this high number with primary FHS to fruits and vegetables found in this study. However, it cannot be excluded that food allergy proceeds to pollen sensitization, and the question of whether the numbers categorized as primary FHS to fresh fruits and vegetables will change into secondary FHS over time remains unsolved.

Additives comprise substances added to food products such as colourings, sweeteners, flavouring and preservatives. Although a large number of different additives are available on the market, a relatively small number are associated with hypersensitivity. Sulphites act as antioxidants that inhibit enzymatic browning of food such as fresh fruits and vegetables or in fermentation processes in wine production [35]. Sulphites are reported as a mediator provoking exacerbation in asthmatic patients [36]. Although several hypotheses are suggested in the sulphite response such as a cholinergic reflex, IgE involvement or a deficiency of sulphite oxidase, the exact mechanisms remain obscure [37–41].

Monosodium glutamate occurs naturally in many foods such as tomatoes, but is also used as flavour enhancers in foods, although the clinical relevance is divergent in different studies [42–45]. Convincing reactions to aspartame (sweetener) following DBPCFC have not been demonstrated [46].

Synthetic colorants such as tartrazine are often added to foods. Although several previous studies suggested a relationship between tartrazine and aspirin, Stevenson et al. [47] were unable to detect tartrazine-induced asthma in any of 150 consecutive aspirin-sensitive asthmatics patients.

Natural dyes (e.g., annatto, carmine and copper chlorophyll) are reported, provoking urticaria, angioedema and even anaphylaxis, further, natural dyes contain proteins capable of inducing direct IgE-mediated response [48–50].

Fuglsang et al. examined the prevalence of intolerance to additives among unselected Danish school children aged 5–16 years based on a questionnaire returned by 4,274 (86%) school children from the local municipality [51]. If positive OCFC, DBPCFC was performed [51]. The children were challenged with a lemonade containing low concentration of additives and if negative, the next dose contained ten times as high concentration of additives as in the first one [51]. In total, the prevalence of intolerance to additives was estimated between 1% and 2% [51]. The estimated prevalence seems high compared to other studies [31, 52], however, Fuglsang et al. included a broad range of additives such as preservatives, synthetic colours, natural colours, acids and flavours in the challenge procedures. The amount of additives used in oral challenge was equivalent to the additive content in candy and soft drinks, making the results very convincing. The most common additives eliciting a clinical reaction during challenge were synthetic colours such as tartrazine, quinoline yellow, patent blue and sunset yellow followed by preservatives (e.g., sulphites and sorbic acid). However, the study also demonstrated that oral challenge with additives is difficult and in daily practice a detailed case history is mandatory.

Young et al. reported an incidence between 0.01% and 0.23% of intolerance to additives [52]. The result seems to be an underestimate as 7.4% of 18,582 suspected hypersensitivity to additives but only a minor part were challenged ($n = 81$) [52].

Epidemiologic Studies of Secondary Food Hypersensitivity

Table 3 lists prevalence studies including secondary FHS. Relatively few studies (Table 3) have investigated the prevalence of secondary FHS confirmed by oral challenges. The prevalence of secondary FHS is correlated to prevalence of pollen sensitization, thus following the allergic march. The prevalence of pollen sensitization peaks in adults, thus secondary FHS is more common in adults compared to children.

Table 3 Self-reported prevalence of secondary FHS in pollen allergic adults based on questionnaire, skin prick tests or oral food challenge

Author	Year	n[a]	Pollen	Prevalence (%)
Bircher et al. [79]	1994	238	Birch, grass and mugwort	39[b]
Eriksson [80]	1978	1129	Birch	24[b]
Hannuksela et al. [81]	1977	388	Birch	36[b]
Ebner et al. [82]	1991	83	Birch	75.9[b]
Osterballe et al. [15]	2005	936	Birch, grass and mugwort	30

[a]Number of participants
[b]Not challenged

The correlation between pollen and fruits and vegetables is explained by the fact that pollen allergens are sharing homologous IgE-binding sites with certain fruits and vegetables allergens such as the major allergen of birch pollen (Bet v 1) cross-reacting with homologous proteins in hazelnut, apple, soybean, bell pepper and celery [53–61].

A recent study from Denmark reported 30% of pollen-allergic (i.e., positive SPT to pollen and symptoms in pollen season) adults (mean age 33.7 years) with secondary FHS [15]. In adults with asymptomatic pollen sensitization (i.e., positive SPT to pollen and no symptoms in pollen season), 7% were diagnosed with secondary FHS confirmed by oral food challenge [15]. Overall, the odds ratio for a clinical reaction allergic reaction to pollen-related foods in symptomatic pollen-sensitized (positive SPT to respective pollen allergen) adults was 3.3 (p-value = 0.003) compared to asymptomatic pollen-sensitized adults [15]. The probability of a clinical reaction to pollen-related foods in different pollen-sensitized groups was significantly different, i.e., 24% if monosensitized to birch, 4% if monosensitized to grass, 10% if monosensitized to mugwort, 35% if sensitized to both birch and grass, 8% if sensitized to both grass and mugwort and 52% if sensitized to both birch, grass and mugwort [15]. The odds ratio of a clinical reaction to pollen-related fruits and vegetables in symptomatic pollen-sensitized adults was as high as four times (birch and grass), the odds ratio of a clinical reaction in asymptomatic pollen-sensitized adults [15]. The most common allergenic food in pollen-allergic adults was hazelnut, affecting 19.2% and followed by apple (16.7%), kiwi (13.3%), celery (7.6%) and brazil nut affecting 7% [15].

Conclusion

In conclusion, previous studies have clearly demonstrated that the general public perceives FHS as a major health problem. However, there is a significant discrepancy between the prevalence of self-reported FHS and the prevalence of FHS confirmed by oral food challenge. Thus, a detailed case history followed by oral food challenge according existing guidelines is mandatory in diagnosing FHS. In future, more epidemiological research is needed to investigate the course of both primary and secondary FHS in children and adults.

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Genetics of Asthma and Bronchial Hyperresponsiveness

Matthew J. Rose-Zerilli, John W. Holloway, and Stephen T. Holgate

Introduction

Asthma is a polygenic disease differentially modulated by heterogeneous gene–environment and gene–gene interactions. For the last two decades, considerable effort has been made to identify the precise genetic factors that lead to susceptibility to this disease. Identification of these factors has advanced our understanding of this disease and has lead to targets for the development of novel therapies to treat patients. The benefits of genetic approaches to study disease mechanisms are exemplified by the recent advances in the understanding of the pathophysiology of other common diseases such as cardiovascular disease and diabetes [1–4] and which are now beginning to have an impact on patient treatment [5–7]. These studies give us insight into the likely outcome of recent and future studies of the genetic basis of asthma.

The recent advances in the understanding of the genetics of asthma and the related phenotype bronchial hyperresponsiveness (BHR) have been driven by successful positional cloning and candidate gene association studies whose aim was to identify genetic factors that underpin inter-patient variability in susceptibility. Since the identification of the first genomic region on Chromosome (Chr) 11 with linkage to an asthma related phenotype by Cookson et al. [8] in 1989, there have been numerous asthma and BHR susceptibility loci found by these approaches [9]. The Online Mendelian Inheritance in Man website (www.ncbi.nlm.nih.gov) lists under the search term: Asthma, susceptibility to (#600807), loci on chromosomes

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_10, © Springer 2009

2, 4, 5, 6, 10, 11 and 13 and a search for BHR reveals additional reported linkage to regions on chromosomes 1, 2, 7, 14. All of these loci are likely to contain one or possibly more genes in which variation may play a small but important role in asthma susceptibility. As a consequence, diligent work to elucidate the precise genetic variations and how these variations contribute to the pathogenesis of this disease will be required. Furthermore this highlights the numerous challenges that researchers face in completely understanding the role of genetic variation in asthma. However, despite these difficulties, there has been considerable progress in the last 5 years in identifying novel genes that underlie these peaks of linkage [10–12]. This progress is likely to be accelerated in the coming months and years as the novel approach of whole genome association (WGA) studies is applied to asthma [13].

A systematic review of all susceptibility genes identified to date for asthma and BHR is outside the scope of this chapter and the majority have already been comprehensively reviewed by Ober and Hoffjan [14,15]. However, this chapter will provide some examples of asthma and BHR genes discovered by positional cloning and candidate gene approaches to illustrate how these studies can aid in our understanding of the disease.

Evidence for Genes in Asthma and Bronchial Hyperresponsiveness

It has been recognised that asthma results from both inherited (familial factors) and environmental exposures for over 350 years (by Sennartus in 1650, cited in [16]; also [17, 18]). The existence of a genetic component to asthma and BHR has been confirmed in a plethora of more recent twin and family studies with current estimates of asthma heritability ranging from 50% to 90% [19–22]. The large range in asthma heritability estimates between studies are due to factors such as differences between the populations studied, age of onset of disease, the type of environment exposure and study design. In several twin studies, the consistent finding is that the non-shared environment between twin pairs is a more important risk factor than the shared environment and that the heritable asthma component may be higher in males (74%) than females (58%) [23, 24].

Approaches to Identify Genetic Factors Underlying Asthma and BHR

There are two well-established approaches to discovering the genomic location of disease susceptibility genes. Positional cloning of disease loci utilising families and the candidate gene approach in both family based and case–control cohorts have been the mainstay of genetic epidemiology for the last decade. However, in the last two years, recent technological advances have enabled researchers for the first time

to perform WGA analysis using hundreds of thousand or even millions of single nucleotide polymorphisms (SNP). In this chapter, we illustrate the differences between the various approaches to identify disease genes with examples of positional cloning and candidate gene analysis in asthma. We will also discuss important considerations in study designs and highlight the exciting novel approach of WGA analysis to identify new susceptibility genes for this complex disease.

Positional Cloning

Positional cloning is a family based co-inheritance analysis of genetic markers spread evenly throughout the genetic region of interest. The region of interest can be genome-wide or a region of a single chromosome. Micro-satellite polymorphisms are the marker of choice for positional cloning because they are highly polymorphic in the general population.

All members of the family group are genotyped and the increased transmission of a particular allele to disease affected individuals indicates genetic linkage with the unknown disease gene. Genetic linkage to a region occurs when the marker and unknown disease contributing gene are adjacent on the same chromosome and no recombination has occurred between them in each new generation. Large regions of chromosomes are inherited as a whole and any genetic polymorphism in that region are potential surrogate indicators of the presence of the disease contributing genes. Linkage is broken down by recombination, therefore in a family group, the resulting linkage region can be relatively large (1–2 Mb in length) as only one to two recombination events occur between generations.

A region of the genome identified as being linked to a disease phenotype will contain several to hundreds of potential disease contributing genes. Traditionally, DNA sequencing of the linkage region in individuals will identify new polymorphisms that may be closer to the gene of interest and can be used as further markers to narrow the linkage region in subsequent analysis. In the era of the Human Genome Project, researchers do not have to sequence large regions of a chromosome to find extra markers as a simple database search will provide a summary of all polymorphism previously identified in that region.

The positional cloning technique has been successful in identifying high penetrance, high-risk genes responsible for Mendelian inherited disorders, such as Huntingtons [25] and cystic fibrosis [26], but the technique does not have the same statistical power to identify low penetrance, low-medium risk genes involved in complex diseases [27, 28]. Linkage analysis has less power than an equivalently sized case–control study as association is only tested in the probands.

In complex diseases, it is often difficult to define an appropriate disease phenotype [29]. There is variability in the severity of the disease in individuals and the age of disease onset may vary, leading to individuals being inaccurately identified as unaffected. Diagnosed individuals may also have apparently identical symptoms resulting from different aetiologies involving various biological pathways.

Researchers may also have difficulty in choosing the best population to study and studies may be confounded by population stratification. In spite of the limitations outlined above, positional cloning has resulted in the identification of several novel asthma and BHR susceptibility genes such as a ADAM33, DPP10, PHF11, HLA-G and IRAK-M [10, 12, 30–32].

At the whole-genome level, positional cloning is a hypothesis-independent approach; it has the potential to identify genes and biological pathways that were not previously implicated in the pathogenesis of the disease (See Table 1 for positional cloned asthma genes). With the advent of genome on a chip technology, researchers can rapidly investigate family or case–control studies for up to millions of SNPs spread over the genome for any associations. These high-density arrays provide greater genome coverage ensuring that the maximum amount of linkage information can be retrieved and the size of the critical linkage region reduced for further analysis. By using cohorts with environmental exposure variables, it may possible to define how genes interact with the environment to cause disease.

Examples of Positionally Cloned Asthma Genes

(i) ADAM33

ADAM33 (Gene ID: 80332; MIM: 607114) was positionally cloned from a genome-wide linkage scan in 460 Caucasian USA and UK families (affected sib-pairs) in 2002 and was the first asthma gene identified through this approach [12]. Strongest genetic linkage at 20p13 was seen with a combined asthma and BHR phenotype (LOD, 3.93) and the D20S482 microsatellite with a 35% in excess allele sharing. The linkage region contained 40 genes that were identified by cDNA cloning and sequencing. Twenty-three candidate genes were then investigated by selective genotyping of 135 SNPs in a case–control study with cases from the linkage cohort and hyper-normal controls (negative personal and familial history of atopy and allergic disease). Analysis of these polymorphisms revealed that the strongest association was in the region of a novel gene, ADAM33.

There have subsequently been numerous studies examining the association of ADAM33 polymorphism with asthma, BHR and related phenotypes in several different ethnic populations. While some studies have not been able to replicate association of asthma to ADAM33 [33–35], the majority of the studies have found significant association with ADAM33 polymorphisms, albeit with different SNPs or haplotypes [36–44]. Non-replication of ADAM33 association may be explained by differences in population and environmental exposures between studies. A recent meta-analysis of ADAM33 association data has shown that variation in this gene could account for 50,000 excess asthma cases in the UK alone [45].

ADAM33 is expressed in mesenchymal cells such as sub-epithelial fibroblasts and smooth muscle cells but not in respiratory epithelium or in cells of the immune system

Table 1 Summary of asthma genes located by positional cloning methods. MA = Maternal affection status and GE = Gene × Environment interactions

Identification Method	Gene Name (GeneID)	Chr	Associated Phenotypes	*Gene Product:* possible functional role in asthma	Interaction GE or MA	Associated Variation	Size of study	Population	Reference of Initial Study	Replication of association
Positional Cloning	DPP10 (57628)	2q14.1	Asthma Atopy (SPT) Asthma severity	*Dipeptidyl peptidase:* Potassium channel regulator with no detectable protease activity. Involved in Cytokine processing (especially in T-cells)		D2S308*3/ *5Multiple SNPs Haplotypes	244 families	Australian UK German	Allen et al [10]	YES [10]
	CYFIP2 (26999)	5q33.3	Atopic Asthma Childhood Asthma	*Cytoplasmic fragile X mental retardation (FMRP) interaction protein 2:* May be involved in differentiation of T-cells		Multiple SNPs Haplotypes	155 families	Korean	Noguchi et al [11]	NO
	HLA-G (3135)	6p21.3	Asthma BHR Atopy	*Class I, Histo-compatibility antigen-G:* Inhibits Th1-mediated inflammation and only the soluble form (HLA-G5) is expressed in asthmatic bronchial epithelial cells.	MA	D6S1281 MOGc Multiple SNPs	129 families	Caucasian Hutterite Dutch	Nicolae et al [31]	YES [31]
	GPRA (387129)	7q14.3	Asthma Total IgE BHR Specific IgE Childhood asthma	*G-Protein coupled receptor:* Bronchial epithelial and smooth muscle surface receptor. May modulate asthma by increasing expression levels in tissues and potential inhibitory effect of GPRA-A on cell growth[66]		Haplotypes	86 families & 103 trios	Finnish Canadian German Italian Chinese	Laitinen et al [60]	YES [60, 67, 69, 70, 148]
	IRAK-M (11213)	12q14.3	Early onset persistent asthma	*Interleukin-1 receptor associated kinase 3:* Negative regulator of TOLL-like receptor/IL-1R pathways. Master regulator of NF-Kappaβ and inflammation.	GE	Haplotypes	100 families	Sardinian Italian	Balaci et al [30]	YES [30]

(continued)

Table 1 (continued)

Identification Method	Gene Name (GeneID)	Chr	Associated Phenotypes	*Gene Product:* possible functional role in asthma	Interaction GE or MA	Associated Variation	Size of study	Population	Reference of Initial Study	Replication of association
	PHF11 (51131)	13q14.3	Total IgE Asthma Asthma severity	*Zinc Finger transcription factor:* Possibly involved in Chromatin mediated transcription regulation. B-cell clonal expansion and regulation of immunoglobulin expression may operate through shared mechanisms at this locus.		Multiple SNPs Haplotypes	230 families	Australian UK European	Zhang et al [32]	YES [32, 149]
	ADAM33 (80332)	20p13	Asthma+ BHR	*Metalloproteinase:* Involved in Airway remodelling by fibroblasts and smooth muscle hyperactivity		D20S482 Multiple SNPs Haplotypes	460 families	Caucasian (UK & US)	Van Eerdewegh et al [12]	YES [36-44].

[46]. It has been shown that ADAM33 is expressed in asthmatic airways and in human embryonic lungs [47] and a recent study has shown increasing expression of ADAM33 and ADAM8 in mild to severe asthmatics [48]. Multiple slice variants of ADAM33 mRNA transcripts have been described in primary cell fibroblasts and intronic SNPs found in the gene may potentially regulate alternative mRNA splicing [49].

ADAM33 is part of the ADAM gene family that is a sub-group of a super-family of zinc-dependant metalloproteinases. ADAM33 has a complex protein organisation of eight domains and is most closely related to ADAMs 12, 15, 19 and Xenopus ADAM13. These genes are a branch of the ADAM family that possess proteolytic activity [50]. Other known functions of ADAMs are to promote myogenic fusion and in the release of proliferative growth factors [51, 52] ADAM members have also been shown to have roles in fertilization, muscle development and neurogenesis [53–56].

In summary, ADAM33 genetic variation is associated with asthma, BHR and reduced lung function in early life (age 3 and 5 years) [57]; it also been shown to have a role in the decline in lung function in later life and in susceptibility to chronic obstructive pulmonary disease (COPD) [58, 59]. The slow progress to date in determining the function of ADAM33 illustrates the difficulties facing research after identifying a novel gene, but it also highlights new opportunities that arise from identifying a novel area of biology relevant to asthma. While the exact function of ADAM33 in both normal and diseased airways biology remains unclear, it is likely that progress will be seen soon.

(ii) GPRA

Recently, G-Protein coupled receptor for asthma susceptibility (GPRA; GeneID: 387129, MIM: 608595) has been identified as an asthma susceptibility gene on chromosome 7p14.3 [60]. This region on chromosome 7 had previously shown linkage to asthma-related phenotypes in several populations (Finnish, Canadian and Australian families) [61–63]. Laitinen et al. [60] positionally cloned asthma candidates on 7p using a hierarchical genotype design. leading to the identification of a 133 Kb segment containing two genes; GPRA (also known as GPRA154 or Neuropeptide S receptor) and AAA1 (asthma-associated alternatively spliced gene 1). While it is unclear if the AAA1 gene encodes a functional protein, the two main transcripts of GPRA (A and B) have alternative 3′ exons encoding proteins of 371 and 377 amino acids, respectively (Genbank AY310326, AY310327) and expression of the two isoforms of GPRA was confirmed by Northern blot and immunohistochemistry analysis. Staining of the bronchus, gut and skin detected isoform A predominantly in smooth muscle cells and isoform B in epithelial cells. In bronchial biopsies, the isoform expression patterns were different between asthmatics and healthy controls, with strong expression of isoform B in asthmatic smooth muscle cells and no expression in healthy samples. GRPA isoform-B staining in epithelial cells was also consistently stronger in asthmatic samples but the expression in healthy controls was more varied. The A isoform showed no consistent differences in staining between the two groups and the authors concluded that one or more polymorphisms in the risk haplotypes might critically alter the balance between the GPRA isoforms. There was up-regulation of Gpra154 mRNA in

ovalbumin-sensitised mice, which is in agreement with the results from human asthma and in another ovalbumin sensitised mouse study. Gpra154 expression was up-regulated in alveolar macrophages from bronchoalveolar fluid [64].

However, studies of Gpra-deficient mice have provided some conflicting results with development of allergic lung disease in these mice being unaltered [65]. An alternative hypothesis has been suggested that GPRA may contribute to the asthmatic phenotype by altering the activity of neurally mediated mechanisms. High levels of GRPA expression in the brain and its recent identification as the neuropeptide S receptor may support this alternative interpretation. Activation of the human GPRA A isoform by its ligand (Neuropeptide S) results in significant inhibition of cell growth [66] and monocytes/macrophages and eosinophils were identified as GPRA positive cells. In peripheral blood mononuclear cells, monocyte activation with lipopolysaccharide (LPS), but not T cell activation with anti-CD3/CD28 antibodies resulted increased Neuropeptide S and GPRA expression [64].

There have been several attempts to replicate association between GPRA and asthma, and as with other asthma genes described in this chapter, they have provided varying results. In 2007, there was another linkage analysis study of Chromosome 7p; this time 117 Italian families demonstrated linkage to allergic asthma phenotypes and several GPRA SNPs were found to associated with elevated IgE levels (SNP 546333, $P = 0.0046$; rs740347, $P = 0.006$) [67]. A Korean case–control study with 439 patients (atopic and non-atopy asthma) and 374 controls genotyped one haplotype tagging SNP 522363 G > C did not find any association with risk of asthma, atopy, serum IgE or log-transformed PC_{20} values [68]. Conversely, there are two studies in European populations that have provided evidence for GPRA in asthma and atopy [69, 70]. Melen et al. identified an increased risk for asthma (OR 1.40) with SNP 546333 and this association was more evident in the joint asthma and BHR phenotype (OR 2.38) [70]. GPRA asthma susceptibility haplotypes have also been associated with respiratory distress syndrome and bronchopulmonary dysplasia in preterm infants [71]. The discovery of GPRA as a candidate asthma susceptibility gene is one of the most exciting recent discoveries as being a G-protein coupled receptor, it is a protein that can be easily targeted by novel small-molecule therapeutics [72].

Candidate Gene Association Analysis

Candidate gene analysis has been extensively utilised in the study of complex diseases and more than 500 studies have now examined the association of genetic variants in over 200 genes with atopic and allergic disease alone [14, 15, 73]. The technique is a hypothesis-dependant approach because rather than utilising a random selection of evenly spaced genetic markers, genes are chosen on the basis of a priori hypothesis about their role in a disease. The selection of a potential candidate gene is based on the involvement of the gene product in biological processes relevant to the disease in question. Evidence for candidate gene selection can be drawn from a

broad range of disciplines, for example, biological function, differential expression, involvement in other diseases with phenotypic overlap, affected tissues, cell type(s) involved and findings from animal models.

Case–control studies are commonly used in the candidate approach. They are essentially a population-based sample of affected and unaffected individuals. Any significant differences in genotype frequency found between the two groups are potentially associated with the disease or susceptibility phenotype. Case–control association studies have greater statistical power to detect genes of small effect than linkage based approaches [74]; this is highly relevant as it is assumed that polymorphisms of milder functional effect in multiple genes in the general population play a role in susceptibility to complex genetic disorders.

Genetic variants showing association with a disease are not necessarily causal because of the phenomenon of linkage disequilibrium (LD). LD is the non-random association of adjacent polymorphisms on a single strand of DNA in a population; the allele of one polymorphism in an LD block (haplotype) can predict the allele of adjacent polymorphisms (one of which will be the causal variant). The size of the LD blocks depends on the recombination rate in that region and the time since the first disease contributing variant arose in an ancestral individual in that population.

The candidate gene approach has been criticised for non-replication of findings, which may be due to poor study design, population stratification and different LD patterns between individuals of different ethnicities. Unfortunately, the genetic association approach can also be limited by under-powered studies and loose phenotype definitions [75]. Therefore it is important that due consideration is given to all aspects of genetic epidemiological study design to ensure that relevant conclusions can be drawn. Candidate gene study design considerations will be discussed later in this chapter. Below we illustrate some of the inherent complexities in the accurate assessment of the role of polymorphisms in a candidate gene in disease susceptibility through the examples provided by studies of the genes IL13 and CD14 in asthma.

Examples of Candidate Gene Studies in Asthma

(i) Interleukin-13

Interleukin-13 (IL-13; Gene ID: 3596, MIM: 147683) is a 12 kDa that has many roles in asthma and allergy [76, 77]. It is produced by activated T-cells to promote B-cell proliferation and IgE synthesis. It also down-regulates the production of TNFα, increases expression of vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells, and enhances the induction of major histocompatibility complex (MHC) Class II and CD23 antigens on monocytes. IL-13 is a key cytokine in asthma not only because of its pro-allergic role but also due to its wide ranging effects on epithelial and fibroblast cells linked to airway wall remodelling. Overexpression of IL-13 in the bronchial epithelium of transgenic mice causes

lymphocytic and eosinophilic infiltration, goblet cell metaplasia, sub-epithelial fibrosis and smooth muscle proliferation associated with marked BHR in humans [78–83]. Numerous positional cloning asthma studies have also demonstrated linkage to chromosome 5q31–33, a region that contains a cluster of pro-inflammatory genes including IL-13, IL-3, IL-4, IL-5 and GM-CSF [84, 85], providing evidence for IL-13 as a positional, as well as a functional candidate gene. As a result, numerous studies have now investigated IL-13 gene polymorphisms for association with a wide range of asthma and allergy phenotypes. A number of functional or potentially functional polymorphisms have been identified in the IL-13 gene.

A polymorphic variant of human IL-13, G + 2044A, is found in approximately 25% of the Caucasian population. This results in the positively charged arginine residue at 110 of the mature polypeptide being non-conservatively substituted with neutral glutamine (R110Q). This variant was first identified by Heinzmann et al. [86] who demonstrated association with asthma in case–control populations from Britain and Japan (Peak OR = 2.31, 95% CI 1.33–4.00); this variant also predicted asthma and higher serum IgE in a Japanese paediatric population. Computer modelling of the variant amino acid showed that it impacts ligand receptor interactions through enhanced charge attraction to IL-13 receptor molecule [86]. This variant amino acid was thought to enhance signalling with IL-13 receptor and this has subsequently been confirmed by in vitro studies [87]. Subsequently, the work of Graves et al. [88], and several more recent studies, have shown strong associations between this IL-13 polymorphism and atopy and atopic diseases such as atopic dermatitis and rhinitis [86, 89–92]. Furthermore in all studies that examined association with total serum IgE levels, the 110Q allele is consistently associated with a phenotype group that includes eosinophilia, IgE and positive skin tests [93].

As well as the R110Q variant, Van der Pouw-Kraan et al. [94] identified a single base pair substitution in the promoter of IL-13 adjacent to a consensus NFAT binding site. Using a sample of 101 asthmatics and 107 controls, they observed an increased frequency of homozygotes in the asthmatic group (13/107 vs. 2/107; $p = 0.002$, OR = 8.3). In additional in vitro experiments, they demonstrated that the polymorphism was associated with less inhibition of IL13 production by CsA and increased binding of NFAT [94]. Further identification and association of IL-13 polymorphisms with asthma and atopy phenotypes in a Dutch population confirmed that IL-13 plays a role in the disease [89]. In this study, it was reported that the −1111C/T promoter variant contributed to susceptibility of asthma and BHR but not to serum IgE levels. DNA resequencing of the IL-13 gene in the Dutch population discovered ten SNPs, four of which were novel polymorphisms. Howard et al. [89] concluded that IL-13 is critical to the pathogenesis of allergen-induced asthma but operates through mechanisms independent of IgE and eosinophils. The authors also genotyped the R110Q SNP but did not replicate the previously observed association with asthma.

The studies of IL-13 polymorphism illustrate many of the difficulties of genetic analysis in complex disease. Replication is often not found between studies and this may be accounted for by the lack of power to detect small increases in disease risk

that is typical for susceptibility variants in complex disease. Differences in genetic make up [95, 96] in environmental exposure between study populations; and failure to 'strictly replicate' [75] in either phenotype (IgE and atopy vs. asthma and BHR) or genotype (different polymorphisms in the same gene) can all contribute to the lack of replication.

Furthermore, given the observation of association with asthma and several components of the IL-4/IL-13 signalling pathway (IL-4, IL-13, IL-4RA, IL-13RA1 and STAT6), it is clear that even when an association is observed; its effect in context of other variation in the biological response pathway should be considered [97–99]. For IL13, strong associations have been shown between IL-13 polymorphisms and atopy-related phenotypes in two studies of children [88, 90]; however neither of these studies examined associations with asthma. In contrast, in adults, IL-13 polymorphisms are associated with asthma but not IgE levels [86, 89]. It is possible that polymorphisms in IL-13 may confer susceptibility to airway remodelling in asthma, as well as to allergic inflammation in early life, showing that the age of subjects may also influence the degree of association observed. Furthermore, the case of IL13 also illustrates the difficulties in identifying the true casual variants in an associated gene given several possible candidates and extensive LD between SNPs.

(ii) CD14

The hygiene hypothesis postulates that increased microbial exposure in early life leads to decreased asthma and allergic disease by the promotion of Th1 over Th2 inflammatory response, resulting in decreased IgE production [100, 101]. Monocyte differentiation antigen CD14 (GeneID: 929, MIM: 158120) is a cell surface molecule preferentially expressed on monocytes/macrophages and functions as a critical pattern recognition molecule for the clearance of bacterial endotoxin (lipidpolysaccaride [LPS]) [102]. Consequently, as CD14 plays a critical role in the LPS response pathway, polymorphism of the CD14 gene that alters expression or function of the protein might be expected to modulate individual response to microbial exposure and hence susceptibility to these conditions. The CD14 gene is located on Chr 5q31.1 and there are two known protein isoforms, a 50–55 kDa membrane bound (mCD14) and soluble protein (sCD14) lacking the membrane anchor that confers LPS sensitivity to cells lacking mCD14 [103]. mCD14 binds LPS and presents to TLR4 (TOLL-like receptor 4) initiating inflammatory gene expression through NF-kappa B and MAPK signaling [104].

In 1999, Baldini et al. [105] discovered a C>T SNP in the CD14 promoter at position -159 from the transcription start site; TT homozygotes had significantly higher levels of sCD14 and were associated with lower IgE serum levels in children that were skin-prick positive for local aeroallergens, although the mechanisms were not clear at the time [105]. There have been inconsistent findings of CD14 polymorphism association studies in asthma, indicating that the levels of environmental endotoxin exposure may alter the effect of CD14 polymorphism [106]. Recently, Nishimura et al. [107] performed a meta-analysis of ten published studies of CD14 polymorphism and asthma and found no overall increased odds ratio risk; but they did conclude that the analysis may not have been sufficiently powered to detect the

modest gene effects expected from a common disease such as asthma [107]. However, another explanation should be considered to explain the variability in observed association between CD14 polymorphism and asthma, namely environmental exposure.

Exposure to endotoxin is known to occur indoors from contact with house dust (HDE, house dust endotoxin). In a study of 327 asthmatic families from Barbados [108], the CD14 TT genotype was protective against asthma in families with low HDE exposure, but the TT genotype was associated with increased asthma risk in families with high HDE exposure. Similarly, in another study, higher house dust endotoxin exposure in children with the −159CC genotype was associated with reduced allergic sensitisation and eczema [109], although non-atopic wheezing, presumably in response to respiratory tract infection, was increased. Taken together, these studies provide support for an 'endotoxin switch', in which there is a dose-dependent response to endotoxin exposure for specific risk genotypes [110]. Exposure to endotoxin is also encountered in occupational settings such as farming. Adults farmers with the CD14 −159TT and −1691GG genotypes have been shown to significantly lower lung function and increased wheezing, compared to other genotypes, possibly due to increased soluble CD14 levels interacting with inhaled endotoxin from the agricultural environment [111].

Animal exposure: The type of microbial exposure during immune system maturation may influence the development of atopy and asthma. In children with the CD14 −159C allele who had regular contact with household pets, serum IgE levels have been shown to be higher than with the T allele [112]. The opposite occurred in children with regular contact with stable animals, where the C allele was associated with lower IgE levels. In another study, early life farm environment and the CD14 −159TT genotype combined to give the lowest risk of nasal allergies and atopy [113].

Environmental tobacco smoke: Exposure to environmental tobacco smoke (ETS) may increase the risk of asthma in susceptible individuals. Interactions between genetic factors in the chromosomal region containing the CD14 gene and BHR and asthma were first identified in 200 families with asthmatic parents from the Netherlands when exposed to ETS [114]. A subsequent study of Puerto Rican and Mexican families showed that asthmatics with CD14 + 1437GG or GC genotypes exposed to ETS had mean FEV1 that was lower by 8.6% predicted, compared to non-exposed [115]. In addition, asthmatics with the CD14 −159TT genotype exposed to ETS had lower serum IgE levels. The mechanism for this interaction could involve exposure to endotoxin found in cigarettes or other ligands of the TLR4 pattern recognition receptor complex. Gender differences may also exist in response to tobacco smoke. Girls whose mothers had smoked during pregnancy or whose parents had asthma had lower mean soluble CD14 levels [116].

Gene–gene interactions: A recent study in 788 asthmatic Korean children reported significantly greater BHR in individuals with risk alleles of both TNFα (−308 G/A) and CD14 (−159 T/C) [117]. It is known that endotoxin-stimulated TNFα production can be modulated by the numbers of $CD14^+$ cells and the level of CD14 expression on immune and inflammatory cells [118–120]. This data suggests

that there might be some synergistic effect between these two risk alleles on BHR and other genes (LTα genes) near TNFα may also be involved as there is extensive LD in that region [121]. Physiologically linked gene–gene interactions need to be considered when attempting to assess the role of any one genetic variant in disease pathogenesis.

Thus, the case of CD14 clearly illustrates that in addition to rigorous study design (adequate power, relevant genes in a pathway, haplotypes of polymorphisms within each gene and relevant phenotypes), genetic studies should ideally include environmental exposure measures to detect gene–environment interaction, and intermediate phenotypes to demonstrate the functionality of the polymorphisms in their population (Fig. 1). While the study of environmental exposure is equally important as genomics in understanding the pathogenesis of asthma, accurate measurement of exposure is a relatively difficult task. Various approaches have employed, including self-reported exposure (e.g., to farm animals or ETS),

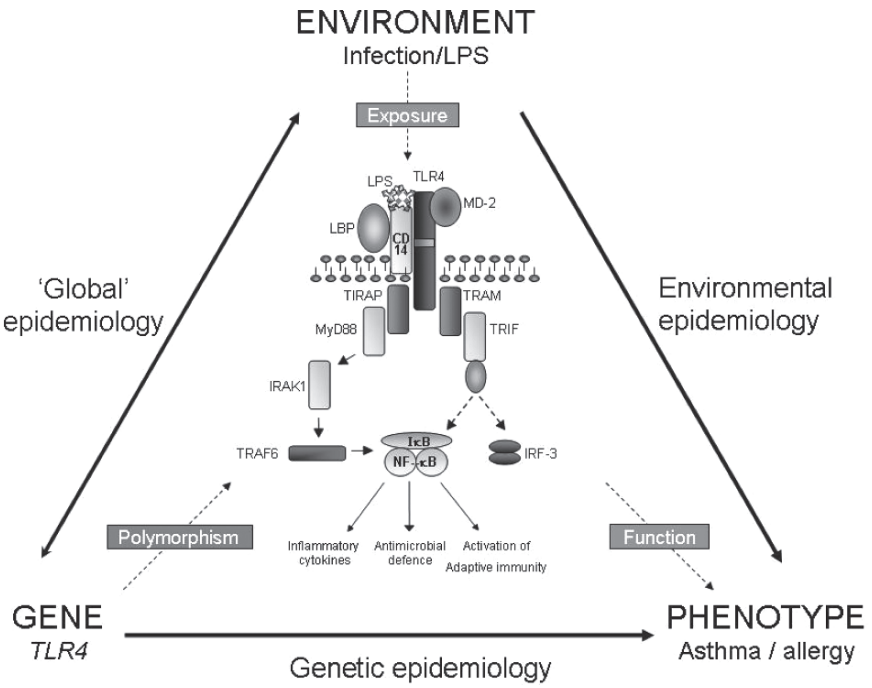

Fig. 1 Approach to understanding gene–environment interaction in asthma and allergy. To understand the biological importance of CD14 in asthma and allergy, studies should examine interactions between gene and phenotype (genetic epidemiology), environment and phenotype (environmental epidemiology), and all three factors (global epidemiology). Supporting evidence from these studies would help to identify causal pathways that lead to the development of asthma and allergy, in genetically susceptible individuals exposed to environmental risk factors. (from Ref. [150], with permission)

correlation with epidemiological data (e.g., concurrent measurement of house dust endotoxin) and controlled exposure in the laboratory (e.g., to air pollutants [122]). Many studies have observed positive associations of specific genetic polymorphisms with differential response to environmental factors in asthma and other respiratory phenotypes [106, 123]. More sophisticated measurements of the effects of environmental exposure are required to bring the environmental side of gene–environment interaction 'up to speed' with advances in molecular profiling.

Combination Approaches: Positional Candidate and Expression Studies

A hybrid of the two approaches described previously is the selection of candidate genes based on their function and/or on their position within a genetic region previously linked to the disease. A good example of the 'positional candidate' approach is the identification of the SOCS5 gene as a potential candidate gene responsible for linkage to BHR susceptibility on chromosome 2p in 364 asthmatic families[124].

A genome-wide scan of European, Australian and USA families with two asthmatic siblings identified nine chromosomal regions with suggestive linkage to asthma and related traits. Further genotyping to refine three regions of linkage to BHR showed strong linkage (LOD score 4.58) to a region on chromosome 2p that overlapped with a marker (D2S2298) that was previously reported to be linked to BHR in a genome-wide scan of 97 German families [125, 126]. The region of linkage was 12.2 Mb in size and contained approximately 75 genes; rather than continue with comprehensive analysis of the region, SOCS5 (suppressor of cytokine signalling 5; GeneID: 9655, MIM: 607094) was selected as an interesting candidate because SOCS proteins are implicated in the control of the balance of Th1 and Th2 cells [127] and SOCS5 is a specific inhibitor of IL-4 signalling [128]. As further supporting evidence for SOCS5 as a potential candidate gene, a transgenic murine model of allergic conjunctivitis has shown that with constitutive expression of SOCS5 there is reduced eosinophil infiltration [129]. The confirmation of SOCS5 as an asthma susceptibility gene awaits further studies.

While this linkage study design is sound and a reasonable candidate gene choice has been made to reduce the considerable work required to narrow a large linkage region of DNA to one single gene to test for association with the disease. It clearly identifies the difficulties facing researchers in identifying the causal genes under a peak of linkage. Without further high-resolution association analysis to further narrow the genetic region carrying the risk allele, researchers can only pursue potential candidate genes based on limited current knowledge rather than directly identifying the causal gene by hypothesis-independent approaches. With the advent of the first WGA studies in asthma, this combined approach may become less prevalent.

Another combination approach is to select potential candidate genes on the basis of their differential expression in diseased versus normal tissue [130–132]. An excellent example of this is the gene encoding tenascin-C (*TNC*), located in a region of the genome previously linked with asthma [126] and those mRNA expression was found to be up-regulated in bronchial epithelial cells in a Th2 cytokine environment [133]. A subsequent case–control study by Matsuda et al. [134] has shown association of TNC polymorphisms with asthma susceptibility. It will not be uncommon in the near future to combine whole gene expression micro-array and WGA data sets in order to elucidate the roles of specific polymorphisms on gene expression in complex disease states [135, 136].

Considerations for Candidate Gene Study Design

A genetic association study design should follow established epidemiological principles in order to have sufficient statistical power to successfully determine any genetic influence on a complex disease. Epidemiological study design can be defined by four terms: what is the biological plausibility of an association, its consistency over different populations, the strength of the association across any sub-group analysis and the existence of a dose–response relationship. The application and adaptation of these considerations to the field of genetic case–control studies have been extensively reviewed in the literature [29, 137–140].

It is crucial that study design strongly adheres to these adapted epidemiological principles to avoid association by random chance alone. The Bonferroni probability value adjustment for multiple independent tests corrects for the effect of performing multiple statistical comparisons that could generate false-positive associations. Approaches to control for multiple testing should be applied to all candidate gene association analyses as association with a single candidate SNP can involve numerous comparisons with asthma-related phenotypes and hence inflate the probability of type I error.

A candidate gene is chosen by examining the evidence from its role in biological pathways relevant to the disease and from animal disease models. Paradoxically, this is also one of the limitations of the method. As gene choice is based on current knowledge and understanding of the disease, genes that are not considered relevant now may be found to be important by whole genome-wide association in the near future. Also, genetic association studies do not necessarily discover the causal locus but a significant association with a genetic variant will narrow the region to search for further understanding of disease pathogenesis or aetiology. Disease-associated genetic variants within genes discovered by genetic epidemiology can then be examined by molecular biological and biochemical experimentation to determine if that variant is the causal loci.

Mendelian disease studies have shown that variants with the highest risk are mostly coding variants (non-synonymous and premature stop codon) that have a

direct functional effect on the gene product. In prioritising polymorphism choice, researchers should consider the merits of intelligent SNP choice by covering regions that may have a possible role in the control of gene expression, splicing, protein function and RNA stability. Mapping haplotypes of the candidate gene with haplotype tagging SNPs (htSNPs) will reduce the practical costs of genotyping numerous SNPs in a region to mark for every possible haplotype and increase the strength of any potential association. In order for the htSNPs to be maximally informative, bio-informatical searches of haplotype data (such as HAPMAP [141], www.hapmap.org) and/or pilot genotyping studies are required to ascertain haplotype patterns in the study population. LD between SNPs in one haplotype may not be the same in different populations; replication of an association using htSNPs may require the genotyping of additional htSNPs to provide informative haplotypes across populations.

A case–control study design is generally used for candidate gene analysis. Retrospective case–control studies are more prevalent as the alternative prospective collection of individuals is rather more time-consuming and therefore costly. Unfortunately, this outweighs the benefit of a more suitable control selection method that the prospective study design offers. There will be little or no population stratification due to control selection in a prospective control group as all the samples were collected at the same time before disease onset, followed up and then sub-divided at a specific time point. A retrospective control group may be 'seeded' with individuals that may go on to develop the disease, this potential heterogeneity within controls could mask any association with a disease-causing genetic variant. In candidate gene analysis, the control selection must be further defined by matching individuals to the cases and performing qualitative and quantitative phenotype measurements to control for any confounding factors.

All case–control studies should have adequate statistical power to correctly detect a genuine association. If there is insufficient power in a study to detect an association, this will lead to a possible false-negative result (type II error). A priori, power calculations should be performed before starting a genetic association study and realistic power probabilities should be set (generally 80%). Statistical power calculations will estimate the required sample size to correctly reject the null hypothesis (i.e. no difference between cases and controls).

In a complex disease like asthma, it is important to carefully define the disease phenotype used in an association study. A binomial category such as "affected/unaffected" may not be the most effective phenotype to test. Asthma is a broad spectrum disorder with a range of interactions including age of onset, severity, atopy, abnormal lung function and environment that contribute unequally and in combination to the full disease phenotype. Fortunately, most of these 'intermediate' phenotypes can be quantitatively measured, providing a more informative and statistically powerful measurement of disease status to test for association. Appropriate phenotype definition and standardisation can be considered to be the most critical stage in the design of complex disease candidate gene association studies.

Whole-Genome Association Studies

The advances of the Human Genome Project and the International HapMap Project [141] in cataloguing and mapping the extent of human genetic variation and the availability of new genotyping methodologies providing high throughput with low cost per genotype call has given rise to the possibility of genome-wide association studies in complex genetic diseases. In these case-control studies, array-based technology is used to genotype SNPs or copy number variations across the genome. Recent results from large disease genetics consortia utilizing WGA Technology have provided exciting gene discovery results for genetic susceptibility to chronic diseases. For example, the Wellcome Trust Case Control Consortium studied ~2,000 cases in seven chronic diseases and a shared set of ~3,000 controls, and discovered genes associated with bipolar disorder, coronary artery disease, Crohn's disease, rheumatoid arthritis, and type I and type II diabetes mellitus, with p values $< 5 \times 10^{-7}$ [142] High-density SNP arrays scale up genetic association studies to the whole genome level and combine the advantages of association studies over positional cloning in families (greater statistical power for a given number of subjects, easier cohort recruitment) with a hypothesis-independent, whole genome approach. The examples provided by the WTCC study together with recent publications for obesity [143], diabetes [144, 145] and breast cancer [146, 147] demonstrate that this approach can be applied successfully to the identification of complex disease genes. The first WGA study of asthma has already identified a novel gene of unknown function; several SNPs were found to regulate ORMDL3 expression and contribute to the risk of childhood asthma [135] (see Table 2). The next few years will likely see the publication of landmark whole-genome association studies in the field of asthma.

Conclusions

The number of novel asthma genes being identified is increasing rapidly and is likely to accelerate with the advent of the whole-genome association study era. Polymorphism in genes such as ADAM33, GPRA and ORMDL3 results in increased disease susceptibility and points to a critical role for these gene's products in the development of asthma. However, while genetic studies are undoubtedly increasing our basic understanding of asthma pathophysiology, it is only the beginning. Validation and replication need to be addressed alongside further molecular genetic studies to help identify the precise genetic polymorphism that is modifying gene expression or function as opposed to those that are merely in LD with the causal SNOften the gene identified may be completely novel and cellular/molecular biology studies will be needed to understand the role the encoded protein plays in the disease and to define genotype/phenotype correlations. By using cohorts with information available on environmental exposures, it may be possible to define how

Table 2 Summary of asthma genes discovered by whole genome association and combination association approach.

Identification Method	Gene Name (GeneID)	Chr	Associated Phenotypes	*Gene Product:* possible functional role in asthma	Interaction GE or MA	Associated Variation	Size of study	Population	Reference of Initial Study	Replication of association
Whole Genome Association	ORMDL3 (94103)	17q12-17q21.1	Childhood onset asthma	*ORMDL3:* Trans-membrane protein anchored in the endoplasmic reticulum. Unknown function.		SNP (rs7216389) & ORMDL3 mRNA expression	994asthmatics and 1243 controls. Replicated in 2320* and 3301† individuals	Caucasian *German † UK	Moffatt et al [135]	YES [135]
Combination Approach	TNC (3371)	9p33	Asthma	*Tenacin C:* Extra cellular matrix glycoprotein. Sub-epithelial marker for asthma severity and response to therapy. May affect the integrity and stiffness of asthma airways		SNP 44513A/T (exon 17)	446 adult asthmatics 658 non-asthmatic controls[134]	Japanese	Linkage: Wjst et al[126] Association: Matsuda et al [134] Expression: Yuyama et al [133]	NO

the genes product may interact with the environment to cause disease. Eventually, the knowledge of the gene's role in disease pathogenesis may make the development of novel therapeutics possible, the ultimate goal for research into the genetics of asthma. We wait with anticipation for the first genomics-derived novel therapy for asthma.

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Genetics of Pediatric Asthma

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Introduction

Atopic disorders, such as asthma, eczema and rhinitis, develop due to the interactions between genetic and environmental factors. Atopy is characterized by enhanced immunoglobulin E (IgE) responses to environmental antigens. There is much evidence to indicate that asthma and atopy are inheritable diseases. Many survey studies have suggested that certain genes are involved in onset of allergic diseases. Since the pathology of asthma and atopy is not simple, it is suggested that many genes are involved in the onset of asthma and atopy. There are two ways to identify causative genes for certain diseases, namely positional cloning and functional cloning. Using these techniques, many genes such as the β-subunit of the high-affinity IgE receptor (FcεR1 β)-chain gene [1], interleukin-4 receptor α (IL-4Rα) chain-gene [2, 3], IL-4 gene, IL-13 gene [4], β2 adrenergic receptor (ADR β 2) gene [5] and a disintegrin and metalloproteinase (ADAM33) gene [6] have been cloned as candidate causative genes for asthma and atopy.

In this chapter, we review the genetic predisposition and genes related to the development of asthma and atopy.

Genetic Predisposition to the Development of Asthma and Atopy

There is good evidence to indicate that asthma is a heritable disease. A number of studies have shown an increased prevalence of asthma and the phenotype associated with asthma among the offspring of subjects with asthma compared to the offspring of subjects without asthma [7].

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_11, © Springer 2009

Numerous studies of twins have demonstrated that concordance rates for asthma, eczema and hay fever are all substantially higher for monozygotic than for dizygotic twins, suggesting a strong genetic contribution. In population-based studies of twins, the estimated effect of genetic factors is about 35% to 70%, depending on the population and the design of the study [7].

In this chapter, we show two of our studies [8, 9] for genetic predisposition in the development of asthma and atopy. In the first study, a questionnaire was distributed in March 1991 to children younger than 16 years of age who were attending kindergarten, elementary or junior high school in two Japanese cities, namely Gifu, with a temperate climate, and Itoman (Okinawa), with a subtropical climate. The

Table 1 Genetic and environmental factors in relation to any allergic diseases as analyzed by multiple logistic regression

	Relative risk (95% confidence interval)	
Independent Variables	Gifu	Itoman
Family history		
No	1	1
Yes	3.58 (2.17–5.91)*	4.22 (2.91–6.12)*
Sex		
Male	1	1
Female	0.93 (0.69–1.27)	0.60 (0.45–0.79)
Age (years)		
0–3	1.72 (0.87–3.40)	0.70 (0.27–1.82)
4–6	1.47 (0.93–2.31)	0.80 (0.44–1.46)
7–9	1.30 (0.81–2.07)	1.10 (0.75–1.62)
10–12	1.15 (0.71–1.85)	1.06 (0.72–1.56)
13–15	1	1
Structure of house		
Made of wood	1	1
Made of reinforced concrete	1.22 (0.87–1.72)	1.15 (0.75–1.78)
Apartment house	1.27 (0.66–2.42)	0.94 (0.60–1.48)
Flooring		
Wooden floor	1	1
Tatami	0.98 (0.64–1.49)	1.91 (1.08–3.38)**
Carpet on tatami	1.17 (0.79–1.72)	1.65 (0.75–3.63)
Carpet on wooden floor	2.00 (1.17–3.42)**	1.71 (0.91–3.23)
Pets		
No	1	1
Yes	0.88 (0.62–1.23)	0.81 (0.58–1.14)

*$P < .01$;
**$P < .05$
n=1,243 (Gifu), 1,953 (Itoman)
Source: Ref [8]

Table 2 Number of children with neither, one, or both parents atopic

		Parents atopic			
Group of children	Total	Neither	One	Both	One+both
Atopic children	256	54(21)	131(51)	71(28)	202(79)
Control children	222	130(59)	81(36)	11(5)	92(41)

Figures in parentheses represent percentage. X^2=72.3; p<0.01
Source: Ref. [9]

number of subjects analyzed was 1,243 in Gifu and 1,953 in Itoman. Multiple logistic regression analysis was performed using SAS (SAS Institute, Cary, NC, USA). Multiple logistic regression analysis showed that in both cities, children of families with a history of allergy had a significantly higher risk (relative risk, 3.58 and 4.22 for Gifu and Itoman, respectively) of contracting an allergic disease (Table 1) and bronchial asthma (except in Gifu).

In the second study, 256 children who had allergic diseases, including asthma, allergic rhinitis and atopic dermatitis, aged 6 months to 15 years (mean 4.7 years), were selected and they were studied along with their family members. As a control group, 222 children without allergic disease, aged 4 months to 15 years (mean 3.4 years) were similarly assessed. Of the 256 children who had allergic diseases, 202 (79%) were found to have a positive family history, in contrast with 92 of 222 (41%) children without allergic disease (Table 2). There was a significant difference between the atopic children and control children in terms of family history.

These results show that there is a genetic accumulation in the development of allergic disorders and asthma. Therefore, the development of allergic disorders and asthma is correlated with some genes. We think that multiple causative genes, but not a single gene, are involved, because there are multiple pathogeneses of allergic reactions.

Genes Related to the Development of Asthma and Atopy

Many candidate genes related to the development of asthma and atopy have been reported, and different genes may be involved in different ethnic groups [7]. Among more than 100 genes by candidate gene association studies, 79 have been associated with an asthma or atopy-related phenotype in two or more independent study samples (Fig. 1) [10]. This figure shows that the genes are associated with asthma or atopy phenotypes in at least one published study. Next, we review the several genes related to the development of asthma and atopy in accordance with the various stages of allergic reaction and development of asthma and atopy.

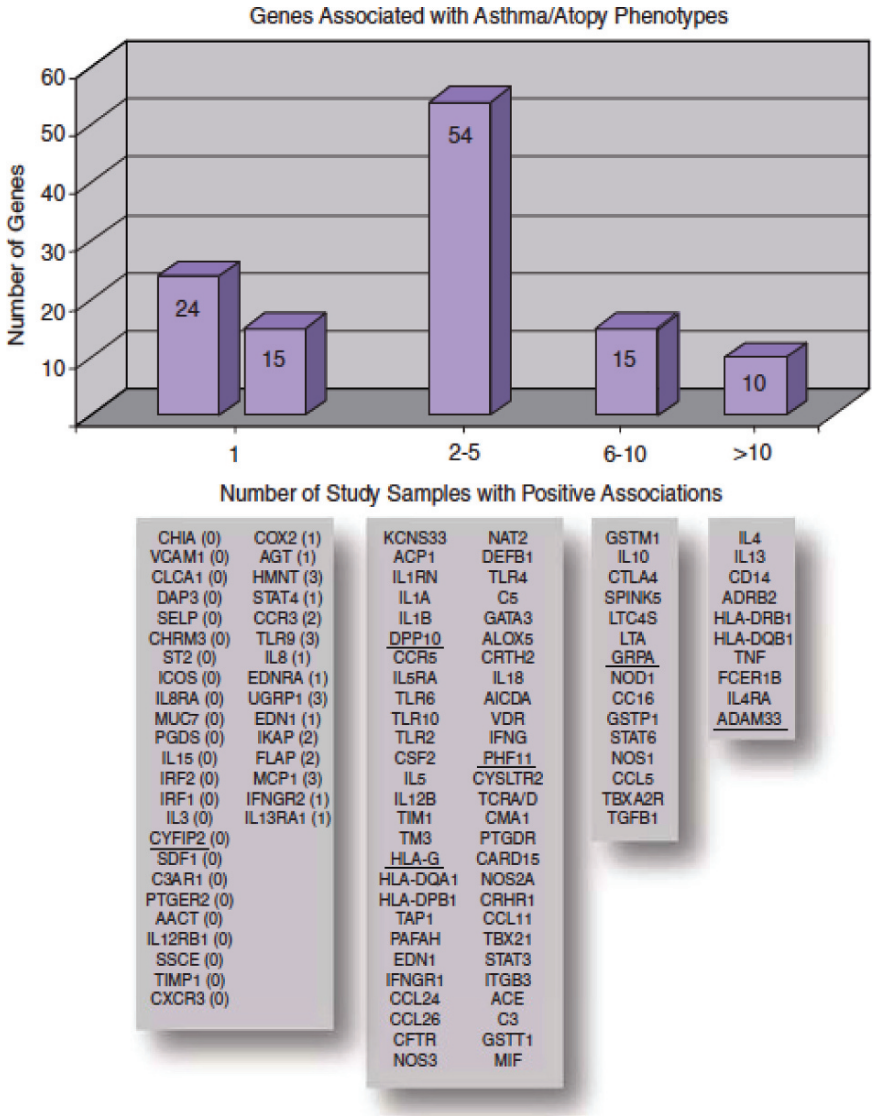

Fig. 1 Genes associated with asthma or atopy phenotypes in at least one published study. Genes are ordered according to their location on the chromosomes. Genes that have been associated in only one population are separated into those without any replication studies reported in the literature and those with subsequent studies that did not replicate the association (numbers in parentheses denote the number of subsequent studies). Genes identified by positional cloning studies are underlined [10]

HLA Genes and Asthma

The HLA genes have been reported to be associated with bronchial asthma [11]. Moreover, the relation between the severity of childhood asthma and HLA type has been reported [12].

Genetic Variation of the Cytokine Signalings in Atopy, Enhanced IgE Production

The production of IgE is upregulated by Th2 cytokines, in particular, IL-4, and is downregulated by Th1 cytokines, in particular, interferon-γ (IFN-γ). Interleukin -12 (IL-12) and IL-18 are the important cytokines that induce IFN-γ and downregulate IgE production (Fig. 2) [13]. In this section, we review the genetic variation of the cytokine signalings in atopy and enhanced IgE production.

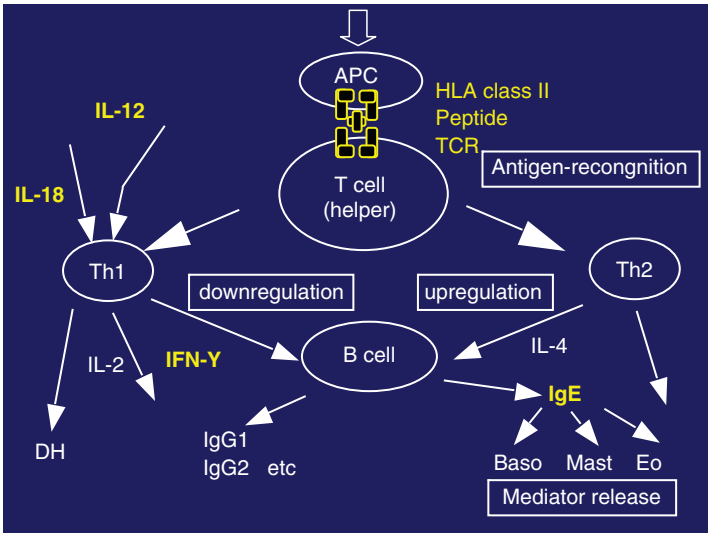

Fig. 2 The Th1 and Th2 lymphocyte balance and upregulation and downregulation of IgE production. IL, interleukin; DH, delayed-type hypersensitivity; IFN, interferon; APC, antigen-presenting cell; HLA, human leukocyte antigen; TCR,T cell receptor; Baso, basophils; Mast, Mast cells; Eo, eosinophils [13]

Genes Related to the Upregulation of IgE Production in Asthma and Atopy

Serum IgE levels of atopic children were plotted against serum IgE levels of their parents (Fig. 3) and a good correlation was found ($p<0.016$) [9]. Therefore, this indicates that IgE production shows genetic accumulation. Several linkage analyses and mutations for candidate genes of atopy (i.e., enhanced IgE production) have been reported. In 1989, Cookson et al. [14] reported a linkage between IgE responses underlying asthma and rhinitis and chromosome 11q. Moreover, Shirakawa et al. (1994) [1] reported that a common variant of Fc ε RI β on chromosome 11, Ile 181 Leu within the 4th transmembrane domain, shows significant association with positive IgE responses. Several associations have been noted between atopy and genes on the chromosome 5 cytokine cluster, including IL-4.

IL-4 and IL-13 Signalings and Atopy

Human IL-4 operates through the IL-4R and thereby signal transducer and activator of transcription 6 (Stat6) activation. Mice deficient in Stat6 or the IL-4R α chain lack IgE production and Th2 inflammatory reactions. IL-4R α is therefore a crucial component required for IL-4 binding and signal transduction. An Ile50Val (numbering for mature peptide) variant of human IL-4R α has been identified. In 1998, Mitsuyasu et al. [3] reported that the Ile 50 Val variant of IL-4R α chain upregulates IgE synthesis and is associated with atopic asthma. Ile50 is associated with atopic asthma but not with non-atopic asthma; Ile50 is specifically and significantly associated with raised total serum IgE levels and mite-specific IgE. The association with atopy was especially strong in children [3].The data from both the mouse and human cell lines strongly suggest that the

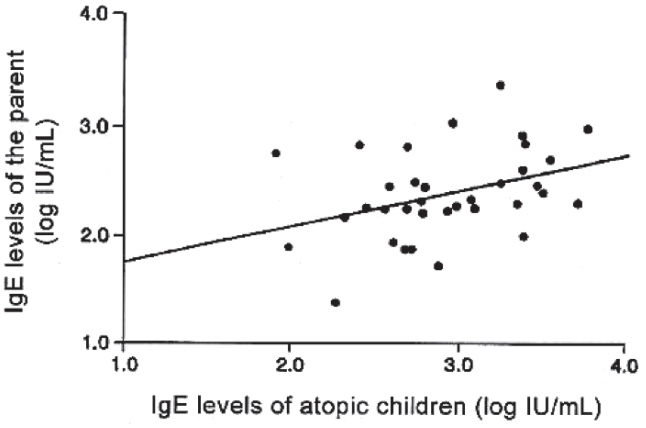

Fig. 3 Relationship between serum total IgE levels of atopic children and the IgE levels of their parents (the highest IgE level of two spouses was used). Children older than 6 years were selected. $P<0.016$ [9]

Ile50 variant of IL-4R α significantly upregulates receptor response to IL-4, with resultant increased activation of Stat6, and hence increased cell proliferation and increased IgE production. Moreover, Shirakawa et al. [4] noted genetic variants of IL-13.

Genes Related to the Downregulaion of IgE Production in Asthma and Atopy

In this section, the genetic defects in the downregulation (brake) of IgE production especially, in terms of IL-12 and IL-18 signalings, are discussed. We found that reduced IFN-γ production by peripheral blood mononuclear cells (PBMC) following stimulation with IL-12 or IL-18 is associated with the heterozygous IL-12 receptor β 2 (IL-12R β 2) chain gene or IL-18 receptor α (IL-18R α) chain gene mutations in atopic subjects [15, 16].

IL-12 Signaling and Atopy

IL-12, which is produced by activated antigen-presenting cells, is a cytokine that consists of two disulfide-linked subunits, p35 and p40. IL-12 plays a central role in promoting Th1-type immune responses and thus cell-mediated immunity. IL-12 also induces IFN-γ production by T lymphocytes and NK cells. The receptor for IL-12 (IL-12R) is composed of two distinct subunits, β 1 and β 2 (Fig. 4) [13, 17, 18]. Although the β 2 chain of the IL-12R is expressed only in Th1 lymphocytes, the β 1 chain is expressed in both Th1 and Th2 lymphocytes [19]. IL-12R β 1 chain does not contain any cytoplasmic tyrosine residues, whereas the cytoplasmic region of IL-12R β 2 chain contains three tyrosine residues. This suggests that the β 2 subunit plays an

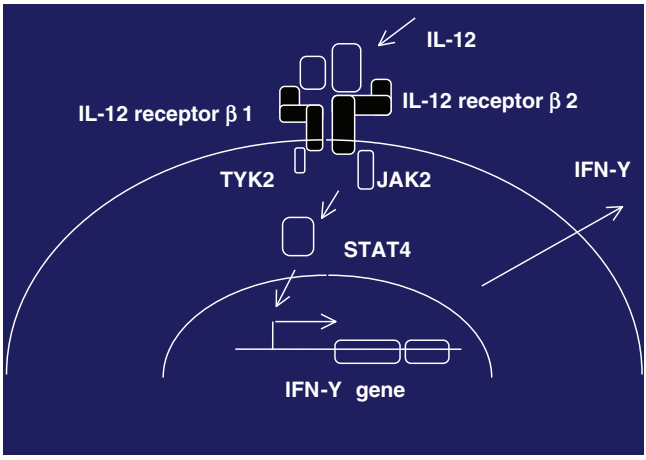

Fig. 4 Interleukin(IL)-12 signaling. TYK2, tyrosine kinase 2; JAK2, Janus Kinase 2; STAT4, signal transducers and activators of transcription 4; IFN, ineterferon [13]

important role in IL-12 signal transduction. IL-12 induces activation of specific members of the signal transducers and activators of transcription (Stat) family of transcription factors and it has been shown that Stat4-deficient mice manifest impaired production of IFN-γ and the phenotype of the IL-12-p40-deficient mouse is similar to that of the Stat4-deficient mouse [20, 21]. Therefore, Stat4 is particularly important. IL-12 induces rapid tyrosine phosphorylation of Stat4 and the formation of nuclear complexes capable of binding to DNA sequences, such as the Stat4-binding site [20, 22]. Tyrosine kinase 2 (Tyk2) is a nonreceptor tyrosine kinase. It was also reported that homozygous Tyk2 mutation caused the hyper-IgE syndrome [23].

We examined the production of IFN-γ in PBMC of atopic patients and healthy controls following stimulation with IL-12 or IL-18. [15, 16] The PBMC of nonatopic healthy controls showed adequate IFN-γ production following stimulation with either IL-12 or IL-18. Although the concentrations of IFN-γ in IL-18-stimulated PBMC were correlated with those of IL-12-stimulated PBMC in atopic patients, there were cases showing different responses to IL-12 and IL-18. The production of IFN-γ following stimulation with IL-12 (or IL-18) was poor, but IL-18 (or IL-12) stimulation elicited detectable IFN-γ production in some atopic patients. The discrepancy in IFN-γ production following stimulation of IL-12 or IL-18 suggests a disturbance in the IL-12 or IL-18 signal cascade in these patients.

It was shown that homozygous nonsense mutation of the IL-12R β 1 chain gene resulted in impairment of immunity against Salmonella and mycobacteria [24]. Moreover, IL-12R β 1 knockout mice showed impaired development of Th1 [25]. In a previous study [15], sequence analysis of the cDNA of IL-12R β 2 revealed three types of distinct genetic mutations (2496 del 91, 1577 A to G (R313G), 2799 A to G (H720R)) in some atopic patients (Fig. 5) [18]. Reduced production of IFN-γ by PBMC following stimulation with IL-12, but not IL-18, is associated with heterozygous IL-12R β 2 chain cDNA mutations in atopic subjects. In these atopic patients, a heterozygous IL-12R β 2 chain cDNA mutation results in decreased tyrosine phosphorylation of Stat4 and subsequently reduced production of IFN-γ following stimulation with IL-12. Such reduced production of IFN-γ could cause insufficient suppression of accelerated IgE production in B lymphocytes by IL-4, resulting in the elevation of serum IgE levels. The 2496 del 91 mutation of IL-12R β 2, which is found all over the transmembrane portion, causes premature termination. The heterozygous missense mutations, 1577 A to G (R313G) and 2799 A to G (H720R), may lead to changes in the conformational structure. Moreover, these heterozygous mutations may play a role via a dominant negative effect. At least, these patients with heterozygous mutations of IL-12R β 2 chain cDNA have not exhibited impairment of immunity against Salmonella and mycobacteria.

The balance between IFN-γ-producing Th1 lymphocytes and proallergic Th2 lymphocytes is important. Heterozyous mutations of IL-12 β 1 or β 2 may result in impairment of the downregulation (brake) of IgE production, whereas homozygous mutations of IL-12 β 1 or β 2 may lead to an obvious impairment of Th1-type cell-mediated immunity in addition to impairment of the downregulation of IgE production. The results of our study [15] indicate that atopic diseases are caused, in part, by impairment of the IL-12 signal cascade, which downregulates IgE production, and that the mutation of the IL-12 β 2 chain gene is one of the causative genes for atopy.

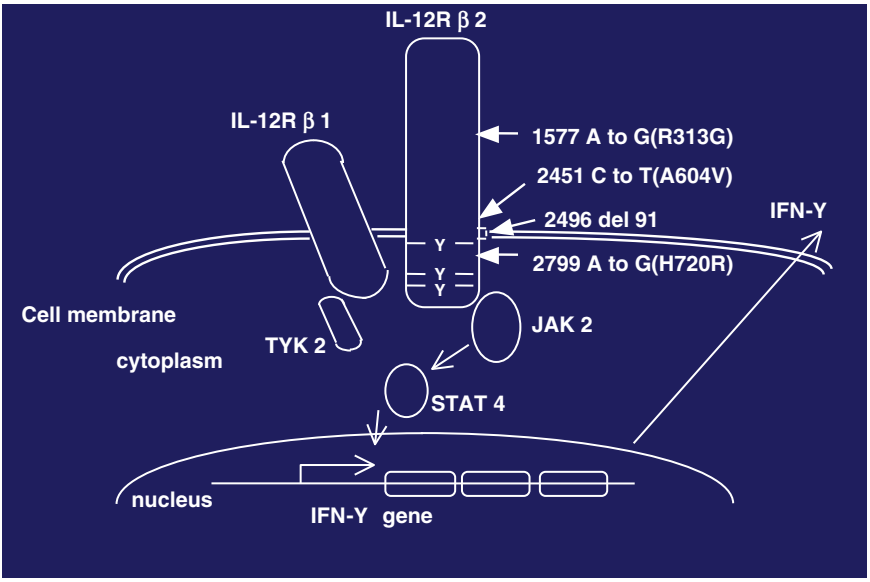

Fig. 5 Interleukin (IL)-12 signaling and mutations of IL-12 receptor β (IL-12Rβ) 2 chain gene. R, arginine; G, glycine; H, histidine; Y, tyrosine TYK2, tyrosine kinase 2, JAK2, Janus Kinase 2; STAT 4, signal transducers and activators of transcription 4. (2451 C to T : by RNA editing) [18]

IL-18 Signaling and Atopy

A variety of biological functions have been associated with human IL-18, including the induction of the proliferation of activated T lymphocytes, enhancement of NK cytotoxity, induction of the production of IFN-γ and granulocyte-macrophage colony stimulating factor (GM-CSF), and promotion of a Th1 response. The activity of IL-18 is via an IL–18R complex. This IL–18R complex is composed of a binding chain termed IL-18R α, a member of the IL-1R family previously identified as the IL-1R-related proteins, and a signaling chain, also a member of the IL-1R family. The IL–18R complex recruits the IL-1R-activating kinase and tumor necrosis factor (TNF)-associated factor 6, which phosphorylates nuclear factor (NF)-κB-inducing kinase, with subsequent activation of NF-κB [26–28].

The IL-18R α chain cDNA of atopic patients was sequenced [16]. We identified a three-base deletion of the IL-18R α chain cDNA (950delCAG), which was generated by alternative splicing, as determined on the basis of genomic sequence data for the IL-18R α chain gene (Fig. 6). PBMC with the predominant expression of 950delCAG significantly showed reduced IFN-γ production after IL-18 stimulation. There was a significant difference in the expression pattern of the IL-18R α chain transcript between atopic patients and nonatopic controls. According to these results, the dominant expression of the 950delCAG transcript of IL-18R α chain cDNA, which was associated with reduced IFN-γ production following IL-18 stimulation and high serum IgE levels, is predisposed to some atopic diseases.

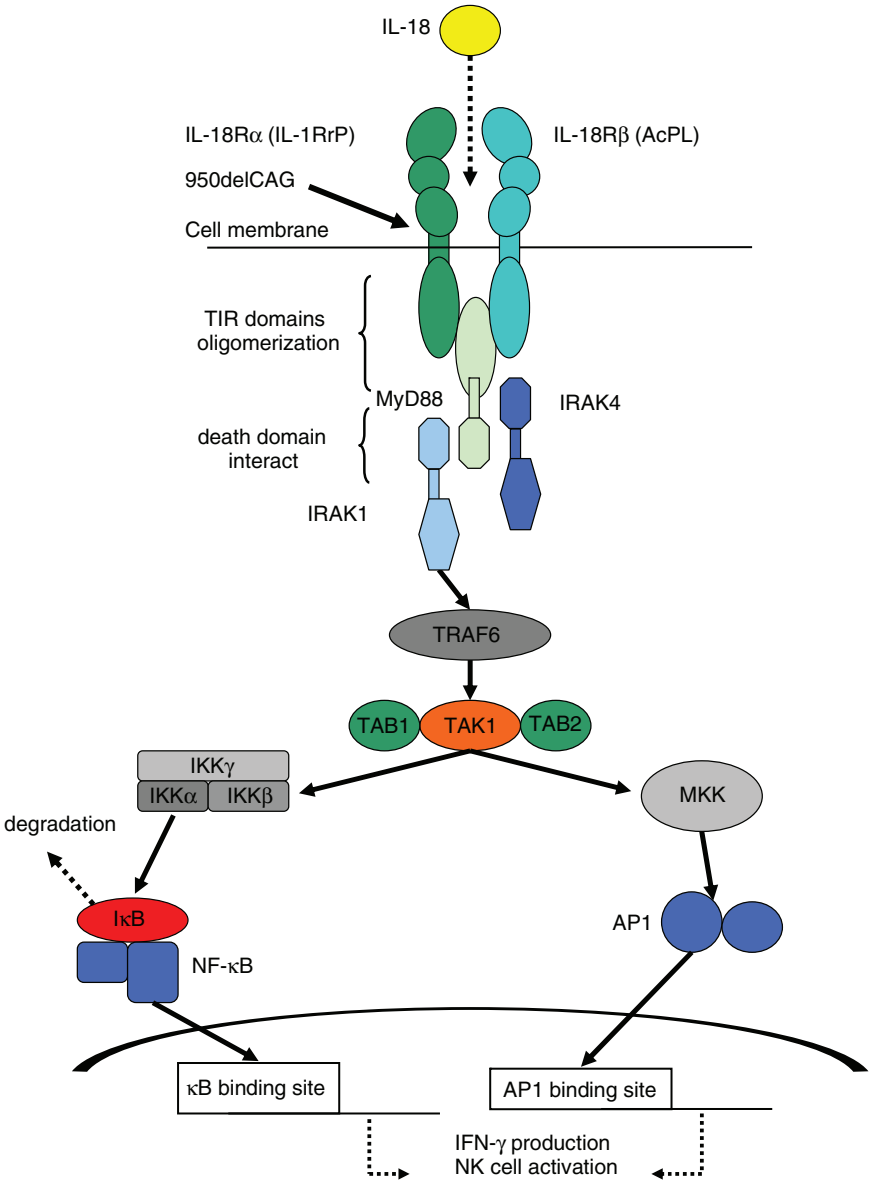

Fig. 6 Interleukin(IL)-18 signaling and 950delCAG in IL-18 receptor α (IL-18Rα) chain CDNA. IL-18Rα, IL-18Rβ ,IL-18 receptor α and β chains, respectively; lkk lκbα kinases; NF-κB, nuclear factor-κB; TRAF-6, tumor necrosis factor receptor-associated factor 6; IRAK, IL-1 receptor-associated kinase

IFN-γR1 Chain and Atopy

We identified a novel heterozygous single-nucleotide substitution 1400 T to C (Leu 467 Pro) in the seventh exon of the IFN-γ receptor 1 (IFN-γR1) chain gene [29]. This substitution was detected in six of the 89 allergic patients, but not in 72 nonallergic subjects. There was a difference in the Leu 467 Pro frequency between allergic and nonallergic subjects ($p<0.05$). Serum IgE levels of allergic patients with Leu 467 Pro were higher than those of nonallergic subjects ($p<0.001$). These results suggest that Leu 467 Pro in the IFN-γR1 chain gene is one of candidate susceptibility genes for atopic diseases.

Genetic Variation of the Mediators and Other Molecules in Asthma and Atopy

LTC4S and Asthma

The locus of leukotriene C4 synthase (LTC4S) is on chromosome 5q35 and has been associated with allergic diseases on the basis of a genome-wide search. Cysteiny1 leukotrienes (cysLTs) play important roles in asthma and can mediate bronchial smooth muscle constriction and increase mucous secretion, vascular permeability, and cellular infiltration [30, 31]. LTC4S converts LTA4 to LTC4 by conjugation to reduced glutathione. A single-nucleotide promoter polymorphis (A-444C) in LTC4S has been associated with aspirin-sensitive asthma in Polish patients [32], although recent studies found no association between this promoter polymorphism and aspirin-sensitive asthma [33]. Very recently, we reported that a novel single-nucleotide substitution 10G>A (Glu 4 Lys) in LTC4S was associated with asthma [34].

nNOS and Asthma

Nitric oxide (NO) is produced by a group of enzymes referred to as nitric oxide synthase: endothelial (eNOS), neuronal (nNOS) and inducible NOS (iNOS). The association of some nNOS marker with asthma or related phenotypes has been reported [35].

Genetic Defects in Target Organs in Asthma and Atopy

ADRβ2 and Asthma

There was no relation between ADRβ2 polymorphisms and asthma prevalence, but the Gly-16 variant was apparently associated with a more severe form of asthma [5]. Subsequently, Turki et al. [36] reported that the Gly-16 allele was found more frequently among subjects with nocturnal asthma than among nonnocturnal asthmatics.

ADAM33 and Asthma

Van Eerdewegh and Holgate et al. [6], performed a genome-wide scan on 460 Caucasian families and identified a locus on chromosome 20p13 that was linked to asthma ($\log_{10}$ of the likelihood ratio (LOD), 2.94) and bronchial hyperresponsiveness (LOD, 3.93). A survey of 135 polymorphisms in 23 genes identified the ADAM33 gene as being significantly associated with asthma using case–control, transmission disequilibrium, and haplotype analyses (P=0.04–0.000003). ADAM proteins are membrane-anchored metalloproteases with diverse functions, which include the shedding of cell-surface proteins such as cytokines and cytokine receptors. The identification and characterization of ADAM33, a putative asthma susceptibility gene identified by positional cloning in an outbred population, should provide insights into the pathogenesis and natural history of this common disease.

Genetic Classification of Atopy

Based on many reports and our results, we present a new genetic classification of atopy on Table 3 [13]. There are four categories of genes that control the expression of allergic disorders, which include (i) antigen recognition, (ii) IgE production (downregulation = brake, and upregulation), (iii) the production and release of meditors, and (iv) events on target organs. In the near future, this genetic classification will facilitate the development of tailor-made (personalized) treatment.

Table 3 A new genetic classification of atopy and genes

Classification	Genes etc.
(1) Antigen-presenting	
	HLA-Pep-TCR
	CD14
	IL-10
	TGFβ1 etc
(2) IgE production	
Downregulation (of IgE production)	
	IL-12Rβ2
	IL-18Rα
	IFN-γR1
	TNF etc
Upregulation (of IgE production)	
	IL-4Rα
	IL-4
	IL-13
	VDJ-Cε
	FcεRIβ etc
(3) Mediators production	
	LTC4 synthase
	TBXA2R
(4) Target organ	
	β2 adrenergic R
	ADAM33 etc

Acknowledgements This study was supported by Health and Labour Science Research Grants for Research on Allergic Disease and Immunology from Ministry of Health, Labour and Welfare.

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Genetic and Molecular Regulation of β_2-Adrenergic Receptors

Ian Sayers and Ian P. Hall

Introduction

β_2-adrenergic receptor agonists are bronchodilators used extensively in the treatment of asthma and other respiratory conditions associated with airflow limitation and obstruction. Both short-acting β_2-adrenergic receptor agonists (SABA; e.g., salbutamol) and long-acting β_2-adrenergic receptor agonists (LABA; e.g., formoterol) have been developed for acute relief from disease exacerbation or maintenance therapy, respectively.

The pharmacological target of β_2-adrenergic receptor agonists is the β_2-adrenergic receptor, a G-protein coupled receptor (GPCR) expressed on multiple cell types in the airways and systemically. In this chapter, we focus on the genetic and molecular mechanisms that regulate the expression and activity of this important drug target in the context of respiratory disease.

β_2-Adrenergic Receptor Structure and Expression

The human β_2-adrenergic receptor gene (*ADRB2*) has been localised to chromosome 5q31–33 and cDNA sequencing identified a 413 amino acid protein with an approximate molecular weight of 46.5 kDa [1]. Analyses identified seven clusters of hydrophobic amino acids in concordance with the classical GPCR structure [1]. Like all GPCR proteins, the β_2-adrenergic receptor has three extracellular loops with an amino-terminus and three intracellular loops with a carboxy-terminus (Fig. 1).

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_12, © Springer 2009

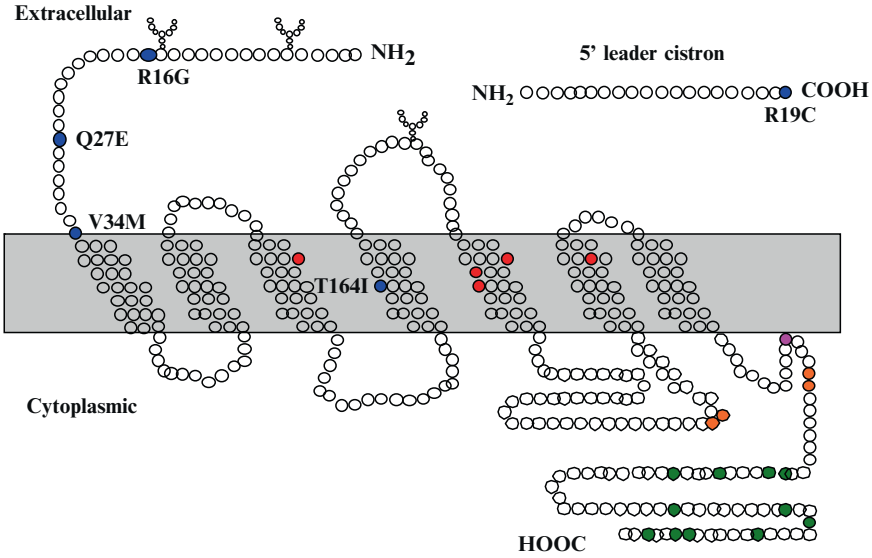

Fig. 1 The structure of the human β_2-adrenergic receptor. Non-synonymous coding polymorphisms are shown in blue (see Table 1). The key amino acids involved in β_2-adrenergic receptor agonist binding (residues Asp113, Ser203, Ser204, Ser207 and Asn293) [8–11] are shown in red. Putative sites of phosphorylation by protein kinase A (PKA) (orange) and β_2-adrenergic receptor kinase (β_2-ARK) (green) [15]. Glycosylation sites at residues 6, 15 and 187 are also illustrated [20] and the palmitylated residue Cys 341, which anchors the carboxy terminus to the membrane is shown (pink) [21]**.** The 5′ leader cistron including the Arg19Cys polymorphism is shown

The β_2-adrenergic receptor is found on several important cell types in the airways including airway smooth muscle (ASM) cells, mast cells and epithelial cells [2–4]. Expression of β_2-adrenergic receptors on peripheral blood mononuclear cells (PBMCs) has also been used as a marker of pulmonary β_2-adrenergic receptor expression [5], however expression of the β_2-adrenergic receptor on the PBMCs is ~700–750 receptors/cell compared to 30,000–40,000 on human ASM cells.

In vivo measurements of pulmonary β_2-adrenergic receptor expression using position emission topography (PET) have been completed [6,7]. These data demonstrated that the β_2-adrenergic receptor density was 10.3±1.8 pmole/g tissue in an asthma group (n = 10) and 10.9±1.9 pmole/g tissue in the control group (n = 30) i.e., not different [6]. Interestingly there was also an inverse relationship between forced expiratory volume in 1 second (FEV_1) and pulmonary β_2-adrenergic receptor density in the asthma group [6]. These data suggest that pulmonary β_2-adrenergic expression maybe directly related to lung function parameters of clinical relevance in asthma.

Agonist Interactions, Receptor Activation and Downstream Signaling Events

Site-directed mutagenesis studies have identified the key agonist contact residues within the β_2-adrenergic receptor (Fig. 1, [8–11]) and these interactions have been modelled for multiple β_2-adrenergic receptor agonists and antagonists [12]. The key amino acids involved in β_2-adrenergic receptor agonist binding include residue Asp113 in the third transmembrane domain, which interacts with the amine group of agonists [8]. Serines 203, 204 and 207 in the fifth transmembrane domain have been shown to interact with the phenyl ring of catecholamines [9,10] and Asn 393 in the six transmembrane domain interacts with the β-hydroxyl group of β_2-adrenergic receptor agonists [11]. The positioning of the Asn 393 residue makes this contact only accessible to the *R*-enantiomers of agonists such as isoprenaline, explaining the stereoisomer-specific effects of *R*- vs. *S*-enantiomers of these compounds. The manner in which a specific β_2-adrenergic receptor agonist interacts with the receptor is dependent on the molecular structure of the agonist, e.g., short-acting agonists including salbutamol access the active site of the receptor directly due to their hydrophilic properties whereas long-acting agonists including salmeterol are taken into the cell membrane prior to interaction with the active site due to lipophilic properties.

Following agonist binding, the receptor adopts a conformation that promotes coupling to heterotrimeric G proteins, particularly Gαs. While still not clearly defined, this is thought to involve the second intracellular loop, the third intracellular loop and the cytoplasmic tail [13]. This coupling results in activation of adenylyl cyclase and the generation of cyclic AMP (cAMP) from ATP. Elevated cAMP activates cAMP-dependent protein kinase A (PKA), which then phosphorylates various membrane and intracellular proteins that result in, for example, smooth muscle relaxation. Key proteins that are phosphorylated by PKA include Gαq-coupled receptors, phospholipase C, inisitol 1,4,5-triphosphate receptor (IP_3R) and myosin light chain kinase (MLCK). These events have the combined effect of diminishing the contractile responses of the cell. In addition to the initiation of cAMP-dependent pathways following β_2-adrenergic receptor activation, other pathways including stimulation of the mitogen-activated protein (MAP) kinase pathway via a Gαi, non-receptor kinase cSRc and G protein RaS mechanism have been described [14].

β_2-Adrenergic Receptor Desensitisation, Sequestration and Down-regulation

Following β_2-adrenergic receptor activation, there is a mechanism of desensitisation, which acts to regulate the activity of the receptor. Several mechanisms contribute to this loss of receptor activity including uncoupling of the receptor from adenylyl

cyclase activity, internalisation of the receptor and phosphorylation of internalised receptors. Agonist-induced phosphorylation of the β_2-adrenergic receptor occurs in the third intracellular loop, leading to decreased coupling of the receptor to Gαs and subsequent adenylyl cyclase activity and desensitisation. G-protein coupled receptor kinases (GRKs) phosphorylate several serine and threonine residues in the β_2-adrenergic receptor cytoplasmic tail. The consensus β_2-adrenergic receptor protein kinase phosphorlayion sites have been confirmed by site-directed mutagenesis (Fig. 1, [15]). Serines 261, 262, 345 and 346 were identified as the putative PKA phosphorlayion sites and mutation to Ala was associated with a reduced desensitisation following agonist exposure [15]. Similarly, mutation of 11 serines and threonines constituting the potential β_2-adrenergic receptor kinase (β_2-ARK) sites in the carboxy tail resulted in a similar phenotype to the PKA variants for the mutant cell line versus wild type although the magnitude of effect was greater (Fig. 1, [15]). More recently, it has been shown that the kinetics of PKA- and GRK-mediated phosphorylation of β_2-adrenergic receptor residues 262 and 355, 356, respectively, are distinct and differentially affected by endocytosis [16]. It was also demonstrated that receptor dephosphorylation can occur at the plasma membrane or in internal compartments [16]. β-Arrestins bind to the phosphorylated receptor and mediate uncoupling by recruiting other proteins.

Following longer exposure to β_2-adrenergic receptor agonist, receptors are internalised or sequestered and are then dephosphorylated [17]. *S*equestration and receptor cycling takes longer to recover from than uncoupling and therefore may be a critical determinant of responsiveness to β_2-adrenergic receptor agonists. Further exposure to β_2-adrenergic receptor agonist results in the net loss of receptors, termed down-regulation, which occurs via mechanisms that are independent of receptor phosphorylation. Ubiquitination of the β_2-adrenergic receptor via a β-arrestin/E3 ubiquitin ligase Mdm2 mechanism targets the receptor for degradation and is thought to contribute to the net loss of surface receptor expression [18]. Multiple studies have demonstrated *in vivo* desensitisation of β_2-adrenergic receptor agonist responses following prior administration of β_2-adrenergic receptor agonist, e.g., in a study of eight normal subjects, Turki and colleagues demonstrated that prior *in vivo* administration of metaproternol led to a decrease in β_2-adrenergic receptor expression (70% reduction) and decreased maximal cAMP responses to isoprenaline (48%) in *ex vivo* airway epithelium cells [19].

Other Post-translational Mechanisms Influencing Human β_2-Adrenergic Receptor Function

The β_2-adrenergic receptor is N-glycosylated at amino terminal residues 6, 15 and extracellular loop 2 residue 187 (Fig. 1, [20]). Using a recombinant Chinese Hamster Fibrobast (CHW) model, it was shown that residue 187 glycosylation is

essential for long-term agonist promoted down-regulation. Mutation of Asn 187 to Gln resulted in the failure of the β_2-adrenergic receptor to enter the lysosomal degradation pathway [20]. Residue Cys 341 of the human β_2-adrenergic receptor is palmitoylated and is thought to act as an anchor, attaching the carboxy terminus to the membrane forming a fourth intracellular loop (Fig. 1). Mutation of Cys 341 to Gly resulted in a non-palmitoylated form of the receptor expressed in CHW cells that had reduced Gαs coupling and adenylyl cyclase activity in response to isoprenaline [21]. These data therefore demonstrate that post-translational modifications including glycosylation, palmitoylation and phosphorylation (see earlier) have key regulatory roles in β_2-adrenergic receptor function.

The ADRB2 Promoter, the 5′ Leader Cistron and 3′-Untranslated Region

The human *ADRB2* gene is located on chromosome 5q31–33 and is intronless [1, 22]. The 1,239-bp open reading frame (ORF) encodes for the 413 amino acids of the mature protein. Within the β_2-adrenergic receptor mRNA, a 5′ leader cistron (5′LC) encoding a short 19 amino acid peptide has been identified (Fig. 1 and 2, [1]). Mutation of the initiation codon of this short ORF leads to an increase of the murine β_2-adrenergic receptor expression by 1.9-fold in the absence of an increase in levels of mRNA in a COS-7 recombinant system [23]. Similarly, truncation of this inhibitory peptide leads to increased β_2-adrenergic receptor expression and a synthetic peptide corresponding to the 5′LC was able to inhibit β_2-adrenergic receptor translation [23]. These data demonstrate that the 5′ leader cistron is a key regulatory mechanism determining the translation level of the β_2-adrenergic receptor.

The transcriptional initiation sites for the human *ADRB2* gene have been mapped to between positions −150 to −172 in a human epidermoid carcinoma cell line, A431 (Fig. 2, [24]). We have previously confirmed this *ADRB2* transcription initiation region in primary human ASM cells (Hall et al., unpublished). These data suggest that the human *ADRB2* gene has transcription initiation close to the translation initiation codon, which in contrast to several other GPCRs, e.g., H1 histamine and CysLTR1 receptors, which we have investigated that have a complex series of 5′-untranslated exons and distal transcription initiation site(s) [25,26]. In addition to the 5′LC, within the promoter region of the *ADRB2* gene is a potential ORF that would generate a protein of 251 amino acid residues (Fig. 2). To date, the functional significance of this transcript has not been explored. Within the *ADRB2* promoter are many consensus transcription factor binding sequences that suggest that transcriptional regulation of the *ADRB2* gene may have a key role in regulating β_2-adrenergic receptor expression and function. In particular, consensus binding sites including cAMP response elements (CRE) and glucocorticoid receptor elements (GRE) have been identified (Fig. 2). Interestingly, the presence of the CRE binding sites suggests that the *ADRB2* gene can auto-regulate expression of the

β_2-adrenergic receptor via a transcriptional mechanism. It has been confirmed that cAMP can induce transcription of the β_2-adrenergic receptor by three- to four-fold in response to 100 nM epinephrine for 30 min [27]. The level of β_2-adrenergic receptor mRNA was dependent on agonist exposure time and correlated with surface receptor expression [27]. Using promoter–reporter constructs, this transcriptional

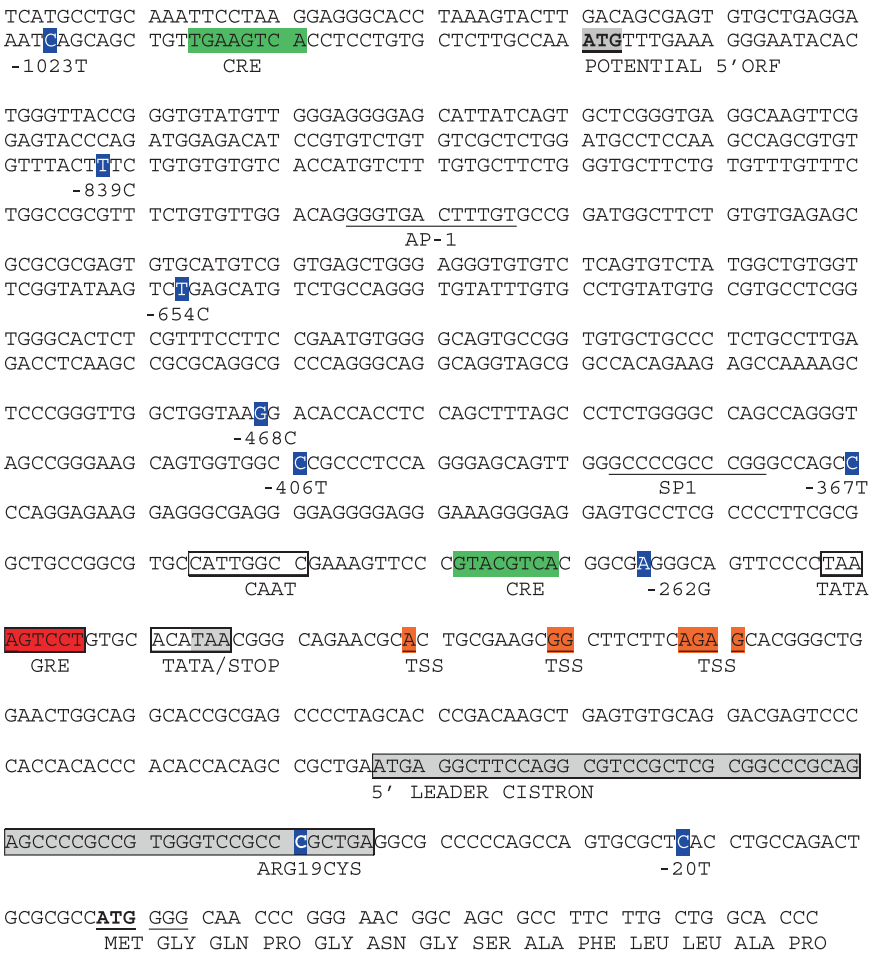

Fig. 2 Representation of the human *ADRB2* promoter region. Approximately 1,000 bp of the promoter region is shown, numbering represents bps relative to + 1 ATG. The position of the 5′Leader cistron is shown, which encodes for a 19 amino acid peptide [1]. The intitiation codon and stop codon of a potential ORF are shown. Polymorphic variation in the region is shown in blue (see Table 1). The identified transcription initiation sites (orange), a potential glucocorticoid response element (GRE) site (WGTYCT, red) and TATA/CAAT box locations are also shown (from [24]). Additional putative transcriptional sites (CRE, AP-1, SP-1) were identified using TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html)

regulation was shown to be contained within the first 300 bp of the promoter region and predicted to utilise the CRE site at −58 (relative to transcription initiation) [27]. Subsequent site-directed mutagenesis of the CRE site between −57 to −51 (relative to transcription) confirmed the role of this site in cAMP-mediated β_2-adrenergic receptor transcription in response to forskolin, which directly activates adenylyl cyclase [28]. Similarly, it has been demonstrated that glucocorticoids can increase β_2-adrenergic receptor transcription in human lung tissue [29]. Dexamethasone treatment of human lung tissue lead to an increase in β_2-adrenergic receptor mRNA (detected at 15 min) and surface receptor expression (maximal between 17 and 24 h) [29]. These effects were shown to be glucocorticoid receptor (GR) dependent using the GR antagonist RU-38486 [29]. β_2-Adrenergic receptor mRNA stability was not effected by dexamethasone, suggesting a transcriptional mechanism potentially utilising the GRE sites described (Fig. 2, [29]). The findings that β_2-adrenergic receptor activation can auto-regulate the transcriptional activity of the *ADRB2* gene and that glucocorticoids can increase *ADRB2* gene transcription have clinical implications [30]. Combined therapy using corticosteroids and LABA has shown greater improvements in clinical outcome measures than monotherapy [31]. These observations may, at least in part, be explained by the synergistic effects of glucocorticoids preventing β_2-adrenergic receptor down-regulation by increasing transcription of the *ADRB2* gene as described. Additionally, it has been demonstrated that LABA, e.g., salmeterol, can increase GR nuclear translocation and GRE-mediated transcription in epithelial cells [32, 33].

mRNA stability has also been identified as a key regulatory mechanism for the expression of the β_2-adrenergic receptor. Transfection experiments in HEK293 cells involving cDNA encoding human β_2-adrenergic receptor with and without the 5′UTR, 3′UTR or ORF suggested a key role for the 3′region in isoprenaline-mediated effects on mRNA expression [34]. Using gel shift assays, the key 3′ sequence (UAAUAUAUU) was identified at 329–337 in the UTR and fusion of this sequence to a the 3′ region of a β-globin gene construct led to agonist-induced destabilisation of β-globin mRNA [34]. Interestingly, it has also been shown that sequence motifs in the *ADRB2* 3′region influence β_2-adrenergic receptor translation by targeting mRNA towards the non-polysomal fractions within the cell [35]. In a recombinant CHO transfection system, the 5′LC and 3′UTR of the β_2-adrenergic receptor gene were shown to be additive in translational suppression of β_2-adrenergic receptor expression [35].

Overall, these data demonstrate the molecular regulation of human β_2-adrenergic receptor expression and function is complex and involves multiple mechanisms including transcription, mRNA stability, translation and post-translational control.

Polymorphic Variation in the ADRB2 Gene

The *ADRB2* gene is intronless and therefore is thought to span ~3 kb on chromosome 5q31–33. Initial mutation screening of DNA from 107 individuals (51 with

asthma, 56 controls) using a combination of temperature-gradient gel electrophoresis (TGGE) and direct sequencing identified nine polymorphisms within the *ADRB2* coding region [36]. These single nucleotide polymorphisms (SNPs) included four non-synonymous polymorphisms: Gly16Arg, Gln27Glu, Val34Met and Thr164Ile (Fig. 1, [36]). The frequency of the position 16 and 27 polymorphism was found to be Arg16/Gly (59)%, Gln27/Glu (29)% in the Caucasian population [36]. The Val34Met and Thr164Ile polymorphisms are rare, with approximate frequencies of <0.001% and 0.05%, respectively [36]. Multiple subsequent studies have identified further polymorphic variants in the *ADRB2* gene including an Arg19Cys polymorphism in the 5′LC [37] and −20T/C, −47T/C, −367T/C, −468C/G, −654G/A, −1023G/A, −1343 and −1429T/A polymorphisms in the promoter region [38] (Fig. 2, Table 1). More recently, an extensive mutation screen of 5.3 kbp of the *ADRB2* region in both Caucasian (n = 419) and African American (n = 240) populations has been completed identifying 49 polymorphic variants [39]. In addition to the SNPs identified, a poly$(C)_{9–15}$ simple sequence length polymorphism was also identified in the 3′-region of the *ADRB2* gene [39], which may potentially have a role in determining mRNA transcript stability and/or translation (see earlier). It is noteworthy to comment that several of these polymorphisms have high frequencies (0.20–0.48) in the Caucasian and other populations and therefore may be expected to have a significant impact at the population level if functional (Table 1). Therefore the *ADRB2* gene is highly polymorphic and polymorphism may be predicted to influence β_2-adrenergic receptor expression and function (Table 1).

Functional Effect of β_2-Adrenergic Receptor Gene Polymorphism In Vitro

Investigations have predominantly focussed on determining the effect of coding region polymorphism on β_2-adrenergic receptor function using relevant *in vitro* outcome measures including surface receptor expression and cAMP production post β_2-adrenergic receptor agonist stimulation (Table 2). Early work utilised a recombinant Chinese Hamster Fibroblast cell line expressing the human β_2-adrenergic receptor variants [40, 41]. Cell lines expressing the position 16 and 27 β_2-adrenergic receptor variants demonstrated that these polymorphisms did not influence agonist binding or cAMP responses. However an enhanced agonist-mediated receptor down-regulation for the Gly16 variant and a resistance to down-regulation for the Glu27 variant were observed [41]. Using the same recombinant system, the Thr164Ile variant receptor produced reduced adenylate cyclase activity and agonist binding [40]. These data were confirmed using a variety of agonists and a 50% reduction in duration of action for the LABA salmeterol was identified [42]. The authors interpreted these findings by suggesting that salmeterol shows reduced exocite binding in the Ile164 variant

Table 1 Common genetic variation in the *ADRB2* gene

Gene position	dbSNP reference	Location	Alleles	Functional change	MAF (Caucasian)	MAF (African)	MAF (Asian)	Source
−3727	rs11746634	5′region	G/C	?	0.41	0.41	ND	PGA_CEPH, YORUB
−3594	rs11168067	5′region	G/A	?	0.41	0.48	ND	PGA_CEPH, YORUB
−3459	rs9325122	5′region	T/C	?	0.42	0.16	ND	[39][a]
−3291	rs11957351	5′region	T/C	?	0.43	0.43	ND	PGA_CEPH, YORUB
−3287	rs11948371	5′region	A/T	?	0.43	0.43	ND	PGA_CEPH, YORUB
−3251	rs11960649	5′region	C/A	?	0.43	0.43	ND	PGA_CEPH, YORUB
−3159	-	5′region	T/C	?	0.14	0.19	ND	[39][a]
−2633	rs1432622	5′region	C/T	?	0.46	0.44	0.45	HapMap-CEU/HCB/YRI
−2387	rs1432623	5′region	T/C	?	0.41	0.41	ND	PGA_CEPH, YORUB
−2274	rs11168068	5′region	T/C	?	0.44	0.45	ND	[39][a]
−2051	-	5′region	4C/5C	?	0	0.05	ND	[39][a]
−1818	rs17778257	5′region	A/T	?	0.33	0.21	0.36	HapMap-CEU/HCB/YRI
−1531	rs2400706	5′region	C/T	?	0.24	0.35	ND	PGA_CEPH, YORUB
−1429	rs2895795	5′region	T/A	?	0.24	0.35	ND	PGA_CEPH, YORUB
−1343	rs2400707	5′region	G/A	?	0.47	0.46	0.33	HapMap-CEU/HCB/YRI
−1023	rs2053044	5′region	G/A	?	0.41	0.38	0.26	HapMap-HCB/YRI. PGA_CEPH
−839	rs17108803	5′region	A/G	?	0	0	0.06	HapMap-CEU/HCB/YRI
−654	rs12654778	5′region	G/A	?	0.34	0.20	0.36	HapMap-CEU/HCB/YRI
−468	rs11168070	5′region	C/G	?	0.41	0.17	0.12	PGA_CEPH, HapMap-YRI/HCB
−406	rs17334228	5′region	C/T	?	0	0.08	ND	PGA_CEPH, YORUB
−367	rs11959427	5′region	T/C	?	0.41	0.08	ND	PGA_CEPH, YORUB
−262	rs33947624	5′region	G/A	?	0	0.06	ND	PGA_CEPH, YORUB
−47	rs1042711	LC	T/C	Cys 19 Arg	0.40	0.12	0.05	CEPH, HapMap-YRI/HCB
−20	rs1801704	LC	T/C	–	0.44	0.14	0.05	PGA_CEPH, HapMap-YRI/HCB

(continued)

Table 1 (continued)

Gene position	dbSNP reference	Location	Alleles	Functional change	MAF (Caucasian)	MAF (African)	MAF (Asian)	Source
46	rs1042713	ORF	G/A	Gly 16 Arg	0.47	0.53	0.47	CEPH, HapMap-YRI/HCB
79	rs1042714	ORF	C/G	Gln 27 Glu	0.50	0.18	0.12	CEPH, HapMap-YRI/HCB
100	-	ORF	A/G	Val 34 Met	<0.001	ND	ND	[36]
252	rs1042717	ORF	G/A	–	0.32	0.35	0.32	CEPH, HapMap-YRI/HCB
491	rs1800888	ORF	C/T	Thr 164 Ile	0.01	0	0	HapMap-CEPH, YRI, HCB
523	rs1042718	ORF	C/A	–	0.19	0.35	0.35	HapMap-CEPH, YRI, HCB
1053	rs1042719	ORF	G/C	–	0.30	0.35	0.42	HapMap-CEPH, YRI, HCB
1239	rs1042720	ORF	G/A	–	0.39	0.58	ND	PGA_CEPH, YORUB
1268	rs28763957	3′ region	C/G	?	0.002	0.05	ND	[39][a]
1269	-	3′ region	Poly $(C)_{9–15}$	?	See Text			[39][a]
1275	rs1042721	3′ region	C/G/A	?	0.29/0.001	0.54/0.06	ND	[39][a]
1278	rs41379548	3′ region	C/A	?	0.005	0.05	ND	[39][a]

Validated polymorphisms with a minor allele frequency (MAF) > 0.05 in the Caucasian or African populations are shown (NCBI *dbSNP*, [39]) (all non-synonymous variants are shown). PGA_CEPH (European, 46 alleles), PGA_YORUB (Sub Saharan African, 46 alleles), hapmap-CEU (European, 120 alleles), hapmap-HCB (Han Chinese, 90 alleles), hapmap-YRI (Sub Saharan African, 120 alleles), CEPH (European, 184 alleles)
LC leader cistron, *ORF* open reading frame, *ND* not determined, *dbSNP* NCBI single nucleotide database

[a] Allele frequencies determined in 419 Caucasians and 240 African Americans (included in MAF (African) in table) [39]

Table 2 The functional significance of *ADRB2* polymorphism in vitro

Polymorphism	System	Outcome measure	Inferences	References
Arg16Gly	Chinese	Radioligand binding affinity	No effect	[41]
	Hamster	Adenylyl cyclase activity	No effect	
	Fibroblasts	Agonist promoted down-regulation	Increased down-regulation for Gly16	
	Human ASM cells	Radioligand binding affinity	No effect	[66]
		Adenylyl cyclase activity	No effect	
		Agonist promoted down-regulation	Increased down-regulation for Gly16	[43]
	Human ASM cells	Magnetic twisting cytometry	Trend towards enhanced acute desensitisation with Gly16	
		Cyclic AMP responses to ISO		
	Human PBMC	Radioligand binding affinity	No effect	[44]
		Cyclic AMP responses to ISO	No effect	
Gln27Glu	Chinese	Radioligand binding affinity	No effect	[41]
	Hamster	Adenylyl cyclase activity	No effect	
	Fibroblasts	Agonist promoted down-regulation	Resistance to down-regulation for Glu27	
	Human ASM cells	Radioligand binding affinity	No effect	[66]
		Adenylyl cyclase activity	No effect	
		Agonist promoted down-regulation	Resistance to down-regulation for Glu27	
	Human ASM cells	Magnetic twisting cytometry	Greater acute and chronic desensitisation with Glu27 (in contrast to [66])	[43]
		Cyclic AMP responses to ISO		
	Human PBMC	Radioligand binding affinity	No effect	[44]
		Cyclic AMP responses to ISO	No effect	
Arg16Gly and Gln27Glu	Chinese	Radioligand binding affinity	No effect	[41]
	Hamster	Adenylyl cyclase activity	No effect	
	Fibroblasts	Agonist promoted down-regulation	Increased down-regulation for Gly16-Glu27	

(continued)

Table 2 (continued)

Polymorphism	System	Outcome measure	Inferences	References
Thr164Ile	Chinese Hamster Fibroblasts	Radioligand binding affinity Adenylyl cyclase activity Agonist induced receptor sequestration	Decreased affinity for all agonists tested with Ile164 Reduced basal and agonist induced activity with Ile164 Lower with Ile164	[40, 42]
Arg19Cys (5′LC)	COS-7 cells Human ASM cells Human ASM cells Human PBMC	Radioligand binding affinity Radioligand binding affinity Magnetic twisting cytometry Cyclic AMP responses to ISO Radioligand binding affinity Cyclic AMP responses to ISO	Reduced receptor expression with Arg19 Reduced receptor expression with Arg19 Greater acute and chronic desensitisation with Arg19 No effect No effect	[37] [43] [44]

ASM airway smooth muscle, *PBMC* peripheral blood mononuclear cells, *ISO* isoprenaline

receptor (Table 2, Fig. 1). Using a recombinant COS-7 system, it was shown that the Cys19Arg 5′LC resulted in reduced receptor expression in line with the previously identified role of the 5′LC in determining translation efficiency [37]. Most of the observations made in recombinant systems have now been replicated using genotyped primary cells including human ASM cells (Table 2). However, a greater acute and chronic desensitisation was observed for the Glu27 in contrast to the recombinant data [43] and polymorphism at the position 16, 27 and 5′LC 19 in PBMC appears to have little effect on β_2-adrenergic receptor expression and cAMP production post β_2-adrenergic receptor agonist [44]. The discrepancies observed for the recombinant expression systems versus primary cells are probably due to cell-specific effects and the investigation of SNPs in isolation rather than in haplotypes, i.e., combinations of SNPs spanning the gene (see later).

The *ADRB2* promoter is highly polymorphic with 22 SNPs with a minor allele frequency (MAF) >0.05 in the first 4 kbp (Table 1). Transcriptional regulation of β_2-adrenergic receptor expression has been shown to have key role in determining basal and inducible receptor expression (see earlier); therefore it may be predicted that promoter polymorphism may influence this regulation. Preliminary analyses of a 549 bp *ADRB2* promoter-luciferase construct containing the −468G, −367C, −47C, −20C (GCCC) haplotype versus a "wild-type" CTTT construct demonstrated a modest 17% reduction in luciferase activity for the mutant construct when transfected into COS-7 cells [38]. In agreement, a subsequent study demonstrated that the (−468G, −376C, −47C, −20T, GCCT) and CTCT haplotypes had a three-fold lower level of transcription compared to the CTTT haplotype in HEK293 cells [45]. The −47 polymorphism (T > C) generates the variant 5′LC (Cys19 > Arg) (Table 1, [37]) and the C allele resulted in reduced luciferase activity [45] in excellent agreement with data suggesting that this allele resulted in reduced receptor expression [37]. Overall these promoter analyses suggested that the expression level was dependent on the promoter polymorphism haplotypes present but that the −47 SNP had a prominent role in determining the level of expression [45]. Interestingly, it has been shown that the −47 Cys19Arg polymorphism also influences the ability of dexamethasone to reverse short-term and long-term desensitisation in primary Human ASM [46]. Recently, an alternative approach to understanding the potential functional role of *ADRB2* promoter polymorphism has been completed by synthesising short oligonucleotides encompassing the promoter SNP alleles and examining electrophoretic mobility shift profiles using ASM and epithelial cell (BEAS-2B) extracts [47]. In all, 19 polymorphisms were examined and allelic differences in nuclear extract binding suggested that many polymorphisms resulted in alterations in binding for both minor and major alleles. In ASM, 10 polymorphisms decreased, two increased and five showed no change in nuclear extract binding. Interestingly there was only ~50% concordance in results obtained from ASM and epithelial cells demonstrating that cell specific effects are of importance [47].

Functional Effect of β_2-Adrenergic Receptor Gene Polymorphism In Vivo

Due to the low frequency of the 34 and 164 coding region variants, most clinical studies of the effect of β_2-adrenergic receptor polymorphism on β_2-adrenergic receptor agonist responses have focussed on the position 16 and 27 polymorphisms (Table 2). In the vasculature, the Arg16 variant was associated with an enhanced isoprenaline-mediated desensitisation and enhanced isoprenaline-induced venodilation in healthy subjects [48]. The Glu27 variant was associated with increased agonist-mediated responsiveness [48]. The enhanced response to β_2-adrenergic receptor agonist in Arg16 carriers has also been observed in respiratory disease. In a longitudinal study of 269 unselected children, the short-acting β-agonist salbutamol (180 µg) gave an enhanced acute response (>15.3% increase in predicted FEV_1 considered a positive response) in individuals carrying the Arg16 genotype [49]. Individuals homozygous for Arg16 were 5.3 times more likely to show a positive response vs. homozygous Gly16 individuals. Heterozygous Gly/Arg individuals were 2.3 times more likely to show a positive response vs. homozygous Gly16 individuals [49]. The Arg16 variant was found to be in 97.8% linkage disequilibrium with the Gln27 polymorphism (inherited together on the same chromosome) prohibiting the identification of the true causative polymorphism [49]. Multiple subsequent studies have been completed correlating patient response to β_2-adrenergic receptor agonists based on position 16 and 27 β_2-adrenergic receptor polymorphism genotypes [50–56]. Limited conclusions have been made due to the heterogeneity of subjects studied (e.g., asthma severity), the small numbers used in some studies, which were analysed retrospectively, the use of different β_2-adrenergic receptor agonists (SABA and LABA) and the potential confounding effect of examining SNPs in isolation. In a prospective study of mild asthma subjects recruited specifically for position 16 genotypes (Gly/Gly, $n = 41$; Arg/Arg, $n = 37$), the effect of genotype on clinical outcomes (primary outcome morning peak expiratory flow rate (PEFR)) following regular scheduled or as needed salbutamol usage was assessed [57]. During the run-in period, when salbutamol was used at a minimal level, Arg/Arg had a significant increase in PEFR (23 l/min) whereas the Gly/Gly did not (2 l/min). During treatment, subjects with the Gly/Gly genotype had an increase in PEFR following regular scheduled salbutamol versus placebo (14 l/min). Carriers of the Arg/Arg genotype had a lower PEFR during treatment versus the placebo arm of the study when salbutamol use was minimal (−10 l/min). These data suggested that carriers of Arg/Arg had increased response (PEFR) to low-level salbutamol during the run-in period, but regular salbutamol is detrimental to asthma control and led to a decrease in PEFR. The use of regular salbutamol led to a worsening of all clinical endpoints including FEV_1 symptoms and relief medication usage in the Arg/Arg group [57]. Overall, the Arg16 variant has been associated with an enhanced acute response to β_2-adrenergic receptor agonists [48,49,53]; decline of asthma control following prolonged use of agonist [54,55,57] and a subsensitivity of

response for bronchoprotection [56]. These findings remain controversial as other studies have not shown a genotype effect on asthma control or acute responses [51,52]**.** The Gly16 variant has been shown to increase agonist-mediated desensitisation [50] and down-regulation [58] compared to the Arg16 variant. Data examining the position 27 polymorphism have shown no effect on acute responses to salbutamol [49] or asthma control [51] or formoterol-mediated protection from methacholine-induced bronchconstriction [52]. While most studies have examined the pharmacogenetic effect of β_2-adrenergic receptor polymorphism on SABAs, more recently the effect on the LABA salmeterol has been explored [59]. This study used data from two trials. One trial examined the effect of taking LABA with inhaled corticosteroids (ICS) and the second trial the effect without ICS. In both datasets, individuals with the Arg/Arg polymorphism had a reduced therapeutic response compared to Gly/Gly individuals once salmeterol treatment was initiated, e.g., in the absence of ICS the PEFR was 51.4 l/min lower in the Arg/Arg (n = 13) vs. Gly/Gly (n = 13) groups [59]. These data confirm that regular use of β_2-adrenergic receptor agonist (SABA and LABA) in Arg/Arg individuals may lead to reduced clinical benefit and potentially adverse effects in asthma control.

ADRB2 Linkage Disequilibrium and Allele Frequencies in Different Ethnic Groups

While analyses *in vitro* and *in vivo* using single SNP markers, e.g., Gly16Arg, have been informative, the polymorphic nature of the *ADRB2* gene prohibits conclusions regarding the identification of the functional/causative SNPs as SNPs can occur on the same haplotype background. The human HapMap project has been established in order to determine the relationship between polymorphic variations spanning the genome and can be used as a tool to examine the relationship between SNPs spanning a specific gene, i.e., determine the extent to which these alleles are inherited together. Figure 3 shows a linkage disequilibrium (LD) plot of the *ADRB2* region on chromosome 5 in the Caucasian population. These data illustrate that there is a high degree of LD spanning the ADRB2 gene and in particular the coding and 5′ regions (high red colour (D'/LOD)). Therefore it may be expected that with the Gly16 > Arg variant, multiple promoter polymorphisms will also be inherited, suggesting that it may be a combination of polymorphisms including the coding region and promoter that ultimately lead to the *in vivo* effects described previously. This high LD pattern is apparent by simply examining the allele frequency of the *ADRB2* promoter and coding region SNPs. It can be seen that predominantly in the Caucasian population, a MAF of ~0.4–0.45 is observed (Table 1). In order to directly address this issue, Drysdale and colleagues completed a study of β-agonist responses stratified based on patient *ADRB2* haplotypes [60]. Twelve haplotypes were identified using 13 SNP markers in the four main ethnic groups. There were

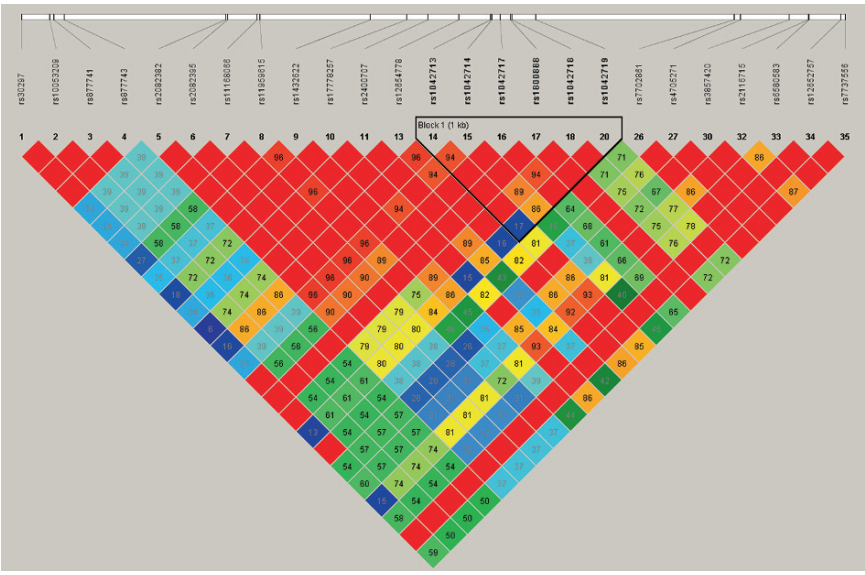

Fig. 3 Linkage disequilibrium plot of the *ADRB2* region in the Caucasian population. The figure represents chromosome 5:148,175,861–148,194,864 and was generated using Haploview Software (HapMap, Build 36). The intensity of shading represents D'/LOD (a measure of linkage disequilibrium). The location of the *ADRB2* coding region is shown (Block 1) including the position of non-synonymous polymorphism; rs1042713 (Gly16Arg), rs1042714 (Gln27Glu) and rs1800888 (Thr164Ile)

clear effects on acute responses (improvement in FEV_1) to salbutamol based on haplotype, e.g., haplotype 2/2 had a greater acute response compared to haplotype 4/4, although the number of individuals studied was small [60]. These two haplotypes differ at eight of the 13 SNPs analysed, suggesting that identification of the causative mechanism for these altered responses will be a challenge. Interestingly, in PBMC, no effect of *ADRB2* haplotypes (based on four SNPs, one promoter and three coding region) was apparent on basal β_2 adrenergic receptor expression or coupling [44]. Studies examining the effect of promoter polymorphism have also used haplotype analyses (see earlier). Therefore, there is extensive evidence that polymorphism within the *ADRB2* gene influences β_2 adrenergic receptor responses, however the precise molecular basis of this phenomenon remains to be resolved.

While the majority of clinical studies to date examining the effect of β_2 adrenergic receptor polymorphism on clinical responses to β_2 adrenergic receptor agonists have used Caucasian subjects, it is increasingly apparent that key *ADRB2* polymorphism allele (and haplotype) frequencies differ significantly between ethnic groups and therefore these groups may be expected to have different pharmacogenetic profiles. Allele frequencies for the common polymorphisms spanning the *ADRB2* gene are shown for representative Caucasian, African and Asian populations (Table 1). These data illustrate that for several known functional polymorphisms, e.g., 5′LC

Cys19Arg, there are significant differences in allele frequencies, i.e., 0.40, 0.12 and 0.05 for Caucasian, African and Asian populations, respectively (Table 1). Recently, the outcomes of the Salmeterol Multicentre Asthma Research Trial (SMART) have been published, which involved the assessment of 26,355 subjects [61]. This study of salmeterol use for 28 weeks was a multi-centre, randomized, double blind, parallel group, placebo-controlled design at 6,163 sites in the United States [61]. Interim analyses demonstrated that there was a significant increase in respiratory related deaths (24 vs. 11, relative risk (RR) 2.16 (95% confidence interval (CI) 1.06 to 4.41)) and asthma-related deaths (13 vs. 3, RR 4.37 (95% CI 1.25 to 15.34)) in the salmeterol versus placebo group. Of particular relevance was the finding that adverse effects were disproportionately high in the African American population, which at this time remains to be resolved but may at least in part be due to genetic factors.

Disease Association Studies

Polymorphism within the *ADRB2* gene has been investigated extensively as disease severity and/or susceptibility markers. Diseases examined include asthma, chronic obstructive pulmonary disorder (COPD), obesity, atherosclerosis, Graves disease and hypertension with variable evidence for and against association. With respect to asthma susceptibility, there has now been two meta-analyses examining the contribution of the Gly16Arg and Gln27Glu polymorphism to asthma relative risk. In a meta-analysis including data from 28 previously published studies, it was shown that neither the β_2 adrenergic receptor position 16 or 27 polymorphism contributes to asthma susceptibility per se, however there was an association between the Gly16 variant and nocturnal asthma Odds Ratio (OR 2.20) and with asthma severity (OR 1.42) [62]. No association was seen for the Glu27 allele and neither polymorphism showed an association with Bronchial Hyper Responsiveness BHR [62]. Interestingly, in an alternative meta-analysis, it was shown that the Gly16 polymorphism was protective for asthma in children (OR 0.53) and the Glu/Glu genotype had a decreased risk of asthma (OR 0.60) [63]. We have recently completed a genetic association study of the key *ADRB2* polymorphisms (including Gly16Arg, Gln27Glu and Thr164Ile) in asthma using approximately 8,000 subjects from the 1958 birth cohort [64]. These data suggested that it is unlikely that *ADRB2* polymorphisms increase the risk of developing asthma; however, the Arg16 and Glu27 alleles may influence disease progression [64].

Summary

The importance of the β_2 adrenergic receptor as a drug target for the treatment of respiratory conditions associated with airflow limitation and obstruction is likely to remain significant. Indeed a new generation of once daily, LABAs are currently

in development for the treatment of asthma and COPD, e.g., Indacaterol [65]. In this chapter, we have described the complexity of molecular mechanisms that regulate β_2 adrenergic receptor expression and function and highlighted the many levels of regulation including transcriptional, translational and post-translational. Similarly, the β_2 adrenergic receptor gene is highly polymorphic and data so far suggest that these polymorphisms add an additional level of regulation to these already complex molecular processes. A greater understanding of the molecular and genetic mechanisms regulating the *ADRB2* locus will lead to the design of safer, more effective therapies that target the β_2 adrenergic receptor for the treatment of respiratory disease.

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Genetics of Hypersensitivity

John W. Steinke

Introduction

Over 100 years have passed since it was first recognized that asthma and allergic diseases have a genetic component. The genetic involvement was suggested from observations that allergic subjects had a higher incidence of positive family histories of disease when compared to families without disease [1, 2]. More recent studies have shown that a child has a 33% chance of developing allergies if one parent has allergies and a 70% chance if both parents are allergic. Evidence for linkage to asthma is not as robust, as there is only a 15% chance of a child developing asthma if one parent has the disease. While the concept of allergic disorders having a familial predisposition has been recognized, defining the genetic mechanism has proven more challenging. It is now accepted that allergies and asthma are not only complex genetic disorders, defined as disorders that have numerous contributing genes, each having variable degrees of involvement in any given individual, but also multi-factorial in origin, involving interaction of genetic and environmental factors. Environmental exposures include allergen exposure, second hand cigarette smoke, pollutants, low birth weight and infectious agents. This review will first discuss gene association studies and explore some of the problems associated with them. The focus will then shift towards the future of genetic studies in asthma and allergy including pharmacogenetics, gene-environmental interactions, gene–gene interactions and epigenetics.

Genome-Wide Screens, Association Studies and Candidate Genes

The classic genetic approach to identifying disease-causing genes involves linkage studies followed by positional cloning. Positional cloning makes use of the presence of highly polymorphic genetic markers whose position on a chromosome is

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_13, © Springer 2009

known. Markers close to the disease gene will be statistically co-inherited with the disease when multiple families are analyzed. This process is labor intensive even with the utilization of today's molecular biology techniques. Completion of the human genome project has allowed access to the map of the human genome in an area where linkage has been established and a list of the genes localized to that chromosomal region obtained. The linkage analysis is repeated to determine whether mutations either in one of the genes in the adjacent regions contribute to the development of allergies and asthma. Often candidate genes can be identified within a linkage region; however, the function of many of the genes identified through the human genome project is not known. It is likely that these unknown genes may provide insight into the asthmatic and atopic disease processes, as they will focus attention on pathways not previously implicated in disease progression.

Over the past decade, more than 18 genome-wide screens utilizing a variety of intermediate phenotypes have been published [3–5]. One of the earliest genome-wide searches for asthma genes was performed using linkage analyses on a very limited number of polymorphic DNA markers to allergen-specific IgE and high total serum IgE. Linkage to chromosome 11q was found in association with maternal – but not paternal – phenotype [6]. Though this study did not directly demonstrate this, analysis of 11q showed that this marker mapped close to the gene for the β chain of the high affinity IgE receptor. While the α and γ chains of the high affinity IgE receptor are sufficient for sending signals to the cell for activation, the β chain acts as an amplification mechanism for this signaling pathway and permits mast cell activation in the presence of fewer cross-linked IgE molecules. These authors have suggested that base exchanges in the cytoplasmic region of the β-chain may be the location of the disease-causing mutations. Significance of the linkage to chromosome 11 has been controversial, as it has been replicated in some studies, while several other groups have not been able to confirm this linkage. The National Heart, Lung, and Blood Institute funded a multi-center Collaborative Study on the Genetics of Asthma (CSGA). Their initial genome screen involved three racial groups (African-Americans, Caucasians, and Hispanics) [7] and in follow-up studies, this group has reported information on individuals of Hutterite ancestry [4]. Together, these studies uncovered ~15 separate promising linkages, including some in previously unsuspected regions of the human genome. Several of these linkages have been confirmed by other investigators in separate populations. These include a locus on chromosome 2 near the IL-1 cluster that contains the genes for CD28 and CTLA-4 and the major histocompatibility complex on chromosome 6. Not surprisingly, the chromosome 5 cytokine gene cluster that includes the genes for IL-3, IL-4, IL-5, IL-9, IL-13, GM-CSF and leukotriene C4 synthase has been linked to allergies and asthma. Genome-wide searches have also supported the presence of potential allergy and asthma genes on chromosome 12 in association with interferon-γ and stat-6.

To date, six genes have been identified using positional cloning. To illustrate the partial success of these studies we will consider the identification of ADAM33. A genome-wide scan, performed on 460 families, identified a relatively strong linkage of asthma and bronchial hyperresponsiveness to markers on chromosome 20p13. A subsequent survey of 135 polymorphisms in 23 genes within this region identified

the ADAM33 gene as being significantly associated with asthma using both association and transmission disequilibrium analyses [8]. This linkage was initially confirmed in two separate genome-screens in UK and US outbred populations [9, 10] and a polymorphism within the gene has been associated specifically with an accelerated decline in lung function [11]. Now more than 10 separate studies have confirmed linkage of this gene to asthma though, as common in genetic studies, a few have failed to replicate this association. ADAM33 is a protease with multiple isoforms that is active at the cell surface and is part of the matrix metalloproteinase family. Its role in asthma is speculative but expression of this protein on airway fibroblasts, myofibroblasts and smooth muscle cells may alter the response of lymphocytes and inflammatory cells by proteolytic release of cytokines and chemokines from precursor molecules that influence cell migration. It might also alter growth factor expression and remodeling responses in the basement membrane to damaged epithelium and smooth muscle of the airway [12].

Whole genome screens are difficult to perform and can be difficult to interpret. As a result, many have adopted the approach of using candidate gene studies to look for the presence of association with asthma and atopy. Candidate genes include the numerous biochemical products known to be abnormally regulated or otherwise function inappropriately that lead to allergies and asthma or influence the severity of disease. Candidate gene studies are performed by aggressively studying a narrow region of the genome with numerous polymorphic markers which saturate the region of interest in a fashion that would not be practical with a genome wide scan [13].

Two examples of genes will be presented that have been replicated in many studies and represent the best examples of well-characterized genes involved in asthma. CD14 is a receptor that has specificity for lipopolysaccharides and other bacterial wall-derived components and it is constitutively expressed on the surface of monocytes, macrophages and neutrophils. CD14 can also exist as a soluble receptor via direct secretion or by enzymatic cleavage of the membrane anchored CD14. Engagement of CD14 is associated with strong IL-12 responses in antigen-presenting cells and is a necessary signal in the formation of Th1 cells from naïve T cells [14]. One hypothesis is that changes in CD14 levels could change the ratio of Th1- to Th2-type cells, altering IgE levels. A C-to-T transition at position –260 (initially incorrectly labeled as position –159) of the CD14 promoter in relation to the transcription start site was found. Individuals homozygous for the T allele were found to have higher sCD14 in the serum and lower total IgE levels in skin-prick test-positive children [15]. The functional role of this polymorphism was further investigated. Members of the Sp transcription factor family bound to the promoter and the affinity of binding was lower for promoters containing the T allele. Using reporter assays, it was found that transcription from promoters containing the T allele was higher than constructs containing the C allele, and that was dependent on the ratio of Sp3 to Sp1 and Sp2 [16]. The IL-13 locus has been one of the most-replicated candidate genes in association with asthma and atopy, with more than 70 reported SNPs in the gene and promoter region [17]. IL-13 is homologous to IL-4 and shares many of the same biological activities on mononuclear phagocytic cells, endothelial cells, epithelial cells and B cells, but due to differential expression of the IL-13 receptors, IL-13 has unique properties distinct from IL-4. Like

IL-4, IL-13 can induce the IgE isotype switch and VCAM-1 expression. IL-13 can induce eosinophilic inflammation, and may be uniquely important in inducing mucus cell hypersecretion, airway fibrosis and airway hyperreactivity (reviewed in [18]). Two SNPs have consistently shown associations with disease in multiple studies: a C-to-T exchange at position –1112 of the promoter and a G-to-A at position + 2044 of the gene. In a Dutch family study, the –1112T allele was associated with asthma, bronchial hyperresponsiveness and skin-test reactivity [19]. Functional studies demonstrated that the C allele at –1112 displayed 30% higher transcriptional activity as compared to the T allele in nonpolarized CD4 + T cells. When primary CD4 + Th2 lymphocytes were examined promoters with the T allele had higher activity. Examination at the molecular level, found that the T allele created a YY1 transcription factor binding site that may function to relieve repression of the normal STAT6 activity on this promoter [20]. Several studies have shown an association of the + 2044A allele and increased IgE levels [21, 22]. The polymorphism results in a non-conservative replacement of the basic amino acid arginine (Arg)130 with a neutral amino acid glutamine (Gln) in the IL-13 protein. Using recombinant proteins, the two forms of the IL-13 protein did not differ in binding affinity to the IL-13Rα1 type receptor, but the Gln130 protein bound to the IL-13Rα2 with lower affinity and was more stable in the extracellular environment [23]. The increased stability may result in higher levels of circulating IL-13 levels in individuals with the Gln130 protein, leading to the observed associations of IL-13 and asthma and atopy.

Pharmacogenetics

Pharmacogenetics is defined as the study of variation in drug response or efficacy due, in part, to genetic differences between individuals. It is hoped that genetic variations in drug target genes can be used to predict clinical responsiveness to treatment or the risk of adverse drug reactions in patients before treatment is started. Data from these types of studies are already being utilized for azathioprine therapy and tumor profiling in oncology. Pharmacogenetics represents the first area where genetic information concerning the allergic response will likely be used in the clinical setting. However, despite the promise, few pharmacogenetic studies have actually been performed in allergic disease. This is due to many reasons including an unwillingness on the part of pharmaceutical companies to pursue such studies, a lack of funding from the government and what is perceived as non-life-threatening responses to the current drugs. All new drug proposals should be required to include a section on pharmacogenetic analysis.

One of the first pharmacogenetic studies was performed by Malmstrom et al. who examined the response of individuals with asthma to the inhaled corticosteroid beclomethasone or the leukotriene modifier montelukast [24]. A wide spectrum of inter-individual responses to each drug was observed as measured by changes in FEV_1 from baseline. Of the patients receiving beclomethasone, 22% failed to show improvement in FEV_1, while 34% receiving montelukast failed to show

improvements in FEV_1 [24]. Recent studies have offered some insight on the variable responses to inhaled corticosteroids. In three independent caucasian asthmatic clinical trial populations (each with more than 300 participants), variation in the corticotropin-releasing hormone receptor 1 (CRHR1) gene was associated with increased response to inhaled corticosteroids [25]. Individuals homozygous for the GAT haplotype displayed a doubling to quadrupling of longitudinal FEV_1 response following treatment with corticosteroids as compared to individuals without the GAT haplotype [26]. An additive effect of the haplotype was observed as individuals heterozygous showed intermediate improvement compared to those homozygous with or without the GAT haplotype.

There have been numerous studies examining genes that are involved in arachidonic acid metabolism and cysteinyl leukotriene production that may explain the variation in response to leukotriene modifiers. Polymorphisms have been reported in the promoters of the 5-lipoxygenase (5-LO) and leukotriene C4 synthase (LTC_4S) genes and the coding regions of the cysteinyl leukotriene receptor type (CysLT) 1 and 2 genes. In the 5-LO promoter, there are variations in the number of binding sites for the transcription factors Sp1 and Egr-1 [27, 28]. The most common and active allele consists of five tandem Sp1-binding motifs with variants having deletions or additions to the number of Sp1-binding motifs [27]. In a controlled trial of the 5-LO inhibitor zileuton, patients with at least one allele containing five Sp1-binding motifs, had an 18.8% improvement in FEV_1 compared to a 1.1% decline in FEV_1 in individuals in whom neither allele contained the five repeats [29]. Despite the functional effect and changes in clinical responsiveness to a leukotriene modifier, the polymorphisms are present in 5% of asthmatic patients and can only account for a small proportion of the variability in response to leukotriene modifier therapy. However, there is renewed interest in this polymorphism with the reintroduction of zileuton to the market for treatment of aspirin-sensitive asthmatics. An A-to-C transversion at position –444 within the LTC_4S gene has been linked to aspirin-sensitive asthma in several studies. Unlike the rare 5-LO polymorphisms, the LTC_4S C allele is found at a frequency of 23% in normal populations, up to 44% in aspirin-intolerant populations and 31% in a population of individuals with chronic hyperplastic eosinophilic sinusitis [30, 31]. Stimulation of eosinophils from carriers of the C allele produced almost three times the levels of LTC_4 as compared to eosinophils from individuals without the C allele [32]. It has also been shown that carriers of the C allele have decreased basal FEV_1 levels and using the transmission disequilibrium test, an association between the C allele and bronchial hyperreactivity to methacholine was observed [33]. From this, one could hypothesize that carriers of the C allele would respond well to leukotriene modifier therapy. Support for this concept came from a small study in which asthmatic patients were given zafirlukast for two weeks. Those who had a C allele displayed a 9% improvement in FEV_1, while those without a C allele had a 12% decrease in FEV_1 [32]. Due to its size, this study needs to be replicated in a larger population. To date no pharmacogentic study has been performed examining polymorphisms in the CysLT receptors. Only associations with asthma and atopy in several distinct populations have been suggested. Given that most of the leukotriene modifier therapy targets the CysLT1 receptor, this represents a likely candidate for pharmacogentic differences.

The best-studied pharmacogenetic response in allergy has been the response of airways to β-agonists. At least 49 genetic variations within the β2-adrenergic receptor gene and surrounding DNA have been identified and grouped into haplotypes [34]. While none of these amino acid substitutions conclusively have been linked to the presence of asthma, they have been associated with response to β-agonists in multiple studies. Retrospective studies suggested that the presence of arginine at amino acid residue 16 (Arg16) was associated with the presence of corticosteroid-dependence, nocturnal symptoms, and loss of bronchodilator responsiveness with long-term administration of albuterol [35]. When studied prospectively, individuals homozygous for Arg16 had lower peak expiratory flow rates and lower FEV_1 when treated with albuterol as compared to those homozygous for glycine at this position [36]. This result has been replicated in a Korean population [37]. In vitro, the Arg16 variant has enhanced agonist-promoted downregulation of receptor expression [38]. It should be noted that in the African-American population, there is an increase in the number of individuals homozygous for Arg16 and this may explain the reported increased morbidity associated with long-term administration of β-agonists in this population [39]. Additionally, polymorphisms in one of the downstream effector molecules, adenylyl cyclase type 9, for the β2-adrenergic receptor have been implicated in albuterol responsiveness in the context of combined inhaled corticosteroid use. Individuals with a methionine at position 772 displayed improved lung function in response to albuterol if they were also on budesonide as compared to individuals with an isoleucine at position 772 [40]. Together these studies suggest that the response to β-agonists is complex and likely involves the interaction of multiple haplotypes on different genes.

Genes and the Environment

As mentioned earlier, the environmental influence on the development of asthma accounts for approximately 50% of the risk. Some of the environmental factors that might contribute to the underlying genetic susceptibilities include endotoxin exposure, diesel exposure, tobacco smoke, inhalant aeroallergens, diet, exposure to viral infections and in utero factors during pregnancy. Incorporation of these risk factors into genetic studies is allowing the interplay between the gene and environment to be elucidated.

Many explanations have been proposed to account for the dramatic increase in asthma that has been observed over the past 20–30 years. One that has gained favor recently is termed the “hygiene hypothesis”. This states that the reduced exposure to childhood infections, or other immune stimuli such as farming and endotoxin exposure, may explain the increased prevalence of allergic diseases in industrialized countries [41]. One component of the hygiene hypothesis is that decreased endotoxin exposure and reduced innate immune responses drive the increased sensitivity to allergens. Endotoxin functions through engagement of the toll-like receptor (TLR) 4 and the costimulatory molecule CD14. As discussed above, polymorphisms have been found in the CD14 gene that lead to a functional change in the expression of the gene [15] and recently, associations of polymorphisms and asthma have been noted

in the TLR4 gene that alter response to endotoxin [42]. Studies examining the CD14 C-260T polymorphism have provided insight into a partial explanation of this gene-environmental interaction. Individuals homozygous for the T allele are protected against the development of asthma in houses with low endotoxin exposure; however, in houses with high endotoxin exposure this genotype was associated with a higher risk for asthma [43]. A similar observation has been found in the Childhood Onset of Asthma Study at three loci: NOS3, FCERB1 and IL4RA [44]. The influence of each gene on the development of asthma was dependent upon a child's daycare attendance. A given genotype in the daycare setting was associated with the highest cytokine response and protection from development of asthma while the same genotype in a child not attending daycare was associated with the lowest cytokine response and protection from development of asthma. The presence of polymorphisms in other TLRs (including TLR 2, 3, 7 and 9) are also likely to have a role in causing (or protecting against) allergic sensitization in response to environmental stimuli. Supporting this is the finding that TLR2 has been identified as a major asthma gene in children of European farmers [45]. TLR2 is a ligand for peptidoglycans and lipoproteins. Associations of SNPs in this receptor are not observed in non-farmers since presumably they do not have the same level of exposures. As industrialization has increased, exposure and infections due to helmiths, tuberculosis and others have decreased.

A major environmental change that has occurred in the past 50 years that might influence the expression of genetic polymorphisms is the increase in airborne diesel particulate matter due to motorized vehicles. These particles contain aryl-hydrocarbons that act on many pathways including the ability to increase production of reactive oxygen molecules. One recent study has shown that a variant of the glutathione-S-transferase (GST) gene modifies the adjuvant effect of diesel particles on allergic inflammation [46]. GSTs can metabolize reactive oxygen species and detoxify xenobiotics present in diesel exhaust particles. Mutations in GSTs that inhibit this function could lead to increased inflammation and response to benign substances such as aeroallergens. These effects are observed in areas where concentrations of airborne diesel particulate matter are high, such as in large cities or areas within 150 m of a freeway [47]. This complex interaction was recently demonstrated in a study from the Children's Health Survey that found a protective effect from developing asthma if individuals were homozygous for the G allele at position –308 in the TNF promoter [48]. The protective effect was higher in communities with low ozone levels compared with communities with high ozone exposure. There was a further reduction in the protective effect of the GG –308 genotype in high ozone communities if individuals also carried either the GSTM1 null or GSTP1 Ile/Ile genotype.

Gene–Gene Interactions Studies

In addition to the environment influencing the expression of genes, it is possible for the expression of one gene to influence the expression of another gene. With studies enrolling larger numbers of subjects and the relative ease of genotyping large

numbers of genes, it has become possible to use statistical modeling to look for these interactions. All of the potential interactions will not be examined, but a few examples will be illustrated. From genetic association and functional studies, IL-13 has consistently been found to be associated with asthma and atopy. It is not surprising that an interaction between IL-13 and other genes has also been found to confer an increased risk for asthma. In a Dutch proband, it was confirmed that polymorphisms in the IL-4 receptor alpha gene, including the S478P change, associated with total serum IgE levels. Additionally, the IL-13 –1112 C/T promoter variant previously shown to be associated with bronchial hyperresponsiveness displayed a gene–gene interaction with the IL-4 receptor alpha S478P allele conveying a fivefold increased risk for developing asthma [49]. A different allele in the IL-4 receptor alpha gene (R130Q) and IL-13 gene (I50V) were found to interact and give an increased risk for asthma [50]. Whether the alleles on each gene in this study were part of a haplotype that included the alleles from the previous study is unclear. A haplotype containing the IL-13 –1112T allele in combination with an IL-13 receptor alpha + 2044A allele was associated with increased total IgE in atopic children [51]. As discussed above, another gene that has been replicated in numerous studies showing a link to asthma is the CD14 gene. In an asthmatic pedigree of African Caribbean individuals, an allele in the CD14 promoter region in association with a marker in the acyloxyacyl hydroxylase gene conferred an increased risk of asthma, IgE and cytokine levels in individuals who carried these alleles [52].

Epigenetic Mechanisms/Studies

Epigenetics can be broadly defined as changes in gene expression patterns that can be inherited and are independent of changes in the DNA sequence, but instead rely on post-translational modifications in the DNA and histone proteins. Epigenetics will influence not only the patterns of genes expressed in the progeny cells but also provides a mechanism for the selective expression of a specific allele from one chromosome while the allele present on the partner chromosome remains silenced. Alterations of the DNA can occur by adding methyl-groups to clusters of CpG residues. Modification of histone proteins can occur by acetylation, phosphorylation or methylation (reviewed in [53]). Together these different types of histone modifications comprise what Allis [54] has termed the “histone code”. This code may represent a mechanism that alters chromatin structure such that differences in the transcriptional on–off state or cell proliferation/differentiation state can be inherited in daughter cells.

Few epigenetic studies have been performed in asthma and allergy. The best-described example involves the differentiation of naïve CD4 + T lymphocytes into functional Th1 or Th2 cells. The cytokine genes in naïve T cells are contained within a condensed chromatin structure with extensive methylation. The first stage of Th lymphocyte differentiation involves chromatin and DNA remodeling into a relaxed state in which Th1- or Th2-associated cytokine genes may be readily transcribed. The factor critical for Th2-specific differentiation is GATA-3 [55], whereas T-bet is essential

for Th1-like lymphocyte differentiation [56]. For Th2 lymphocyte differentiation, the combination of antigen stimulation and engagement of the IL-4 receptor results in Stat6 activation [57]. Stat6 is responsible for specific demethylation of DNA around the chromosome 5q cytokine gene cluster, which includes the genes for IL-3, IL-4, IL-5, IL-9, IL-13, and GM-CSF. Stat6 activation also leads to elevation of GATA-3 transcription. Once translated, GATA-3 protein both stabilizes its own expression and leads to activation of the 5q gene cluster [58]. This ability of GATA-3 to stimulate and thus perpetuate its own transcription is one mechanism for the largely irreversible nature of differentiation of Th2-like lymphocytes. Th1 differentiation results from engagement of the antigen and IL-12 receptors. T-bet expression and the subsequent demethylation of the IFN-γ locus on chromosome 12 are controlled by Stat1 [59]. Histones in the cytokine loci for Th1 cells are unacetylated in naïve T cells. In addition to demethylation of DNA, when signals are transmitted through the T cell receptor, histones H3 and H4 become rapidly acetylated. This acetylation is maintained by cytokine signaling and T-bet expression [60]. Similar to GATA-3 on Th2 lymphocytes, T-bet expression keeps the chromosome 12 cytokine locus in an open configuration accessible to the transcription machinery. In this example, the progenies are subsequent generations of T cells within an individual. However this does not explain the familial link to asthma and rise in incidence of the disease. Evidence for this type of phenomena has been provided by a study from Li et al. on the transgenerational link of smoking and asthma. It was found that there was an increased risk (odds ratio 2.1) of an unexposed child developing asthma if the grandmother smoked during the mother's pregnancy [61]. They hypothesize that tobacco products alter the DNA methylation patterns in fetal oocytes and the changes in immune function and detoxification can be passed on to subsequent generations, increasing the risk for asthma. While interesting, much work is needed to verify this concept.

Conclusions

With a worldwide increase in the prevalence of asthma and allergic diseases and the soaring healthcare cost associated with treating affected individuals, further understanding of the factors associated with this disease is needed. Our initial attempts to characterize the genetic components of the disease relied on simple models of gene transmission. Despite a large amount of time and money, these studies have failed to provide answers in terms of cause of disease and treatment plans based on this genetic information. New models combining large populations and complex statistical analysis are beginning to unravel the subtle complexities of this disorder. Additionally, while often ignored, the influence of genes on response to treatment is now recognized as being of major importance and likely to be controlled by a few genes. We may now be able to see in the near future the promise of genetics delivered to the clinical setting with the ability to analyze a patient's genetic repertoire and tailor a specific treatment regime for each patient.

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Functional Genomics of Allergic Diseases

Donata Vercelli

Introduction

Allergic inflammation and its most common phenotypes (asthma, allergy and atopic dermatitis) are one of the most eloquent examples of human complex diseases, disorders caused by a constellation of genetic hits that are individually mild but lead to major phenotypic effects when they act on multiple steps along a mechanistic pathway. The literature is rich in association and linkage studies pointing to candidate genes that might act as critical determinants of allergy/asthma susceptibility. However, the abundance of single nucleotide polymorphisms (SNPs) in the human genome, and the complex patterns of linkage disequilibrium (LD) found at most genetic loci, prevent the tools of genetic epidemiology from deciphering the contribution of individual polymorphisms to increased disease risk. As a result, the mechanisms underlying the associations between patterns of genetic variation and disease phenotypes are in most cases unclear. Functional genomics studies provide a powerful tool to understand how genetic factors affect the pathogenesis of, and the susceptibility to, complex diseases such as allergic inflammation.

Functional genomics is still in its infancy. Indeed, as yet there is no universally accepted approach to defining the impact of genetic variants on gene expression and/or function. Interestingly, the more we experiment, the more we realize how subtle, even devious, the effects of genetic variants can be, and how lightly we must tread on the uncharted ground of functional genomics. Here we shall briefly review some of the results our group recently obtained studying the functional genomics of interleukin (IL)13, a major candidate gene for allergic inflammation [1, 2], and we shall discuss how our findings have contributed to advancing the field of functional genomics.

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_14, © Springer 2009

IL13 Association Studies

Genetic epidemiology provides the questions functional genomics needs to answer. *IL13* is no exception. For some years now, we have known the *IL13* locus on chromosome 5q31 contains a block of common SNPs that spans the third intron (+1923CT), the fourth exon (+2044GA) and the 3′ untranslated region (+2525GA, +2580CA and +2749CT) [3]. Two SNPs in the promoter (-1512AC and -1112CT) are also in strong, albeit not complete, LD with the downstream polymorphisms. In view of the central role IL-13 is known to play in the pathogenesis of allergic inflammation [1, 2], it is not surprising robust associations were found between genetic variation in *IL13* and allergic/asthmatic phenotypes. In fact, *IL13* is one of the most replicated genes in the asthma/allergy literature [4].

Association studies have focused mostly on *IL13* + 2044GA in the coding region and *IL13*-1112CT in the promoter. *IL13* + 2044GA is strongly associated with increased total serum IgE [3, 5–9], asthma [10], atopy [11], atopic dermatitis [5, 11, 12] and a grouped phenotype including eosinophilia, IgE and positive skin tests [13]. *IL13*-1112CT (also known as -1055 and -1111) is associated with asthma, bronchial hyperresponsiveness (BHR) and skin test responsiveness [14, 15], total IgE [9, 16], sensitization to food and outdoor allergens [16, 17] and latex allergy [18]. Most of these associations were found in Caucasian and/or Asian populations. More recently, *IL13*-1112CT was found to be associated with asthma/atopy in a small African American population sample [19]. Interestingly, a significant gene–gene interaction was detected between an IL4RA coding variant (S478P) and *IL13*-1112CT. Individuals with the risk genotype for both genes had increased risk to develop asthma [20] and food sensitization [21] compared to individuals with both non-risk genotypes. Another study assessed the combined effect on asthma and IgE levels of allelic variants arrayed along the Th2-dependent pathway. Combining polymorphisms in all major genes (*IL13*-1112CT, *IL4*-589CT, *IL4RA*148AG and *STAT6*2892CT) in a stepwise procedure, the risk for high serum IgE levels increased by 10.8-fold and the risk for the development of asthma increased by 16.8-fold compared with the maximum effect of any individual SNP [22]. Another study of Chinese asthmatic and control children revealed significant interactions between *IL13* and *IL4RA* for asthma, and *IL13* and the gene for thymus- and activation-regulated chemokine (TARC) for total plasma IgE [23]. Collectively, these studies reiterate the crucial role of *IL13* and its variants in modifying the risk of allergic inflammation.

When Genetic Variation Affects Gene Function: Functional Studies of IL13 + 2044GA

IL13 + 2044GA (rs20541) is found in approximately 25% of the Caucasian population [3] and is expected to result in the non-conservative replacement of a positively charged arginine (R) with a neutral glutamine (Q) at position 130

(numbering including the signal peptide; also referred to as position 110 when numbering does not include the signal peptide [7, 10, 24, 25]). Since the R130Q substitution occurs in α-helix D, the region of IL-13 which is thought to interact with IL-4Rα/IL-13Rα1 heterodimers [26], *IL13* + 2044GA has the potential to affect IL-13-dependent signaling events.

To examine the impact of *IL13* + 2044GA on the functional properties of IL-13, we directly compared the activity of recombinant wild-type (WT) IL-13 and IL-13 R130Q on primary human cells involved in the effector mechanisms of allergic inflammation [27]. We found that IL-13 R130Q was significantly more active than WT IL-13 when inducing STAT6 phosphorylation, CD23 expression in monocytes and IgE switching in B cells. Moreover, IL-13 R130Q was neutralized less effectively than WT IL-13 by an IL-13Rα2 decoy, a property which could contribute to enhanced activity of the minor variant in vivo. It is important to note that neither IL-13 variant engaged T cells, suggesting increased allergic inflammation in carriers of *IL13* + 2044A depends on enhanced IL-13-mediated Th2 effector functions rather than increased Th2 differentiation [27]. Collectively our data indicate that natural variation in the coding region of *IL13* may be an important genetic determinant of susceptibility to allergy.

Some Lessons We Learnt

Performing these functional studies taught us several important lessons. The first was that, when modeling naturally occurring protein variants with recombinant molecules, the system chosen to express the recombinant proteins is critical for the experimental outcome. Indeed, *E. coli*-expressed IL-13 was significantly less active than eukaryotic IL-13 at physiologic concentrations, and was prone to C-terminal truncation [27]. These problems likely reflected the lack of glycosylation typical of proteins expressed in bacteria and were particularly acute for IL-13, which is highly glycosylated in its native state [28]. As a result, all functional studies had to be performed using recombinant IL-13 variants expressed in eukaryotic cells, even though this approach was more cumbersome and time-consuming.

Another problem arose from the specificity of the anti-IL-13 antibodies required for detection and quantification of the cytokine variants. The R130Q substitution was found to affect the recognition of IL-13 epitopes, resulting in underestimation of the minor variant. Therefore, concentrations of eukaryotic IL-13 R130Q had to be adjusted using a correction factor developed through a combination of in vitro IL-13 translation and Western blotting analysis [27]. Other studies of protein variants generated by non-synonymous coding polymorphisms might encounter similar difficulties. Our experience suggests bacterially expressed recombinant proteins should be chosen for functional genomics experiments only if they fully recapitulate the activity of native molecules. Antibody-based detection methods also require validation of the antibodies' ability to recognize distinct protein variants with comparable efficiency.

These considerations may appear too technical, but they are useful because they provide a rationale for the discrepant results reported by different groups. For instance, Arima et al. recently compared the activities of WT IL-13 and IL-13 R130Q [24] and found them to be indistinguishable. Of note, these investigators used recombinant IL-13 expressed in prokaryotes and a transfected B cell line overexpressing IL-13Rα1. The pitfalls of prokaryotic recombinant proteins are discussed above. Utilization of target cells overexpressing a receptor might mask subtle differences in the affinity of its ligand, because the overall strength of ligand–receptor interactions will be dictated more by the artificially increased number of receptors than the affinity of individual ligand-binding events. Reliance on eukaryotic IL-13 proteins and primary human cells therefore provides a more sensitive approach to detect subtle differences in the properties of natural protein variants.

Some Conclusions

SNPs in coding regions represent the majority of disease alleles in Mendelian disorders, and common disease variants are likely to show a similar trend [29]. Our results show IL-13 R130Q, a common variant encoded by *IL13* + 2044A and associated with elevated serum IgE levels and other allergy-related phenotypes in individuals of multiple ethnic backgrounds [4], is significantly more active than WT IL-13 in enhancing essential effector pathways of allergic inflammation in primary human cells.

Structure/function analyses provide mechanistic insights into the increased activity of IL-13 R130Q. The replacement of R130 with a glutamine occurs in α helix D, a region of the molecule critical for its interactions with IL-13 receptors. Alanine-scanning mutagenesis recently revealed R130 to be important for IL-13 binding to IL-13Rα2 [26], the decoy receptor expressed both as a cell-associated and a soluble protein, which binds IL-13 with high affinity but does not signal [30]. IL-13Rα2 is a key negative regulator of IL-13 responses in vivo [31] and its expression is strongly enhanced by IL-13 itself [32], pointing to the existence of complex feedback loops designed to tightly control IL-13-dependent events. Consistent with this scenario, IL-13 R130Q was neutralized by a soluble IL-13Rα2-Fc chimera much less effectively than WT IL-13 [27], suggesting the minor IL-13 variant might to some extent escape the dampening mechanisms, which normally restrain the activity of WT IL-13 in vivo.

In comparison with the often drastic effects obtained by genetic manipulation in animal models, the functional differences between the common and the minor IL-13 variant may appear too modest to influence disease susceptibility. Several considerations argue against this conclusion. Similar results were obtained in several other functional studies of human polymorphic genes such as *CD14* [33], *IL3* [34] and *LTA* [35], all of which show subtle effects of individual common risk alleles. Furthermore, functional differences between the IL-13 variants became manifest within a physiologically relevant concentration range. Finally, *IL13* + 2044GA is in

partial LD with a promoter SNP, *IL13*-1112CT, which results in increased *IL13* transcription in CD4+ Th2 cells [36]. The transcriptional enhancement conferred by *IL13*-1112T is relatively modest as well, but the increase in IL-13 activity caused by the RQ replacement, combined with the concomitant increase in transcription of the -1112T allele, might effectively synergize to amplify IL-13-dependent events. The functional impact of SNP–SNP interactions within the same gene could be further amplified by gene–gene interactions along the same pathway, e.g., when IL-13 R130Q is expressed in carriers of gain-of-function variants in IL4RA [25] and/or STAT6 [22].

When Genetic Variation Affects Gene Expression: Functional Studies of IL13-1112CT

The human *IL13* promoter harbors two common SNPs, *IL13*-1512AC (rs1881457) and *IL13*-1112CT (rs1800925, also referred to as -1055 and -1111) [3]. The *IL13*-1112TT genotype was found to be more prevalent in individuals with asthma and atopic dermatitis and has been associated with increased risk of sensitization to food and outdoor allergens in several studies [14, 15, 17, 21]. Associations between the *IL13*-1112T allele and allergic phenotypes, such as high IgE serum levels, BHR and positive skin tests, were also demonstrated [3, 14, 15].

While these results strongly suggest genetic dysregulation of *IL13* expression and/or function may be a critical determinant of susceptibility to allergy and asthma, genetic epidemiology cannot define the contribution of the promoter SNPs to allergic inflammation susceptibility because these polymorphisms are highly linked to other SNPs in the locus, including *IL13* + 2044GA. Stratified analysis of *IL13* haplotypes in a large Caucasian population did suggest an effect of *IL13*-1112CT on IgE levels independent of *IL13* + 2044GA [16], but assessment of the impact of *IL13*-1112CT on the regulation of *IL13* expression requires dedicated functional studies.

For this purpose, we used a combination of in vivo, in vitro and *in silico* approaches [36]. We started with a comparative analysis of the *IL13* promoter. Genomic segments strongly conserved during evolution frequently exhibit regulatory properties [37], implying SNPs located in such regions are likely to be functional. Although a human/mouse sequence alignment revealed poor conservation of the region containing *IL13*-1112CT, phylogenetic shadowing, a method recently developed to analyze sequence conservation profiles among closely related species [37], showed *IL13*-1112CT falls within a peak of high intra-primate conservation that spans approximately 80 bp and predicts the existence of a primate-specific *cis*-regulatory element. Of note, this element maps to the vicinity of a region that exhibits constitutive DNA hypomethylation and hypersensitivity to DNase I digestion in human naïve, Th1 and Th2 CD4+ T cells [38], suggesting this region may be endowed with regulatory properties. These findings provide indirect but suggestive evidence for a potential role of *IL13*-1112CT in the regulation of *IL13* expression.

To examine more directly whether *IL13*-1112CT affects *IL13* transcription, we generated luciferase reporter constructs driven by a 2.7 kb *IL13* promoter fragment carrying either the major (C) or minor (T) allele at position -1112. Initially the -1112/Luc reporter constructs were transfected into the Jurkat T cell line, a well-established model to study transcriptional regulation of cytokine genes. In this model, the allergy-associated *IL13*-1112T allele was significantly less active than the major allele. However, this finding was inconsistent with the reported associations between the T allele and allergy/asthma susceptibility, which point towards increased *IL13* activity. Therefore, we reassessed transcription of the *IL13*-1112 alleles in primary T cells using nucleofected CD4+ T cells freshly isolated from normal peripheral blood. Activation of CD4+ T cells upregulated transcription of both allelic variants to a comparable extent, a result that was again inconsistent with the association between *IL13*-1112T and increased susceptibility to Th2-dependent inflammation.

Faced with these puzzling results, we reasoned that the true transcriptional impact of the -1112 polymorphism might only become apparent within a polarized cytokine/nuclear environment leading to high-level *IL13* expression. Jurkat cells and non-polarized CD4+ T cells upregulate *IL13* mRNA levels in response to activation, but only a minority of these cells expresses detectable levels of intracellular IL-13 protein. Thus the majority of luciferase activity in non-polarized CD4+ T cells was generated from a nuclear environment inadequate to promote optimal *IL13* expression.

Since *IL13* is typically expressed by polarized CD4+ Th2 cells, and these cells play a critical effector role in human and experimental allergic inflammation, we examined the transcriptional effect of *IL13*-1112CT in two independent, primary Th2 cell models: human neonatal naïve CD4+ T cells differentiated in vitro under Th2-polarizing conditions and murine D10.G4.1 Th2 cells [39]. In both cases, nucleofection of Th2 cells with *IL13*-1112C and T reporter constructs led to significantly higher activation-dependent transcriptional of the -1112T allele. Our results demonstrated that the nuclear environment dictates the transcriptional outcome of genetic variation. In the context of a Th2 milieu that drives high *IL13* expression, but not within non-polarized CD4+ T cells, the -1112T allele conferred higher activity to the *IL13* promoter, consistent with the reported association between this allele and increased susceptibility to allergic inflammation [40].

To identify the mechanisms underlying higher transcription of *IL13*-1112T in Th2 cells, we used electromobility shift assays (EMSA) to compare and contrast patterns of DNA–protein interactions occurring at the *IL13* -1112 promoter variants in distinct T cell nuclear environments. We reasoned that such comparisons could provide an indirect but powerful tool to tease out the interactions involved in increased transcription of the -1112T allele.

Using oligonucleotides corresponding to the C or T allelic variants of *IL13* promoter, we demonstrated that both the C and the T allele-bound STAT6 and STAT1 contained in nuclear extracts from activated primary Th2 cells (in which the -1112T allele was transcriptionally more active). However, the -1112T probe selectively bound an additional complex containing YY1. When we analyzed nuclear factor binding to the polymorphic *IL13* promoter region using nuclear extracts from non-polarized primary CD4+ T cells (in which the T allele was transcribed less

actively), we found again equivalent interactions of the C and T alleles with STAT6 and STAT1. Interestingly, in CD4+ T cell extracts the T allele selectively bound not only constitutively expressed YY1, but also NFAT2. Finally, nuclear extracts from Jurkat T cells (which, like fresh CD4+ T cells, supported weaker transcription of the -1112T allele) showed strong specific binding of STAT1 to both alleles and selective binding of YY1 and Oct-1 to -1112T. However, no STAT6-containing complex was detected, consistent with deficient STAT6 activity in Jurkat T cells [41]. Thus the higher activity of the *IL13*-1112T allele in Th2 cells correlated with a unique pattern of DNA–protein interactions marked by the combination of STAT6 and YY1 (Fig. 1), providing a molecular rationale for the differential transcription of the -1112 alleles in distinct T cell nuclear environments.

Our next task was to define the mechanism(s) underlying the increased activity of the -1112T allele in Th2 cells. YY1 is a ubiquitously expressed nucleoprotein that can either activate or repress transcription [42]. Since the sequences flanking

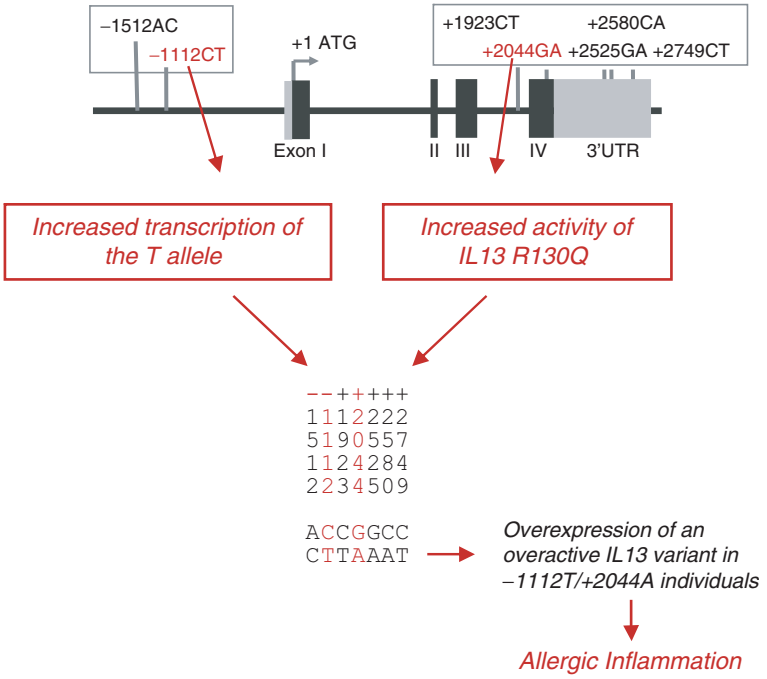

Fig. 1 Distinct *IL13* variants may synergize and increase allergy susceptibility. (Top) Common SNPs in *IL13*. (Middle). *IL13*-1112CT (*rs1800925*) in the *IL13* promoter results in increased *IL13* transcription in Th2 cells, whereas *IL13* + 2044GA (*rs20541*) is a non-synonymous SNP that leads to the expression of a gain-of-function variant, IL-13 Arg130Gln. (Bottom) Linkage disequilibrium at the *IL13* locus is such that both SNPs are frequently, albeit not invariably, found in the same individuals. Co-occurrence of the rare alleles at -1112 (T) and + 2044 (A) is expected to result in overexpression of an overactive IL-13 variant, which may contribute to enhanced susceptibility to allergic inflammation

the YY1 core motif (TCAT) vary, many promoters contain YY1 sites that overlap those for other transcriptional regulators [42]. This topology fosters interactions between YY1 and other factors, the outcome of which depends on the function of the proteins involved. *In silico* analysis of the *IL13* promoter sequence and mutational EMSA analysis by nucleotide transversions demonstrated that the YY1 motif created by -1112T overlaps the 3′ end of a STAT palindrome that is present on both alleles and binds STAT6 or STAT1 in Th2 cells. In view of the dual role of YY1 in transcriptional regulation, two models may explain the role of YY1 in the increased activity of the *IL13*-1112T allele in Th2 cells. Both YY1 and STAT proteins may act cooperatively as transcriptional activators. Alternatively, STAT binding to the *IL13*-1112 region may repress transcription, as reported for the human *IL4* promoter [41], and YY1 may relieve STAT-mediated repression by displacing STAT or recruiting STAT corepressors.

We reasoned that understanding the role played by YY1 in the increased activity of the *IL13*-1112T allele required the functional characterization of the STAT motif overlapping the YY1 site. A combination of independent approaches (mutation of the STAT site in reporter vectors, neutralization of STAT6-dependent signaling in Th2 cells, and STAT6 overexpression in Jurkat cells) clearly showed that the STAT6 motif upstream of the polymorphism plays a strong negative regulatory role in the context of the -1112C *IL13* promoter. Binding of YY1 to the site created by *IL13*-1112T relieves STAT6-mediated repression, leading to increased activity of the -1112T allele in Th2 cells. Chromatin immunoprecipitation analysis confirmed that STAT6 and YY1 bind the endogenous *IL13*-1112 promoter region in primary human Th2 cells, further supporting a critical role of these factors in the transcriptional outcome of *IL13*-1112CT.

The last piece of the *IL13*-1112CT puzzle was provided by the analysis of the correlation between *IL13*-1112 genotypes and levels of IL-13 production in a large population sample. We assessed IL-13 secretion in subjects enrolled in the Tucson Infant Immune Study, a large prospective study of the development of immunological markers of asthma risk [43]. We focused on 174 women at the third trimester of pregnancy, unselected for atopy and asthma. Mitogen-activated peripheral blood mononuclear cells from *IL13*-1112TT homozygotes secreted significantly higher levels of IL-13 compared to -1112CC and CT individuals. The effect was strengthened after adjusting for ethnicity and *IL13*+2044 genotype in a multivariate linear regression. These data strongly support the contribution of *IL13*-1112CT to increased *IL13* expression *in vivo*.

Some Conclusions

Gene–environment interactions in the nucleus were the most unexpected and interesting finding of our analysis of *IL13*-1112CT. This polymorphism enhances *IL13* promoter activity in primary human Th2 lymphocytes, cells programmed for high *IL13* expression, but has opposite effects in non-polarized CD4+ T cells. Thus, the nuclear milieu can determine the functional outcome of genetic variation.

Gene–environment interactions in the nucleus are a phenomenon we previously observed for *CD14*-159CT, a SNP which results in distinct patterns of *CD14* promoter activity in monocytes and hepatocytes depending on the Sp1/Sp3 ratio [33]. However, the data on *IL13*-1112CT are in a sense more remarkable because differential *IL13* expression was observed not in distinct cell types but in distinct CD4 + Th cell phenotypes expressing distinct transcriptional milieus. That the gain-of-function associated with the *IL13*-1112T allele only emerged in differentiated Th2 cells eloquently shows how subtle the functional impact of genetic variation can be, and how essential it is to choose experimental models able to capture it. Furthermore, these results suggest *IL13*-1112CT is likely to influence risk of allergy and/or asthma primarily in the context of an established Th2 response. Thus this polymorphism may contribute to the maintenance and/or exacerbation of allergic inflammation more than to its inception.

More generally, gene–environment interactions in the nucleus may offer a rationale for the common but disquieting finding that many published associations cannot be replicated [44–46]. If the functional outcome of genetic variation contributing to disease risk is determined not only by the genetic, but also by the biological context, as our data indicate, the conditions under which biological samples are collected for phenotyping may become critically important, and failing to account for gene–environment interactions in the nucleus may hamper detection of susceptibility loci. Interestingly, there are now many examples of established associations with different functional variants within the same gene or with opposite alleles at the same SNP in different populations [40]. For example, IgE levels are associated with *IL13*-1112CT in some populations [7, 8], and with *IL13* + 2044GA [3, 5, 7] or *IL13*-1512AC [9] in others. It is tempting to speculate that these seemingly contradictory results might represent an outcome of gene–environment interactions in the nucleus.

Similar to the results obtained for *IL13* + 2044GA, the impact of *IL13*-1112CT on transcriptional activity was relatively modest. This reflects the nature of single nucleotide variations, subtle differences that alter fine-tuning or sensitivity thresholds of promoters and regulatory elements rather than impose the drastic effects of loss- or gain-of-function mutations seen in Mendelian disorders. Indeed, the magnitude of the effect was similar to other regulatory polymorphisms such as the SNP in *SLC22A* associated with rheumatoid arthritis and loss of transcriptional activity [47] and the variant *CD14* and *TGFB* promoters [33, 48]. Since the functional effects of individual polymorphisms may be small, risk for complex diseases is substantially increased by synergism between multiple SNPs arrayed along a regulatory pathway.

Studying functional genomics in an evolutionary framework may deepen our understanding of the role a given gene and its variants play in physiology and disease. Comparative analysis of the *IL13* promoter showed the *IL13*-1112T allele that increases risk for allergic disease is the ancestral allele. In contrast, the derived -1112C allele (the one currently most common among Caucasians) is protective. Furthermore, this analysis revealed the topology of STAT6 and YY1 motifs resulting in increased *IL13* promoter activity has been fully conserved through at least 30 million years of evolutionary history, and all the replacements found in the STAT motif in Old World and New World monkeys occurred within the 3 N spacer, not in the TTC/GAA palindrome critical for DNA–protein interactions. These findings

and their relevance to common diseases are best interpreted in the framework of the ancestral-susceptibility model [49], according to which ancestral alleles reflect ancient adaptations to the lifestyle of ancient human populations. In that context, derived alleles were deleterious. With the shift in environment and lifestyle that has occurred in modern populations, ancestral alleles can increase the risk of common diseases, as exemplified by variants involved in energy metabolism and sodium homeostasis [49]. An equivalent role of *IL13*-1112CT among immunity genes is suggested by its current associations with allergy and asthma susceptibility in Western environments [40] in the face of strong associations between *IL13*-1112T and protection from *Schistosoma hematobium* in Africa [50] and severe malaria in Thailand [51]. While it is unclear why *IL13*-1112C rose abruptly in frequency to become the common allele in most human populations, the *IL13* locus shows signatures of a recent selective sweep in the Caucasian and Chinese populations [52]. We speculate that a genetically determined propensity for high *IL13* expression may have become detrimental through deleterious effects on reproduction. Indeed endometriosis, which increases the risk of infertility, has been associated with elevated *IL13* mRNA and protein expression within the ectopic endometrium [53]. *IL13* may therefore be the first immunity gene that conforms to the ancestral-susceptibility model.

Final Comments

The lessons that emerged from the studies discussed above are somewhat sobering. Experimental strategies which are successful in classical immunology may not be readily applicable to functional genomics work, whose targets are inherently elusive. When studying the effects of human genetic variation, we actually explore complex interactions between polymorphic genes (and their products) and the cellular milieu. Both genes and environments need to be faithfully modeled, because the effects of genetic variation are likely to be context-dependent. Thus functional studies need to recreate as much as possible the biological conditions under which natural genetic variation exerts its subtle effects, and these conditions may be different for different polymorphisms. Therefore, even at this early stage of functional genomics studies, it is clear that unraveling the molecular mechanisms whereby natural genetic variation shapes the pathogenesis of complex diseases will require more adequate conceptual frameworks as well as novel experimental and analytical tools.

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Genetic Markers for Differentiating Aspirin-Hypersensitivity

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Introduction

The ingestion of acetylsalicylic acid (ASA) can induce allergic reactions such as ASA-intolerant asthma (AIA), ASA-induced acute or chronic urticaria/angioedema (AIAU or AICU), anaphylaxis, and, in rare cases, hypersensitivity pneumonitis [1, 2]. Among these, AIA and AIU are most prevalent. Although the pathogenic mechanism of AIA is not completely understood, a chronic overproduction of cysteinyl leukotrienes (Cys-LTs) derived from cyclooxygenase (COX) inhibition has been consistently found to be associated with the condition [3, 4]. Although recent reports have suggested that an overproduction of Cys-LTs may play a role in AIU development [5, 6], knowledge about the pathogenic mechanism of AIU is limited. Here, we summarize recent data regarding the molecular genetic mechanisms that govern AIA and AIU, with the objective of identifying genetic markers that can be used to differentiate between the two conditions.

Demographic Characteristics of AIA and AIU

Acetylsalicylic acid-intolerant asthma is a clinical syndrome, characterized by eosinophilic rhinosinusitis, nasal polyposis, ASA sensitivity, and a moderate to severe degree of asthmatic symptoms [7, 8]. This condition most commonly occurs in middle-aged female asthmatic patients with chronic rhinosinusitis and/or nasal polyps [9]. The lysine-ASA bronchoprovocation test has been widely used to confirm the diagnosis of AIA in Europe and Asia [10, 11], whereas the oral provocation test has been more commonly applied in the USA.

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_15, © Springer 2009

Acetylsalicylic acid ingestion can induce swelling and aggravate wheals in patients with acute and chronic urticaria. Patients experiencing acute ASA-intolerant urticaria (AIAU) are defined as those showing urticaria and angioedema when exposed to ASA/non-steroidal anti-inflammatory drugs (NSAIDs). In chronic ASA-intolerant urticaria (AICU), chronic urticaria and/or angioedema symptoms are aggravated with exposure to ASA. AIU, which includes both AIAU and AICU, can be confirmed by an oral ASA challenge test. Chronic urticaria patients are classified into two groups: those exhibiting a positive response to oral ASA challenge (diagnosed as AICU), and those exhibiting a negative response, which is defined as ASA-tolerant chronic urticaria/angioedema (ATCU). The proportion of patients with chronic urticaria who develop exacerbation after ASA administration ranges from 20% to 30% [6]. Our recent study [12] demonstrated that AICU patients tended to be relatively young and to exhibit a high atopic rate as well as a high serum total immunoglobulin E (IgE) level. No significant differences in the prevalence of thyroid autoantibodies, the prevalence of anti-nuclear antibodies, or other clinical parameters were noted between AICU and ATCU patients.

A recent study reported that the prevalence of serum specific IgE to staphylococcal superantigens, particularly toxic shock syndrome toxin 1 (TSST-1), was significantly higher in AICU than in ATCU or normal controls, whereas the colonization rate of *Staphylococcus aureus* was similar between the two conditions. Moreover, patients with high specific IgE to these superantigens showed a higher serum total IgE level and atopy rate. These findings suggest that the Th2 immune response to these superantigens may be involved in the pathogenic mechanism of a subtype of AICU [13].

Differential Contributions of Genetic Polymorphisms to ASA Hypersensitivity

Genetic Studies of AIA

An association between the human leukocyte antigen (HLA) allele HLA-DPB1*0301 and AIA was first reported in a Polish population [14] and was later recognized in a Korean population [15]. The frequency of DPB1*0301 was significantly increased in AIA patients when compared with normal and asthmatic controls, suggesting that the immune recognition of an unknown antigen may be part of the pathogenesis of AIA [15]. The patients with DPB1*0301 tended to be females, having lower forced expiratory volume at 1 s (FEV_1) levels, but higher prevalence of rhinosinusitis and/or nasal polyps than those lacking DPB1*0301. Interestingly, these are also the typical clinical features of AIA [15]. Furthermore, the presence of DPB1*0301 was significantly associated with a requirement for a higher dose of leukotriene receptor antagonist in the long-term management of AIA [16]. When combined, these results suggest that HLA-DPB1*0301 may be an important genetic marker for the AIA phenotype. Furthermore, a genetic interaction

between tumor necrosis factor α (TNFα) −1031T>C (or −863C>A or −857C>A) and HLA-DPB1*0301 synergistically increased susceptibility to AIA, suggesting that a TNFα promoter polymorphism may significantly increase susceptibility to AIA via a genetic interaction with HLA-DPB1*0301 [17].

It is widely recognized that Cys-LT biosynthesis is associated with the development and progression of AIA [3, 4]. The activity of leukotriene C4 synthase (LTC4S), a key enzyme for Cys-LT synthesis, may be genetically regulated in AIA pathogenesis. The *LTC4S* −444A>C polymorphism has been reported to be positively associated with the AIA phenotype in a Polish population [18, 19]. Specifically, patients carrying the C allele exhibited a higher risk for AIA development by increased binding of the histone H4 transcription factor-2 to the promoter polymorphism, both in vitro and in vivo. However, this association has not been identified in other groups examined, including Japanese, American, and Korean populations [20–22].

Some reports have suggested a possible involvement of the 5-lipoxygenase gene (*ALOX5*) in AIA. For example, the Drazen research group reported an association between a promoter polymorphism of the *ALOX5* gene, consisting of a variable number of tandem-repeated GC-rich motifs, and increased binding of Sp1 transcription factors [23]. Subjects exhibiting the wild-type genotype (five repeats) showed a significantly higher capacity to produce Cys-LTs when compared with those showing the mutant genotype (three, four, or six repeats). Furthermore, the mutant genotype was reported to be positively associated with increased severity of airway hyper-responsiveness in a Korean population [24]. Specifically, AIA patients carrying a mutant genotype ($n > 5$ or $n < 5$ repeats) showed increased airway hyper-responsiveness when compared with AIA patients with the wild-type genotype.

In an earlier study, we screened a Korean population for ten single nucleotide polymorphisms (SNPs) of key enzymes involved in arachidonate metabolism; these included 5-lipoxygenase (*ALOX5*; −1708G>A, 21C>T, 270G>A, 1728G>A), ALOX5-activating protein (*ALOX5AP*; 218A>G), *COX-2* (−162C>G, 10T>G, 228G>A), *LTC4S* (−444A>C), and cysteinyl leukotriene receptor 1 (*CysLTR1*; 927T>C). We reported a lack of association between *ALOX5AP, COX-2*, and *CysLTR1* polymorphisms and the AIA phenotype; however, we suggested the possible involvement of ALOX5 haplotype 1 (G-C-G-A) in AIA development [22].

Recently, we reported a significant genetic association of two types of Cys-LT receptors, *CysLTR1* and CysLTR2, in AIA patients [25, 26]. We found three SNPs of the *CysLTR1* promoter (−634 C>T, −475A>C, and −336A>G) that were significantly associated with the AIA phenotype, particularly in males. These promoter polymorphisms exhibited significantly higher capacity to increase promoter activity in epithelial and mononuclear cells. In addition, four SNPs of the *CysLTR2* gene (c. −819T>G, c. 2078C>T, c. 2534A>G, and c. 2545+297A>G) were identified in a Korean population [26], and the rare alleles at these sites showed significant association with a greater percentage fall in FEV_1 after ASA provocation, indicating greater ASA sensitivity.

A case control study of 63 candidate genes in a Japanese population [27] showed that a functional SNP of the prostaglandin E2 (PGE2) receptor subtype 2 gene (*EP2*) was associated with increased risk of AIA. This may result from a reduction in the PGE2 braking mechanism in inflammation. Although a novel promoter polymorphism of *COX-2* (−765G>C) was not associated with AIA, the CC homozygote of this polymorphism was associated with increased PGE2 production by creating an E2F transcription factor binding motif [28].

Using direct sequencing, we also screened for genetic variations in the prostanoid receptor genes *PTGER1, PTGER2, PTGER3, PTGER4, PTGDR, PTGIR, PTGFR*, and thromboxane A2 receptor gene (*TBXA2R*), and selected 32 tagging SNPs among the 77 polymorphisms with frequencies >0.02 on the basis of linkage disequilibrium for genotyping [29]. A haplotype analysis of each gene revealed that seven SNPs were significantly associated with the AIA phenotype: −616C>G and −166G>A in *PTGER2*, −1709T>A in *PTGER3*, −1254A>G in *PTGER4*, 1915T>C in *PTGIR*, and −4684C>T and 795T>C in *TBXA2R*. The frequency of *PTGIR* haplotype 3 (G–G–C–C), which includes 1915T>C, differed significantly between the AIA and ATA patients. These findings suggest that genetic polymorphisms in *PTGER2, PTGER3, PTGER4, PTGIR*, and *TBXA2R* are important in the pathogenesis of AIA. Further studies are needed to clarify the hypothesis of COX-2 and prostaglandin imbalance in the pathogenic mechanisms of these conditions.

TBXA2R encodes a receptor for a potent bronchoconstrictor, thromboxane A2 (TBXA2). A study conducted on a Korean population showed that the *TBXA2R*+795T>C polymorphism augmented the bronchoconstrictive response to inhaled ASA, which may contribute to AIA [30]. It is possible that oral ASA administration leads to the uncoupling of TBXA2-dependent negative feedback mechanisms and thus increases the production of Cys-LTs, explaining the effect of increased TBXA2 production on the pathogenesis of AIA [30]. TBXA2-dependent regulation of LTC4S activity may be an important pathophysiological mechanism of AIA.

There was no association between two common polymorphisms of *FcεR1β* (−109T>C and E237G) and the AIA phenotype [31]. However, the *FcεR1β* −109T>C polymorphism was significantly associated with IgE specific to *Staphylococcal enterotoxin B* [31], suggesting that this gene/environment interaction may contribute to the development of AIA. This same study also reported that the *FcεR1β* −109T>C polymorphism may increase FcεR1β expression in mast cells, leading to enhanced release of proinflammatory mediators in the asthmatic airway and thereby contributing to increased susceptibility to AIA.

TBX21 encodes the transcription factor T-bet (T-box expressed in T cells), which influences naive T lymphocyte development and has been implicated in asthma pathogenesis. The −1993T>C SNP in the *TBX21* promoter was shown to be significantly associated with increased risk of AIA owing to increased transcriptional activity [32]. This genetic variation can cause inappropriate Th1 responses in the airways, leading to severe airway inflammation in combination with antigen-specific Th2 responses. Furthermore, the report suggested that the Th1 response may play as great a role in AIA pathogenesis as the Th2 response. Another study [33] reported that the *TGFβ1* −509C>T polymorphism was not significantly associated with the AIA

Table 1 Summary of genetic association studies of AIA.

Gene	Locus	SNP	Phenotype	*N*	Year of publication
HLA	6p21.3	DPB1*0301	AIA	76 AIA	2004
PTGER2	14q22.1	uS5	AIA	396 AIA	2004
TBX21	17q21.32	−1993T>C	AIA	72 AIA	2005
ALOX5	10q11.2	ht1(GCGA) $(GGGCGG)_{4,6}$	AIA AHR*	93 AIA 107 AIA	2005 2006
FcεRIβ	11q12.1	−109T>C	IgE to SEB	107 AIA	2006
TBXA2R	19q13.3	795T>C	FEV1 fall by ASA-BPT	93 AIA	2006
CYSLTR1	Xq13.2–21.1	−634C>T	AIA	105 AIA	2006
TNFα /HLA	6p21.3	*TNFα −1031T>C/DPB1*0301*	AIA	163I AIA	2006
CYSLTR2	13q14.2–21.1	c. −819T>G, c. 2078C>T, c. 2534A>G	FEV1 fall by ASA-BPT	115 AIA	2006
PTGER2	14q22.1	−161C>G	AIA	108 AIA	2007
		166G>A	AIA	108 AIA	2007
PTGER3	1q31.1	−1709T>A	AIA	108 AIA	2007
PTGER4	5q13.1	−1254A>G	AIA	108 AIA	2007
PTGIR	19q13.32	1915T>C	AIA	108 AIA	2007
ADAM33	20p13	ST + 7, V-1, V5	AIA	102 AIA	2007
TGFβ1	19q13.2	−509C>T	Rhinosinusitis	203 AIA	2007

HLA, human leukocyte antigen. AHR, airway hyperresponsiveness. ASA-BPT, acetylsalicylic acid-bronchoprovocation test.

phenotype; however, a significant association with the prevalence of rhinosinusitis in AIA patients, but not in ATA patients, was observed. When augmented by the presence of rhinosinusitis, the frequency of carriers of the *TGFβ1* −509C>T T allele (CT and TT genotypes) was significantly higher in AIA patients than in ATA patients, with a significant difference in the serum TGFβ1 level.

The A-disintegrin and metalloprotease (ADAM) 33 gene was reported to be associated with the asthma phenotype and airway hyper-responsiveness in asthmatic patients in various ethnic groups [34, 35]. In a Japanese population of AIA patients, sequence variations (ST + 7, V-1, and V5) in *ADAM33* were associated with susceptibility to AIA [36]. Table 1 summarizes the current knowledge of genetic associations in AIA.

Genetic Studies of AIU

The first study suggesting an association between HLA and the AIU phenotype, which was conducted in a Korean population, demonstrated a strong association of two HLA alleles (HLA-DRB1*1302 and HLA-DQB1*0609) with AIU [37]. When clinical parameters were analyzed according to the presence of these two alleles, patients carrying HLA-DRB1*1302 or HLA-DQB1*0609 were found to be significantly

younger (by approximately 10 years) than those lacking either allele, indicating that patients with these alleles develop AIU at an earlier age. There were no significant differences in the other clinical parameters examined, including atopy, total serum IgE, and circulating autoantibodies, between these two groups. Moreover, recent data showed that the prevalence of serum specific IgE to staphylococcal superantigens was significantly higher in AICU patients than in ATCU patients and normal controls, with specific IgE to TSST-1 being the most prevalent form in AICU patients (25.8% vs. 6.5% in controls and 13.7% in ATCU patients). Furthermore, significant associations were noted between the prevalence of specific IgE to the staphylococcal superantigens SEA and SEB and the DQB1*0609 and DRB1*1302 HLA alleles in the AICU group [13]. This suggests that patients with either of these two HLA alleles may be more susceptible to developing Th2 immune responses to staphylococcal superantigens, which could contribute to the development of AICU. Thus, the HLA alleles DRB1*1303 and DQB1*0609 may be strong HLA markers for predicting the AICU phenotype in Asian populations. However, a study conducted using a low-resolution technique in an Italian population reported the Class I allele (HLA-B44) as a risk factor for AICU, whereas HLA-Cw4 and Cw7 were associated with lower risk of AICU [38]. Further studies are needed to clarify the significance of HLA markers in AICU patients.

Leukotrienes are believed to participate in the pathogenesis of AIU. Immunopharmacological studies demonstrated that mast cells and basophils are activated to a greater extent in patients with AIU [39]. Mastalerz et al. [40] showed that the overproduction of Cys-LTs was significantly associated with a polymorphism at −444A>C of the *LTC4S* gene in AICU patients, with the frequency of the C allele being significantly higher among AICU patients compared with ATCU patients. Moreover, AIU was aggregated in families carrying the *LTC4S* −444C allele [41]. However, no such association was found in a Spanish population [42]. We also investigated the genetic polymorphisms of candidate genes encoding enzymes involved in leukotriene synthesis in a Korean population. We examined nine SNPs of five leukotriene-related genes: 5-lipoxygenese (*ALOX5*; −1708G>A, 270G>A, and 1728G>A), 5-lipoxygenase-activating protein (*ALOX5AP*; 218A>G), cyclooxygenase 2 (*PTGS2*; −162C>G, 10T>G, and 228G>A), *LTC4S* (−444A>C), and *CysLTR1* (−634C>T), showing that a polymorphism of *ALOX5* (−1708G>A) and of *CysLTR1* (−634C>T) had genotype frequencies that differed significantly between AICU and AIA patients [43]. The frequency of the *ALOX5* −1708A allele was significantly higher and that of the *CysLTR1* −634 T allele was significantly lower in the AICU group compared with the normal control group. These findings were confirmed in vivo by a functional study showing that the *CysLTR1* mRNA level significantly increased after ASA challenge in AIA patients but did not change significantly in AIU patients [44]. These results suggest that ALOX5 and CysLTR1 play different roles in two major ASA-related conditions, namely, AICU and AIA.

Eleven known SNPs of the genes encoding high-affinity IgE receptor I [FcεRIβ; −109T>C, Rsal_Int2, I181L(A>C), E237G(A>G) Rsal_Ex7], histamine *N*-methyl transferase [*HNMT*; T105I(C>T)], histamine receptor H1 [*HRH1*; −17C>T, D349N(G>A)], and histamine receptor H2 (*HRH2*;

543G>A, 826C>T) and their haplotypes were compared among AIU patients, patients exhibiting other drug allergies, and normal controls. No significant differences in allele, genotype, or haplotype frequencies of any of the SNPs from *FcεRIβ gene, HNMT, HRH1, and HRH2* were observed among the three groups, suggesting that the polymorphisms of the *FcεRIβ* gene and the three histamine-related genes do not contribute to the development of the AIU phenotype [45]. We also investigated the functional variability of the *HNMT* gene according to genetic polymorphisms in AICU patients and found that the *HNMT* 939A>C polymorphism was significantly associated with AICU [46]. Moreover, an in vitro functional study demonstrated that an A-to-G conversion at position 939 in the 3′ UTR increased both mRNA stability and protein expression. Thus, genetic variants of the *HNMT* 939A>C polymorphism may affect mRNA stability and protein expression, resulting in altered histamine metabolism and thereby contributing to the development of AICU. Given that the bioactive histamine level is also regulated by the synthesizing enzyme histamine decarboxylase (HDC), further investigation into the genetic contribution of HDC in association with HNMT is needed.

Recent studies demonstrated a significant association between two promoter polymorphisms of *FcεRIα* (−334C>T and −95 T>C) and the AICU phenotype [12], although no such association was found in a similar study conducted on a Polish population [47]. FcεRIα is the first receptor to bind with IgE antibodies. The rare allele of the −344C>T polymorphism was significantly associated with higher serum total IgE in AICU patients when compared with other subjects [12]. Furthermore, in an in vitro functional study using a reporter plasmid carrying the −344T allele, this allele exhibited significantly higher promoter activity than the −344C allele in the rat mast cell line RBL-2H3. Specifically, the transcription factor myc-associated zinc finger protein (MAZ) preferentially bound to the −344C>T polymorphism. In addition, AICU patients carrying the T allele exhibited higher histamine releasing activity of IgE antibody than those with the homozygous CC genotype, whereas the two groups showed no significant differences in calcium ionophore-induced histamine releasing activity [12]. These findings suggest that the −344C>T polymorphism of the *FcεRIα* promoter may be associated with increased expression of FcεRIα on mast cells and enhanced release of histamine, which in turn contributes to the development of AICU. Table 2 summarizes the current knowledge of genetic associations with AIU.

Table 2 Summary of genetic association studies in AIU.

Gene	Locus	SNP	Phenotype	*N*	Year of publication
HLA	6p21.3	DRB1*1302	AIU	188 AIU	2006
		DQB1*0609	AIU	188 AIU	
ALOX5	10q11	−1708G>A	AIU	101 AIU	2005
FcεRIα	1q23	−344C>T	AICU	95 AICU	2007
HNMT	2q22.1	939A>C	AICU	110 AICU	2007

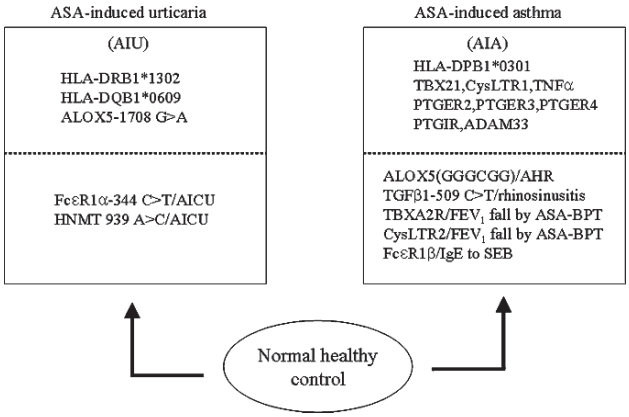

Fig. 1 Potential gene markers for differentiating acetylsalicylic acid hypersensitivity.

Conclusion

Further information about genetic polymorphisms of candidate genes and supporting functional studies would help to elucidate the molecular mechanisms of the two major ASA-related conditions, namely, AIA and AIU. Such information would also aid in the identification of useful genetic markers for differentiating between AIA and AIU, which should lead to the development of new diagnostic markers and additional therapeutic targets on the basis of genetic information, as shown in Fig 1.

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Molecular Biology of Allergens: Structure and Immune Recognition

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Introduction

Allergens are defined as environmental agents that induce IgE-mediated immediate hypersensitivity reactions following inhalation, ingestion or injection. In some texts, allergens are described as 'innocuous' or 'harmless', which is certainly true for the majority of non-sensitized individuals. However, for patients with hay fever, asthma or atopic dermatitis (AD), the majority of whom are sensitized to pollen or indoor allergens, exposure to allergens is far from harmless. Equally, local and systemic anaphylactic reactions to insect venom or food allergens are serious, and potentially life-threatening, problems for allergic patients. Little is understood about why certain allergens are associated with specific allergic conditions: why pollens cause hay fever, why asthma is strongly associated with indoor allergens and why peanut is such a potent cause of anaphylaxis. From the immunological point of view, it is important to distinguish between complete ('true', sensitising) allergens and incomplete (non-sensitising) allergens. Non-sensitising allergens are able to interact with IgE antibodies (which may or may not result in allergic symptoms), but are unable to induce the production of IgE antibodies. Their role as allergens fully depends on their cross-reactivity with complete (or sensitising) allergens. A good example of a non-sensitizing would be the apple allergen, Mal d 1, which is strongly cross-reactive with birch pollen, Bet v 1, but does not itself cause sensitization. While non-sensitizing cross-reacting allergens are of interest both from the clinical as well as from the immunological point of view, we focus in this chapter on allergenicity, the process that results in allergen-specific IgE synthesis.

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_16, © Springer 2009

Studies of allergen structure and function, exposure levels and aerodynamics have provided insights into features that predispose to allergenicity. Most inhaled allergens are 10–60-kDa proteins or glycoproteins that become airborne on particles, e.g., pollen grains, mite feces, animal dander that are 5–50 μm in diameter and contain ~1 ng allergen per particle. Continued exposure to 1–2 μg/g of a major allergen in house dust will cause sensitisation for IgE responses in some, but not all, genetically predisposed (atopic) individuals [1]. House dust allergens comprise a significant proportion of the protein in house dust. One could predict that an entirely novel protein with similar properties to all those described above would ultimately cause sensitisation in atopic individuals.

Structural and molecular studies have revealed that allergens are a diverse group of proteins with different structures and biological functions [2–7]. Most common allergens have been cloned, sequenced and manufactured as recombinant proteins in high-level expression systems. Recombinant allergens provide essential tools for research and are increasingly being used to develop new allergy diagnostics and vaccines [5, 8–11]. There are now over 50 three-dimensional allergen structures in the Protein Database (PDB) and allergens are found in ~150 protein families in the Pfam protein family database (www.sanger.ac.uk/Software/Pfam). Breiteneder has argued that this is a relatively small number, given that over 8,000 protein families reside in Pfam [12, 13]. However, the 150 allergen protein families that have been identified still represent a huge degree of diversity at both the structural and biological level. Such diversity precludes any common structural feature, e.g., amino acid sequence motif or protein structure, which makes an allergen an allergen. From the molecular standpoint, glycoproteins need special consideration because the glycan structure is determined largely by the host that is used for the expression of the glycoprotein. Expression of glycoproteins in yeast, molds, plants, invertebrates or vertebrates results in different glycoproteins with often strikingly different IgE reactivity [14].

The ability of diverse proteins to be allergens must relate to immunologic, environmental and host factors that influence IgE responses, as well as adjuvant-like effects. Some of these factors have been widely investigated over the past 10 years. They include observations that the proteolytic enzyme activity of dust mite allergens can potentiate IgE responses [15, 16]. Mite cysteine and serine protease allergens can damage lung epithelia, cause production of pro-inflammatory cytokines and may act as gatekeepers to allow access of other non-enzymatic allergens to antigen-presenting cells [17–19]. Recent studies suggest that mite feces contain other elements, including endotoxin, bacterial DNA and mite DNA that could also influence IgE responses and inflammation [20]. The effect of allergen dose on IgE responsiveness came to the fore following studies, which showed that children living with cats (and exposed to > 20 μg/g Fel d 1) had a lower prevalence of IgE antibody to cat [21, 22]. This was associated with high levels of IgG4 antibody to Fel d 1, in what has been termed a modified Th2 response (i.e., a class switch to IgG4, but not to IgE). It is common to find individuals with high levels of IgG1 and IgG4 antibody to Fel d 1, without IgE, and, paradoxically, this form of IgE-selective tolerance is associated with high-level exposure to Fel d 1. To complicate matters even further, it has

recently been proposed that the degree of "foreignness" of the allergen relative to the human may also affect immune recognition. Cat and dog allergens are widely distributed in the environment, and have the expected aerodynamic properties of allergens, and yet appear to be rather weak allergens. There are over 50 million cats (and dogs) in the USA and it is surprising given their prevalence and the ubiquitous distribution of these allergens in the environment that the rate of sensitization to these allergens is not higher. Many individuals may develop tolerance in response to the high-dose exposure. Another explanation is that because mammalian allergen sequences are more closely related phylogenetically to human sequences than, for example, mite or cockroach sequences, they are less 'foreign' and by inference less likely to stimulate the immune system [20].

In this chapter, we will explore some of these new 'frontiers' and use selected examples to illustrate that some allergens are more important than others. Major allergens have in the past been designated based on sensitization levels of > 50% in a panel of allergic patients with IgE antibody to the source material. Intuitively, one would expect that a major allergen is one that makes a difference. Objective evidence can distinguish those allergens that make a difference from those that do not. Understanding which allergens are important influences decisions about allergen selection for immunodiagnostics and for new therapeutic strategies. Molecular biology has provided the tools for manipulating allergen genes and proteins. The new frontier is how to harness this exciting technology to better understand the sensitization process and to more effectively treat allergic disease.

Allergen Structure and Biologic Function

Molecular Biology

The molecular biology of allergens has followed a familiar path over the past 20 years: (i) cloning and sequencing of allergens; (ii) high-level expression of recombinant allergens; (iii) determination of three-dimensional structures by X-ray crystallography or nuclear magnetic resonance spectroscopy (NMR); (iv) generation of mutants or "hypoallergenic" variants with reduced IgE binding activity; and (v) clinical trials of recombinant allergen vaccines [5, 10, 23–28]. Additionally, epitope scans of overlapping linear peptides (usually 6–15 amino acids) derived from the amino acid sequence are often tested for IgE binding and/or T cell stimulatory activity. As a result, the sequences of over 500 allergens have been determined and more than 50 allergen structures have been deposited in the PDB (Table 1). Initially, most allergens were cloned by screening cDNA expression libraries with pooled IgE antibodies from selected allergic patients. Polymerase chain reaction (PCR) and, more recently, phage display techniques have also been used [29]. Subsequently, allergen homologues were identified using degenerate primers whose nucleotide sequence was derived from the previously cloned allergens. The first allergens to

Table 1 Protein database files for structures of common allergens

Allergen	PDB file number(s)			
Indoor				
Bla g 2	1YG9			
Bos d 2	1BJ7			
Der p 1	1XKG	2AS8		
Der p 2	1A9V	1KTJ		
Der f 2*	1AHK	1AHM	1WRF	1XWV
Fel d 1	1PUO	1ZKR	2EJN	
Mus m 1	1MUP			
Rat n 1	2A2G	2A2U		
Outdoor				
Bet v 1*	1B6F	1BTV	1BV1	1FM4
Bet v 2	1CQA			
CCD**	2MYR	1E6S	1FX5	1LTE
Jun a 1	1PXZ			
Ole e 6	1SS3			
Phl p 1	1N10			
Phl p 2	1BMW	1WHO		
Phl p 5	1L3P			
Phl p 6	1NLX			
Phl p 7	1K9U			
Che a 3	2OPO			
Foods				
Ara h 6	1W2Q			
Bos d 5	1BSO			
Prua v 1	1E09	1H2O		

be cloned were those for which the natural allergen had been purified and shown to be important, e.g., Der p 1, Der p 2, Bet v 1 and Amb a 1 [30–33]. Cloning identified many other allergens for which the natural counterpart had not been purified. The repertoire of 21 mite (*Dermatophagoides pteronyssinus*) allergens currently listed in the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature (www.allergen.org) includes many allergens that were defined based on the recombinant allergen sequences alone (similarly with other allergen sources) [7]. Cloning and/or PCR also defined a large number of isoallergens: multiple molecular forms of the same allergen that share extensive amino acid sequence homology (>67%) and IgE cross-reactivity [34, 35]. The 40 or more Bet v 1 sequences represent 31 isoallergens that show 73–98% sequence identity. This form of genetic variation appears to be a particular feature of the Group 1 tree pollen allergens. Polymorphic variants of the same allergen, termed isoforms, show > 90% amino acid sequence identity and are again highly prevalent in Birch pollen (42 isoforms of Bet v 1) and also dust mite (23 isoforms of Der p 1 and 13 isoforms of Der p 2) [7]. Because isoforms differ in only a few amino acid substitutions, analysis of immunoreactivity to isoforms can be useful in defining antibody binding sites and T cell epitopes on allergens [36].

Recombinant allergens have been produced in high-level expression systems in *Escherichia coli*, *Pichia pastoris*, baculovirus and tobacco plants [23, 37–41]. Most allergens have been expressed in *E. coli* as the mature protein or as fusion proteins (with glutathione *S*-transferase or maltose binding proteins), or with histidine tags, to aid purification. Some allergens (for example, Phl p 1, see Fig. 1) are not properly folded in prokaryotic bacterial systems or are produced in inclusion bodies which require solubilisation in guanidine or urea and refolding prior to purification [42]. In these cases, eukaryotic systems such as yeast or baculovirus may be more suitable for high-level expression of allergens with correct folding. The yeast, *P. pastoris*, is especially useful for allergens that do not express in *E. coli*, such as the Group 1 mite allergens. The original *P. pastoris* vector used the AOX1 promoter, which required feeding cultures with methanol to induce allergen expression [39, 40, 43, 44]. This can be avoided with the newer pGAPZ vectors in which the allergen is constitutively expressed into the culture medium. The advantages of the *P. pastoris* system are high-level expression (up to 100 mg/l) and that the protein of interest is the major protein secreted into the medium and is more easily purified. *P. pastoris* can also be used in fermentors and scaled up into bioprocessing systems that facilitate the production of gram quantities of protein.

Recombinant allergens have several advantages when compared with natural allergen extracts or purified natural allergens. Unlike natural allergenic products, recombinant allergens are homogeneous and do not contain non-allergenic proteins. They are also less likely to contain endotoxin, bacterial products or viruses. To some extent, these advantages are shared by purified natural allergens. However, trace contamination with other allergens can occur in purified natural allergen preparations. One advantage of purified recombinant over natural allergens is the

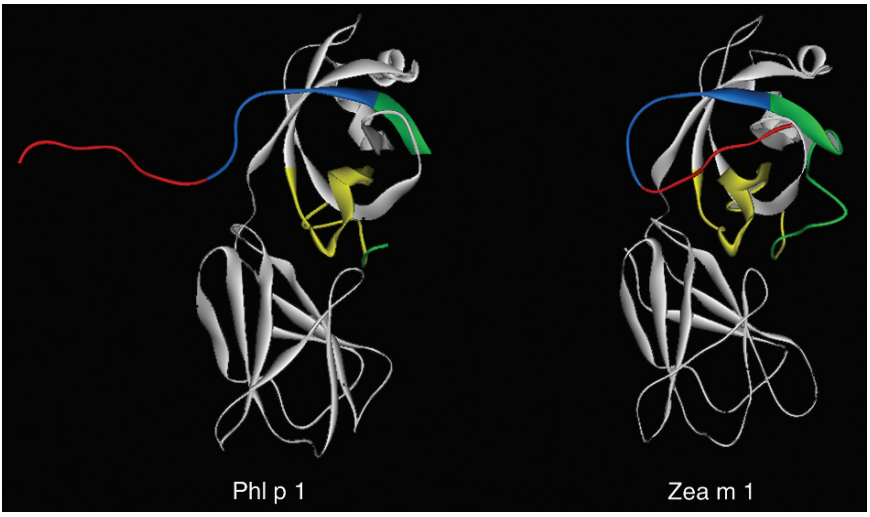

Fig. 1 Comparison of the three dimensional structures of Phl p 1 (with largely unfolded N terminal domain, PDB-code 1N10) and Zea m 1 (with a folded N-terminal domain) PDB-code 2HCZ [128]

availability of the source material. Obtaining large quantities of natural source material on a consistent basis can pose problems for natural allergen purification, especially to obtain gram quantities of pure protein. In contrast, recombinant allergen production can be scaled up to these levels and can be done under good manufacturing practice (GMP) conditions.

The other key advantage of recombinant allergens is that, unlike natural allergenic products, they can be precisely formulated into cocktails for diagnostic or therapeutic use at defined concentrations and dosage levels. It was recognized early on that a cocktail of two to four major allergens could be effectively used for diagnostic purposes either in vitro or in vivo [45]. Typically, formulations containing ~10 μg/ml each allergen could be used for skin prick testing and several studies showed good correlations between skin testing with purified natural and recombinant allergens [26, 46, 47]. However, the future of allergy diagnostics lies more in the use of purified allergens in *in vitro* diagnostics, rather than skin prick testing [5, 48, 49]. For example, a streptavidin-CAP assay has been developed using biotinylated allergens that enable IgE antibodies to specific allergens to be routinely measured by fluorescent enzyme immunoassay (FEIA) [50]. As with other diagnostic tests, FEIA uses a separate test to measure each IgE response in procedures that use relatively large amounts of serum. Recently, static or suspension microarray systems have been developed that enable IgE antibodies to multiple allergens to be measured simultaneously. Microarrays provide a profile of IgE responses to specific allergens. One commercial test uses a static allergen array and can measure IgE antibodies in four sera to ~75 purified allergens at the same time. Results obtained with the microarray correlate with FEIA using allergen extracts and the microarray uses only 30 μl serum [51] Similarly, fluorescent multiplex array technology has recently been developed which measures total IgE and specific IgE to ten purified allergens simultaneously using 20 μl serum [52]. Multiplex technologies are especially suited to large population surveys or birth cohorts for monitoring IgE responses to multiple allergens, and for pediatric studies where serum is often is short supply.

Structure and Function

Amino acid sequence homology searches allowed allergens to be assigned to different protein families based on their degree of sequence similarity and, in many cases, this allowed the biologic function of the allergen to be established. Thus Der p 1 was identified as a cysteine protease through its homology to papain and actinidin, and Der p 3, Der p 6 and Der p 9 were identified as serine proteases [53–55]. Structural data were used to show that these allergens had the appropriate amino acid residues at the enzyme catalytic sites and biologic experiments were performed to show that the purified allergens had the respective enzyme activity. The X-ray crystal structures of both the pro-enzyme and mature forms of Der p 1 have recently been determined at high resolution using *P. pastoris* expressed allergens [56, 57] (Fig. 2). The pro-enzyme has an 80 amino acid pro-peptide containing four

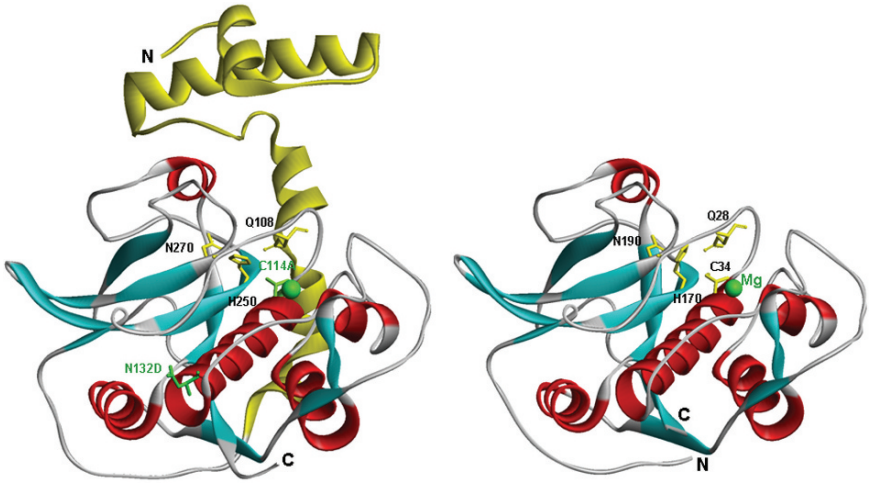

Fig. 2 Tertiary structures of the pro-enzyme and mature forms of rDer p 1 expressed in *Pichia pastoris*. The pro-region of Der p 1 comprises 80 amino acids in three α-helices (*left panel*), which are cleaved to form the mature Der p 1 cysteine protease allergen (*right panel*) [56,57]

alpha helices, which appear to be unique among the C1 family of cysteine proteases. The pro-peptide covers a large surface area of Der p 1 and inhibits binding of IgE antibodies [56, 58, 59]. Both structures also revealed that Der p 1 has a magnesium ion binding site, the function of which is not known. The crystal structure of mature Der p 1 suggests the possibility of dimer formation, which was proposed to stabilize the molecule and facilitate its persistence in the environment, even though natural Der p 1 is largely monomeric as assessed by size exclusion chromatography. The reversed situation was observed for the cat allergen Fel d 1. Natural Fel d 1 is a dimer of a heterodimer (chain 1 + chain 2). The first crystal structure (1PUO) was based on a recombinant protein in which the C terminus of chain 2 was linked to the N-terminus of chain 1. In this structure, only crystallographic contacts were observed rather than the expected stable interface. In a recently published structure (1EJN), which was based on a construct of chain 1 linked to the N terminus of chain 2, a properly assembled structure of the expected size was found, i.e., corresponding to the natural four-chain structure (Fig. 3).

To some extent, allergens segregate among protein families that are according to whether they are indoor allergens, outdoor allergens, plant and animal food allergens, or injected allergens:

- Indoor allergens (mite, animal allergens, cockroach, molds)

Proteolytic enzymes (serine and cysteine proteases), lipocalins (ligand-binding proteins), tropomyosins, albumins, calcium binding proteins, protease inhibitors [5, 60]

- Outdoor allergens (grass, tree and weed pollens, mold spores)

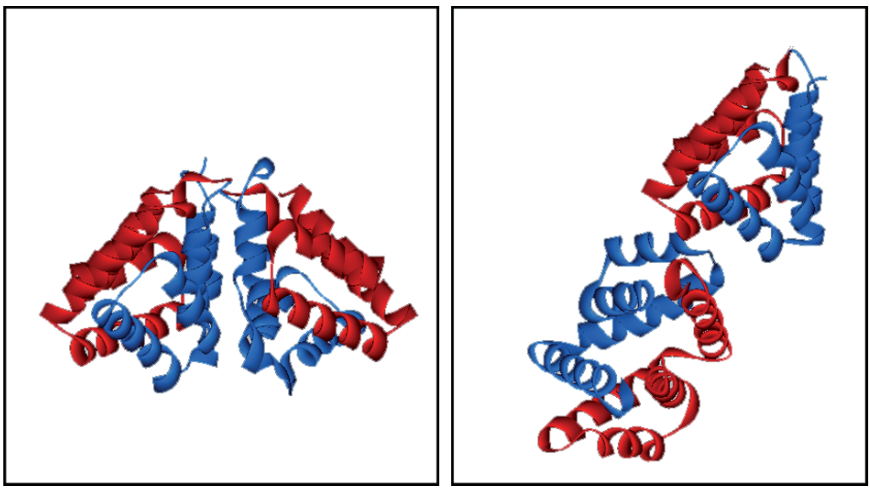

Fig. 3 Cat allergen Fel d 1: new structure. *Left panel*: Fel d 1 structure 1PUO as initially resolved from allergen expressed as chain2-chain1-single-chain construct (improperly dimerized) [91]. *Right panel*: latest Fel d 1 structure 1EJN derived from Fel d 1expressed as a properly dimerized chain1-chain2-single-chain construct [92]

Plant pathogenesis-related (PR-10) proteins, pectate lyases, β-expansins, calcium-binding proteins (polcalcins), defensin-like proteins, trypsin inhibitors [3, 13, 61, 62]

- Plant and animal food allergens (fruits, vegetables, nuts, milk, eggs, shellfish, fish)

Lipid transfer proteins, profilins, seed storage proteins, lactoglobulins, caseins, tropomyosins, parvalbumins [63–65]

- Injected allergens (insect venoms and some therapeutic proteins)

Phospholipases, hyaluronidases, pathogenesis-related proteins, asparaginase [66, 67].

Allergens belonging to these protein families are likely to have biologic functions that are important to the host. Proteolytic enzymes are involved in digestion, tropomyosins and parvalbumins in muscle contraction and profilins in actin polymerization in plants. The mouse lipocalin allergen, Mus m 1, is produced in the liver of male mice, secreted in large amounts in the urine and serves to mark the territories of male mice [68]. The cockroach lipocalin allergen, Bla g 4, is produced in accessory glands of the male reproductive system and has an as yet unknown reproductive function [69, 70]. Crystallographic studies showed that Bet v 1, a plant pathogenesis-related (PR-10) protein, contained a hydrophobic pocket that could bind brassinosteroids and functions as a plant steroid carrier. The PR-10 proteins are important in plant defense, plant growth and development [71].

In addition to biological function, the molecular biology of allergens has also explained the structural basis for clinical symptoms to apparently unrelated allergens, especially conditions such as oral allergy syndrome. Tree pollen allergic patients

frequently have oral symptoms (itching at the back of the throat) upon eating apples and other soft fruits. These patients are primarily sensitized to PR-10 allergens or profilins in the pollen and the response is mediated by the presence of structurally homologous allergens in fruits and vegetables. Mite allergic patients undergoing immunotherapy in Italy have experienced anaphylactic reactions on eating snails, which are thought to be due to cross-reactivity between tropomyosins [72, 73]. Bird fanciers may develop clinical sensitivity to chicken egg due to cross-reactivity between airborne allergens derived from the caged pet with proteins present in chicken egg yolk, which result in an atypical egg allergy: reactivity to egg yolk with little (if any) reactivity to egg white [74, 75].

Adjuvant Effects that Influence IgE Responses

One of the most important aspects of the biologic function of allergens is whether function can influence the ability of allergens to cause IgE responses or Th2 responses and inflammation, in general. Over the past 10 years, a significant body of evidence has been gathered, which suggests that the cysteine and serine protease activity of mite allergens (Der p 1, Der p 3, Der p 6 and Der p 9) potentiates IgE production through cleavage of CD23 from activated B cells and CD25 from T cells [76, 77] (Table 2). The enzymatic activity of these allergens disrupts the lung epithelium through cleavage of tight junction membrane proteins (occludin and claudin-1), which increases bronchial permeability and enables access to sub-epithelial, dendritic antigen-presenting cells [18]. Der p 1 also causes release of pro-inflammatory cytokines from bronchial epithelial cells (IL-6, IL-8, GM-CSF), and Th2 cytokines from mast cells and basophils (IL-4, IL-13) (Table 2) [19, 78]. Cytokine release from epithelial cells by mite protease allergens is mediated by protease-activated receptor 2 (PAR-2) [17, 79, 80]. Most recently, animal experiments showed that production of total IgE and IgE anti-Der p 1 was significantly reduced in mice immunized with rDer p 1 that was inactivated using the cysteine protease inhibitor E-64 [76, 81]. The hypothesis that there are synergistic effects of mite allergens on IgE production, Th2 responses and inflammation is attractive because it provides an explanation for the strong epidemiological association between mite allergy and asthma [1, 82]. Deposition of mite fecal particles in the lung releases a package of enzymes that can contribute towards both the immediate and late phase reactions that characterize the asthmatic response.

This theory falls short in explaining why other asthma-associated allergens are not proteolytic enzymes. Cockroaches are an important cause of asthma in inner city populations in the USA and in other parts of the world [83–85]. However, none of the allergens that have been cloned from German or American cockroach are proteolytic. The most important cockroach allergen in terms of IgE sensitization is Bla g 2, which elicits IgE responses in ~60% of cockroach allergic patients. Although Bla g 2 belongs to the aspartic protease family of enzymes, it has critical substitutions in the catalytic site and other parts of the molecule that render the protein inactive as

Table 2 Immunobiologic effects of proteolytic enzyme allergens produced by dust mites

Der p 1:				
• Cleaves CD23 from activated B cells				
• Cleaves CD25 from T cells,				
• Causes detachment of bronchial epithelial cells from lung segments				
• Disrupts the architecture of bronchial epithelium by disruption of intercellular tight junctions				
Mite proteinases (Der p 1, Der p 3, Der p 6 or Der p 9)				
• Induce pulmonary epithelial cell detachment				
• Induce production of proinflammatory cytokines				
(IL-8, IL-6, MCP-1 and GM-CSF) in vitro				
• Induce IgE-independent mast cell and basophil degranulation, and release of IL-4 and IL-13 in vitro				
Foods				
Prua v 2	2AHN			
Pru p 3	2ALG	2B5S		
Ric c 3	1PSY			
Zea m 1	2HCZ			
Injected allergens				
Api g 1	2BK0			
Hyaluronidase	1FCQ	1FCU	1FCV	2J88
Ves v 2	2ATM			
Ves v 5	1QNX			

Additional structures are available for Der p 2 (2F08) and Bet v 1 (1FSK, 1LLT and 1QMR)
CCD complex carbohydrate determinant

an enzyme. These substitutions are apparent in the X-ray crystal structure and the lack of enzyme activity has been confirmed in functional assays (Fig. 4) [86–88]. Bla g 2 belongs to a sub-group of inactive aspartic proteases, termed pregnancy-associated glycoproteins (PAG), whose biologic function is unknown. Bla g 2 has a deep cleft within the molecule, which may serve to bind a ligand of some kind. Non-enzymatic ligand-binding allergens associated with asthma do not conform to the protease theory. Other examples include Der p 2, which is a lipid binding protein, homologous to MD-2 and Niemann-Pick disease C2-type protein [89, 90], and mammalian allergens, which are predominantly lipocalins and albumins. Fel d 1, the major cat allergen belongs to the secretoglobin protein family, which suggests that its function is to control inflammation at mucosal surfaces (Fig. 3) [91, 92].

An obvious implication from the structure and function data is that we should look for other potential adjuvants, co-factors or biologic effects that may play a role in influencing IgE responses and/or asthma. Let's take another look at the mite fecal particle. Platts-Mills and colleagues have recently shown that in addition to proteolytic enzymes, mites feces also contain endotoxin, bacterial DNA and mite DNA, elements which are known to act a potential adjuvants [20]. Endotoxin binds to Toll-like receptor 4 (TLR-4) on antigen-presenting cells and low-dose endotoxin exposure favors the development of Th2 [93, 94]. Conversely, both bacterial DNA and mite DNA bind to antigen-presenting cells through TLR-9, are relatively

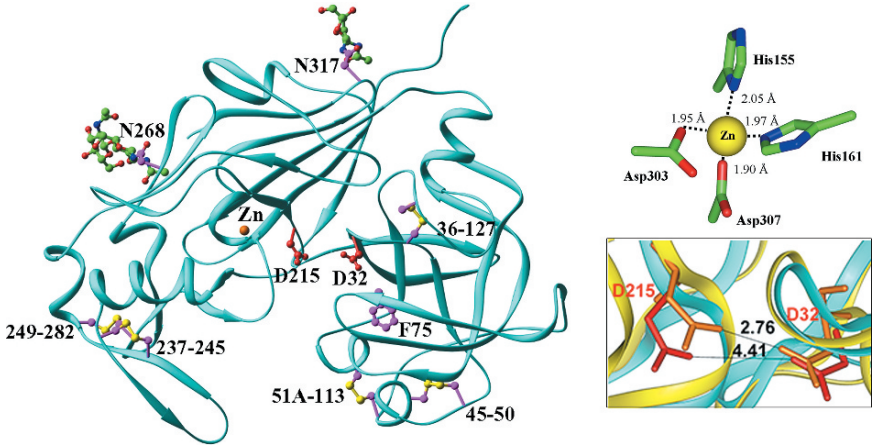

Fig. 4 Crystal structure of cockroach allergen, Bla g 2, an inactive aspartic protease (1YG9). *Left panel*: Bla g 2 structure showing the region of the catalytic site (D215, D32), the zinc ion, disulphide bonds and N-glycosylation sites. *Right upper panel*: residues involved in zinc ion binding. *Right lower panel*: inter-atomic distances and aspartate positions of Bla g 2 (blue ribbon) and pepsin (yellow ribbon). Reprinted with permission from Ref. [88]

unmethylated and contain immunostimulatory motifs that favor Th1 responses [95]. The balance between these various adjuvants coupled with host immune response genes may determine whether or not an individual makes an IgE response. Accurate measurements of enzyme activity, endotoxin and DNA in mite feces have not yet been made, but estimates of the doses that can be delivered to the lung will be important in establishing the relevance of these adjuvants in generating local Th1 or Th2 responses. In contrast to mite and cockroaches, DNA from animal allergens (cat, dog, rat, mouse) is fully methylated and these allergens are not enzymes. The evolutionary distance of these species from humans is much less than for mites, cockroaches, pollens and fungi and it has been proposed that evolutionary distance plays a role in determining immune responsiveness: these allergens are more closely related to human proteins and, therefore, inherently less immunogenic [20]. This may have credence with respect to IgE, but not necessarily with IgG antibody responses, which are common in humans who are persistently exposed to animal allergens. The weak immunogenicity of lipocalins has also been suggested to favor IgE induction [96]. At a structural level, mammalian lipocalin allergens are no more conserved than lipocalins from other species. The lipocalin family comprises over 50 proteins that show only 20–25% amino acid sequence homology. Lipocalins have a conserved tertiary structure comprising a C-terminal α-helix and an eight stranded anti-parallel β-sheet barrel, with three structurally conserved regions that form the ligand-binding pocket. The amino acid residues in this pocket are conserved irrespective of the host species of the lipocalin [97, 98].

A further adjuvant that should be considered in relation to AD is staphylococcal enterotoxin B (SEB). Most AD patients have high levels of IgE antibody to mite

and other inhaled allergens to which they are exposed. The patients also mount strong Th2 responses to allergen and have allergen specific T cells ($CD4^+$ and $CD8^+$) in the peripheral blood and in the skin. Application of mite allergen to abraded skin for 48 hours can reproduce eczematous lesions in patients with AD [99]. Recent studies using HLA Class II tetramer cell sorted populations to present a Der p 1 epitope to T cells have shown that SEB enhanced T cell responses to Der p 1. The SEB-promoted HLA class II expression on antigen presenting keratinocytes and amplified T cell cytokine production, principally IL-4 and IFN-γ [100]. The SEB acts as a potent adjuvant for allergen specific Th2 cells by promoting class II expression on epithelial cells (through IFN-γ) and by IL-4 mediated amplification of $CD4^+$ T cells. Thus bacterial superantigens should also be considered as adjuvants in the immune response to allergens.

Surprisingly, the most relevant adjuvant for the production of IgE seems to be IgE itself. In the presence of IgE antibodies, the production of IgE antibodies to other epitopes is facilitated largely via mast cell induced local IgE production [101–104, 105]. Some of these newly induced IgE antibodies are directed to epitopes on the same allergen that the pre-existing IgE antibodies recognize. This is an example of classical epitope spreading, which does not require the involvement of new Th2 cells. However, based on the spectrum of proteins from a single allergen source material that is recognized by a typical allergic patient, the epitope spreading extends beyond this initial allergen and involves epitopes on other antigens that happen to be present in that microenvironment. In order to recruit Th2 help for this new specificity, new Th2 cells need to be involved. Since this process of extended epitope spreading seems to be common, we have to assume either that Th2 recruitment is not a severely restrictive requirement, or, alternatively, that allergen-specific Th2 involvement is not required for this extended epitope spreading. Cells that are activated via an allergen–IgE interaction might provide the signals needed for isotype switching in the presence of pre-existing IgE.

Allergen-Specific Immune Responses

It is interesting to note how diverse (and occasionally contradictory) current ideas on the nature of allergenicity are. This obviously reflects our lack of critical information, largely due to the absence of animal models that closely mimic human sensitization. As already alluded to, allergens have been proposed to special immunogenic properties or carry a special “danger signal” [106]. On the other hand, allergens have been suggested to lack features that make other proteins strong immunogens.

In this section, we will give possible reasons why allergy is not simple. We will argue that the IgE isotype switch is not really exceptional and is not the only rate-limiting step towards IgE production. Furthermore, we will discuss why it is unlikely that allergens are an exclusive set of proteins with distinctive features, even if some features may enhance a protein’s allergenic potential.

We have two indisputable facts. First, most immune responses do not induce a noticeable IgE antibody response. This is true for many common microbial pathogens. Second, the majority of the human population (possibly only a small majority, but it is still generally assumed to be more than 50%) does not develop an allergy and those who do develop an allergy do not become allergic to every antigen, not even to every allergen. The 1,000–10,000 lower plasma level of IgE compared to IgG and the equally lower relative incidence of IgE myelomas compared to IgG myelomas all indicate that the production of IgE antibodies is a rare event compared to the production of IgG. Why is IgE production such a rare event? Most allergist/immunologists would argue that the requirements for a class switch to IgE are only rarely met. In this view, the isotype switch is the rate-limiting step that protects most of us from developing allergies. Since even patients with an allergy do not develop IgE antibodies to all antigens in their environment, the implication is that allergens are exceptional antigens that somehow overcome the barriers that usually prevent the IgE isotype switch.

However, if the class switch to IgE was an exceptional event and if allergens were exceptional antigens, allergy should be simple. It is not. Hundreds of different proteins have been found to be allergens. Moreover, an allergic patient will not produce a single IgE antibody to a single allergen molecule, but will typically react to several allergens from the allergenic source (which strongly argues against the notion that allergens are very exceptional proteins) and produce IgE to several epitopes per allergen. This implies that in a number of B cells the IgE isotype switch has been induced, rather than in a single clone that managed to pass the very restrictive switch requirements. This multi-clonal response is hard to reconcile with the concept of a heavy roadblock on an otherwise smooth the differentiation pathway towards IgE that would only allow B cells to pass under exceptional conditions.

A hint regarding the nature of the second rate-limiting step (subsequent to the isotype switch) came from work by Brinkmann and Heusser, who showed that clones resulting from IgE-switched B cells are much smaller than clones resulting from IgG-switched B cells [107]. Another hint came from mouse experiments, in which IgE-switched memory B cells proved virtually undetectable following regular IgE induction protocols (but were easily detectable following administration of heterologous antibodies to IgD, a procedure known to induce high circulating IgE levels) [108]. An analogous observation has been observed in human peripheral blood: in this compartment IgE switched cells are not only rare, but the few that can be found prove to be pre-plasma cells rather than B memory cells [109]. Molecular biology also provided an intriguing anomaly that is relevant in this context. Karnowski et al. found that the IgE-switched B cell has a problem in producing membrane-anchored antibody, because of a structural defect in the mRNA [110]. This lack of membrane immunoglobulin expression is likely to compromise the proliferation and survival potential of the epsilon-switched B cell.

In addition to these indications that the route from naïve B cell to IgE-producing plasma cell contains (at least) two rate-limiting steps (not only a demanding isotype switch to epsilon, but also a compromised survival/proliferation potential of IgE-switched B cells), information is becoming available that indicates modulating

effects of the type and "matrix" of the allergen on the type of immune response upon allergen exposure. Best known is the "modified Th2 response" [21]. In the original description, this terminology was used to classify a subgroup in population studies: subjects with IgG4 antibodies to allergen (cat allergen, in this case), but without IgE antibodies. The rationale to use allergen-specific IgG4 as the readout was that IgG4 antibody production requires activation of allergen-specific Th2 cells (as does IgE). The authors drew attention to this subgroup of subjects, because it convincingly demonstrated that not all Th2 responses result in IgE synthesis. Two additional observations are relevant. First, subjects with a modified Th2 response were predominantly found in the sub-group with the highest allergen exposure. Previous experiments suggested that this effect was not due to a modulating effect of antigen dose on the Th1/Th2 balance [111] Second, no modified Th2 responses were found for mite allergens, suggesting that there was a dichotomy between allergens that induce modified Th2 responses and those that do not [112]. Or, more likely, allergens can be ranked according to their potential to induce a modified Th2 response rather than a "non-modified" Th2 response, with cat at one side of the spectrum and mite at the other. An alternative description of these phenomena has been presented elsewhere in which the focus is on the classification of allergens (rather than on the classification of immune responses) [113]. Allergens that are likely to induce a 'non-modified immune response', such as the 'classical' atopic allergens from mites and pollen, are characterized by their low propensity to induce IgG4 (but also IgG1) antibodies in the absence of an IgE response. In contrast, allergens on the other side of the spectrum induce 'regular' immune responses, usually with IgG antibody in the absence of IgE. So, the original concept has been fine-tuned in two ways: firstly, the IgG response is not exclusively focused on IgG4, but includes IgG1 as well, and may even lack IgG4. It is important to stress that not all IgG assays are able to make this kind of distinction. In contrast to reports claiming similar (or even increased) levels of IgG antibodies to pollen or mite allergens in the absence of IgE antibodies, reports that indicate a striking lack in IgG reactivity in the absence of IgE antibodies are based on high-affinity assays using fluid-phase, radiolabeled purified major allergens [114, 115]. Secondly, IgE responses may occur occasionally with 'modified Th2 allergens'. This description allows a statistical classification of allergens based on the relative prevalence of IgG antibodies in the presence and absence of IgE antibodies to that allergen (in populations with similar levels of allergen exposure).

How could these differences in allergen-induced immune responses be explained? Our hypothesis is based on the additional observations (i) that IgE responses occur either via a direct isotype switch (i.e., from mu to epsilon) or via an indirect switch (from mu via an intermediate isotype, often gamma4 to epsilon; (ii) that mice showing that a weak antigenic stimulus tend to result in a direct switch to epsilon, whereas upon a strong antigenic stimulus IgE production occurs mainly via an indirect switch [116]. According to our working hypothesis, classical atopic allergens (e.g. from pollen or mites) are weak antigens that fail to give B cell responses most of the time, but may occasionally induce a weak response in several (if not all)

isotypes including IgE, particularly in people with hyperreactive B cell system because of their genetic predisposition. Such immune responses do not result in active germinal centers, but may occur in extra-nodal tertiary lymphoidal structures, for example, in the airway mucosa [102–105, 117, 118]. Allergens at the other end of the spectrum are more likely to induce a brisk immune response, which results in a more selective and expansive immune response, involving active germinal centres in secondary lymphoid tissues. In order to explain why there is so little IgE production, we assume that individuals are protected by the activity of the germinal centers for the removal of IgE-switched B cells. Some IgE may be produced in this situation, but mostly outside the germinal centers via allergen-specific IgG4-switched memory B cells.

Allergens That Make a Difference

Some allergens are more important than others. Previously, allergens have been classified as ‘major’ or ‘minor’ based on the prevalence of IgE sensitisation in a selected population of allergic patients (usually > 50% prevalence defines a major allergens and < 20% is minor). This criterion is dependent on the sensitivity of the IgE detection method. As the sensitivity of these assays has increased, so has the number of ‘major’ allergens. To be entered into the WHO/IUIS Allergen nomenclature, all that is needed is to show that the allergen elicits an IgE response in five patients (the objective of the nomenclature is to name allergens, not to assign their importance) [7, 34]. However, it is clear from many studies that some allergens play a pre-eminent role in causing immune responses in atopic individuals, are better marker proteins for immunologic, clinical and epidemiologic studies, and are usually considered to be high-profile targets for allergy diagnostics and therapeutics. Table 3 lists eight criteria for defining the properties of these ‘allergens that make

Table 3 Eight criteria for defining allergens that make a difference

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

a difference'. Examples of allergens that we consider to fulfill most of these criteria are as follows:

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

The mite Group 1 and Group 2 allergens cause sensitisation in > 80% of mite allergic patients and absorption of these allergens from mite extracts can significantly reduce allergenic activity. They have been consistently used as markers of the immune response to mite in patients with rhinitis, asthma and AD and assays for Groups 1 and 2 are routinely used for environmental exposure assessment. IgE responses to Groups 1 and 2 can account for a significant proportion (10–20%) of total IgE. Similar data fulfilling our criteria has been obtained for Fel d 1. Absorption of Fel d 1 from cat extracts removes 60–90% of the allergenic activity. Fel d 1 has been used for exposure assessment and, because it is the most dominant allergen produced by cats, Fel d 1 has been used in clinical trials to develop new cat vaccines [11]. Rat n 1 and Mus m 1 are dominant rat and mouse allergens and are the allergens that are targeted in studies of occupational exposure. Can f 1 is not included in the list because even though this allergen has been useful for studies of dog allergy, it does not fulfill the criteria listed in Table 3 and development of a vaccine for dog would require more thorough evaluation of Can f 1 and other dog allergens. The same arguments apply to cockroach allergens. While ~60% of cockroach allergic patients make IgE responses to Bla g 2, and the allergen appears to be potent based on exposure levels, it has been difficult to assess IgG responses and T cell responses to Bla g 2. Certainly, Bla g 2 appears to be the most important cockroach allergen identified to date, but more comprehensive data are needed.

Among pollen allergens, Bet v 1 is pre-eminent in importance: 95% of birch pollen allergic patients are sensitised to Bet v 1, there is a wealth of immunologic data about the allergen, and clinical trials to develop recombinant vaccines using Bet v 1 or Bet v 1 derivatives are underway. Recombinant Bet v 1 is almost indistinguishable from the natural molecule and is produced under GMP conditions for therapeutic purposes. Amb a 1 has been used as a surrogate immunologic marker for ragweed since the classic studies of King, Norman and Lichtenstein in the 1960s [119]. Gleich first demonstrated that IgE to Amb a 1 could account for a high proportion of total IgE [120]. Natural Amb a 1 has been produced under GMP and coupled to CpG nucleotides for use in immunotherapy trials [121]. The timothy grass pollen allergens, Phl p 1and Phl p 5, have sensitization rates of 60–90% among grass pollen allergic patients, have been produced under GMP conditions and were recently used in a successful trial of allergen immunotherapy [28]. Ara h 1 and Ara h 2 comprise a high proportion of the extractable proteins in peanut

(10–15%) and cause sensitization in 60–90% of peanut allergic patients. They are the most extensively characterized peanut allergens and are being used in the formulation of an enteric vaccine [122].

The Final Frontier

From the perspective of an allergic patient, the end-game of molecular biology of allergens should understandably be the development of safer and more effective allergy vaccines. This an exciting time because much progress has been made over the past 20 years and, especially in Europe, new approaches to allergy vaccination are being tried and tested. In 2000–2004, the WHO/IUIS Allergen Standardization Committee, embarked on a program to develop new allergen standards based on purified allergens (the EU CREATE program) [123]. The aim was to develop international standards whose potency and purity could be verified worldwide using standard immunochemical and proteomic techniques. Purified natural and recombinant allergens were directly compared for allergenic activity and structural properties, and Enzyme-Linked ImmunoSorbent Assay (ELISA) systems for each allergen were validated. Not surprisingly, the allergens selected for this study were 'allergens that make a difference': Der p 1, Der f 1, Der p 2, Der f 2, Bet v 1, Phl p 1, Phl p 5 and Ole e 1. Overall, there was a good correlation between allergenicity of recombinant and natural allergens and, as a result, two allergens (Bet v 1 and Phl p 5) were chosen for the production of a recombinant allergen standard. These standards are currently being prepared under the auspices of the European Directorate for the Quality of Medicines (EDQM).

Purified allergen standards are essential to enable vaccine manufacturers to formulate new products. Another essential pre-requisite is the production of allergens under GMP conditions, which to date includes recombinant Bet v 1, Phl p 1, Phl p 2, Phl p 5, Phl p 6 and natural Amb a 1. Purified allergens, derivatives, hypoallergenic forms and peptides are now being tested in clinical trials. Perhaps the most promising were the results of a double-blind placebo controlled study using a cocktail of recombinant timothy pollen allergens in a conventional subcutaneous immunotherapy regimen. The treatment was designed to achieve a maintenance dose of 5–10 μg each allergen and the clinical effect was striking: a 39% reduction in symptom scores in the actively treated group, compared to placebo, which was accompanied by a 2–3 log increase in allergen-specific IgG1 and IgG4 levels [28]. Moreover, the prevalence of adverse reactions was low (10% active, 6% placebo) and limited to mild local reactions. Less striking were the results of trials using Bet v 1 fragments (*E. coli* expressed half-molecules) and trimers. Administration of these derivatives did not result in compelling changes in IgG or IgE antibody levels or in clinical efficacy [124]. The use of purified natural Amb a 1 coupled to immunostimulatory sequences (AIC) offered great promise. The allergen conjugate masked cross-linking of IgE by Amb a 1 and triggered TLR-9 receptors on dendritic cells, thereby enhancing a shift from Th2 to Th1 responses. A pilot study showed that administration of a six-dose regimen of AIC, with a maintenance

dose of 12 μg, reduced symptoms in the subsequent ragweed season and that this symptomatic improvement was maintained for a second year following treatment [121, 125]. However, no significant differences were seen in nasal symptom scores in a multi-centre Phase III clinical trial comparing low or dose AIC (drug name TOLAMBA) with placebo. This, as yet unpublished, study involved approximately 240 subjects in each group. The lack of an effective clinical outcome has been attributed to enrollment of allergic patients into the study who were only mildly sensitive to ragweed. Such are the vagaries of clinical trials.

Other vaccine products in the early stages of testing include tolerogenic T cell peptides, chimeric human Fc gamma/allergen proteins (which inhibit IgE cross-linking on mast cells), molecular antigen translocating (MAT) molecules (target allergen to the major histocompatability complex [MHC]) and enteric vaccines using recombinant peanut allergens expressed in *E. coli* [11, 122, 126, 127]. Over the next 5 years, some of these vaccines will enter clinical trials and it is possible that in future the number of allergen-specific options therapeutic options available to allergic patients will increase. Already in Europe, there is a choice of subcutaneous immunotherapy or various sublingual immunotherapy products using natural allergens. Recombinant allergens offer greater sophistication in targeting specific allergens, more uniform dosing and a more strategic and mechanistic approach to treatment. Ultimately, this should result in vaccines with greater efficacy that will more closely resemble pharmaceuticals than biological products and which will significantly improve the treatment options for allergic patients.

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Role of Allergens in Airway Disease and Their Interaction with the Airway Epithelium

Irene Heijink and Henk F. Kauffman

Introduction and Background View

The epithelial surface of the airways is an ingenious system for exchange of gases, inhaled oxygen for exhaled carbon dioxide, and an important contact organ with inhaled bioorganic substances from the outside world. By a sensitive intercellular contact system, the epithelial cell layer carefully selects which (small) ions and bioorganic molecules are allowed to be transferred over the epithelial layer. Contact between the outside world and the lung tissue is critical for transfer of bioorganic molecules over the epithelial layer. Integrity of the epithelial cell layer is therefore one of the major hallmarks for a balanced ecology of the immune system. Disturbed interactions with inhaled bioorganic molecules from the outside world may finally lead to hyperresponsiveness of the airways to environmental factors in asthmatic patients. Generally, this bronchial hyperresponsiveness (BHR) in asthmatic reactions maybe in part due to airway remodeling as a result of failure intercellular interactions that determine the integrity of the epithelial layer and/or disruption of integrity by (aggressive) components present in inhaled biological substances (antigens/allergens). When the epithelial barrier is disrupted, a repair response will be initiated, in which epithelial cells adopt a migratory phenotype to cover the area of damage. In addition, the epithelial cells will be activated with respect to secretion of growth factors and also proinflammatory cytokines in order to alarm the environment. Subsequently, cells will proliferate and finally redifferentiate to form a functionally intact epithelial barrier. The repair response maybe aggravated by a genetically determined Th2-type immunological response. The release of growth factors and airway inflammation are basic to the airway remodeling as is seen in allergen-driven asthmatic reactions. In this chapter, we will describe the characteristics of aeroallergens and their interaction with the airway epithelial cells of asthmatic individuals with emphasis on the vulnerability of the epithelial cell layer due to

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_17, © Springer 2009

integrity/connectivity, resulting in a continuous state of repair and remodeling of airways in asthmatic patients.

Integrity of the Epithelial Membrane; Environmental Factors and Genetic Variations in Structural Cell Adhesion Molecules in Asthma

Epithelial integrity is maintained by intercellular contact formation, which decreases permeability and prevents environmental agents to pass the epithelial barrier. Cell–cell contact is mediated by homophilic interactions of the adhesion molecule E-cadherin in so-called adherens junctions. E-cadherin is linked to the cytoskeleton through its association with catenins (α-, β-, and p120-), which stabilizes cell–cell contacts. In addition to and more apical of the adherens junctions, tight junctions provide intercellular contact formation. Tight junctions are macromolecular assemblies of proteins that form contiguous rings at the apices of epithelial cells. Inhaled substances including diesel exhaust particles and allergens may act to disrupt the epithelial barrier by destruction of epithelial junctions. Generally, these harmful agents are effectively neutralized in healthy subjects. However, in asthmatic patients, it is increasingly recognized that epithelial cells behave abnormally, showing structural aberrancies [1, 2]. Increased permeability of the bronchial epithelium to house dust mite allergen has been observed [3]. The airway epithelial barrier is often disrupted in asthma patients, with evidence for shedding of ciliated cells and downregulation of E-cadherin at the sites of epithelial detachment. In animal models, reduced E-cadherin-mediated intercellular contact during the asthmatic response was correlated with increased permeability and BHR [4–7]. Downregulation of E-cadherin is known to be an important component of epithelial-to-mesenchymal transition (EMT), a process involved in cell invasion/migration, tissue remodeling and repair. Whether EMT occurs in the asthmatic airways and contributes to the increased number of myofibroblasts and airway remodeling in asthma, however, is currently not known.

Furthermore, the basis of this abnormal phenotype of the airway epithelium in asthma is still undefined. Possibly, the bronchial epithelium lacks the ability to inactivate allergens due to genetic variance in the expression of protease inhibitors, e.g., serine protease inhibitor SPINK5 [8], although this has remained controversial [9]. Alternatively, the extent of epithelial damage in asthma maybe due to an increased vulnerability of the epithelium or an inadequate repair mechanism with inability to restore cell–cell contacts in response to damaging stimuli. This is supported by the increased expression of repair mediators, e.g., CD44, epidermal growth factor receptor (EGFR), and TGF-β at sites of ciliated cell detachment [10–12]. It is of interest to note that TGF-β can induce downregulation of E-cadherin and that a polymorphism in the TGF-β promoter region has been associated with the diagnosis of asthma [13]. Additional genetic studies have shown associations for structural cell adhesion molecules involved in epithelial integrity

and the underlying mesenchymal cell phenotype. One of the important candidates that is thought to play a role in both epithelial cell integrity and airway remodeling is the family of "a disintegrin and metalloproteinase" (ADAMs). This family of molecules serves to regulate formation of cell–to cell and cell–matrix contacts and have been shown to be important in the regulation of cell proliferation, cell survival, cell migration, and airway remodeling. Recently, it has been shown that several single-nucleotide polymorphisms (SNPs) in ADAM33 are strongly associated with asthma and BHR [14–19]. Furthermore, ADAM8 was shown to be important in the activation of airway inflammation by allergens and *Aspergillus fumigatus* in a mouse model of asthma [20]. Additional ADAMs of which variable gene expression may have implications for asthma are ADAM9 and 10. These ADAMs are of particular interest with respect to their regulation of E-cadherin. Studies in mouse keratinocytes and fibroblasts have demonstrated that ADAM10 is responsible for the shedding of E-cadherin [21]. Furthermore overexpression of ADAM9 enhances growth factor-mediated recycling of E-cadherin in human colon cancer cell line HT29 cells [22]. Besides ADAMs, metalloproteinases (MMPs) are determinant factors in epithelial integrity, repair, and invasiveness. They play a crucial role in remodeling of the extracellular matrix, induce release of growth factors, e.g., TGF-β and EGF, and have also been implicated in E-cadherin ectodomain shedding. MMP-9 is the predominant MMP in asthma although MMP-2, -3, and -12 are elevated as well. During acute asthma exacerbations, the ratio of MMP-9 and its inhibitor, tissue inhibitor of metalloproteinase-1 (TIMP-1), is increased, which may promote airway remodeling [23]. A polymorphism in MMP9 has been associated with childhood asthma [24] and an association between a polymorphism in the TIMP-1 gene and asthma has been observed in Australian women [25].

Together, interaction of environmental factors with the epithelial cell layer is determined by both the integrity of the epithelial cell structure, which is in part dependent on genetic heritance, and the characteristics of components present in the inhaled bioorganic substances.

Innate Defence to Allergens

Allergens are continuously inhaled generally as particulate materials, e.g. pollens, bacterial and fungal spores, fungal mycelium fragments, house dust mite fecal pellets, etc. Allergen-containing particles generally range from 50 ∝M (grass pollen, fungal (Alternaria) spores to 5 μM particles (spores of fungi and bacteria). Larger-sized particles predict deposition in both upper airways (nose) and the upper part of the lower airways, while the smaller particles become entrapped in smaller airways. Particles that are deposited on the mucosal surface will be eliminated by combined activities such as transportation, innate binding to soluble components in the mucosal layer, and innate recognition by immune- and nonimmune cells (tissue cells) of the airways [26].

After deposition on the airway mucosal surface, particles are trapped in the mucus and eliminated by transportation to the oropharyngeal cavity by ciliary action and swallowed. Although ciliary clearance is apparently a passive mechanism, the rate of transportation is influenced by factors released from the particles (enzymes, toxins) that may facilitate the ciliary movement. During this transport phase, the particles start to release their allergenic molecules. Most allergens are soluble components that are rapidly released from their particles, mainly glycoproteins and often different proteases, generally within minutes. Other particles, e.g., spores from fungi release their bioactive molecules at a low rate (several hours), generally during the phase of germination. Elimination of large quantities of inhaled allergens during peak exposure (e.g., spring time for tree pollen) is an energy-dependent process and may become a challenge for both patients with airway disease, e.g., asthma and maybe associated with respiratory mortality [27]. Furthermore, disturbed mucociliary clearance in asthma maybe derived from the hypersecretion of mucus that is observed in asthma patients in conjunction with the apparent shedding of ciliary cells.

The release of allergens and antigens may initiate a second innate defence reaction by components released by airway epithelial cells that actively neutralize nonself particles (microorganisms, pollens, house-dust mite fecal pellets, enzymes).

These components include molecules with antibacterial and antifungal properties (lysozyme, defensins, cathelicidins, secretory leukoprotease inhibitor (SLPI), and elafin) [28–30]. A second group of innate soluble components, the calcium-dependent collectins, are produced by epithelial type II cells and nonciliated bronchiolar cells such as the pulmonary surfactant proteins, SP-A, SP-D, and serum-derived mannose-binding protein (MBP). This group of molecules interact with a variety of carbohydrates, e.g., mannose, fucose, glucose, and inositol that are found at the surfaces of bacteria, fungi, and viruses [31–33]. The role of these defensive molecules and their role in diminishing the allergen-induced inflammatory reactions has been described recently [34]. The in vitro and in vivo data described in this latter review indicate that surfactant molecules may play an important role both in the elimination of allergens by direct binding to glycosylated moieties and by downregulating of inflammatory reactions by inhibition of different cell types.

Recognition of Allergens by Innate Receptors Expressed by Airway (Epithelial) Cells

Protease Versus Nonprotease Innate Recognition Systems

Inhaled air contains large quantities of allergen-containing particles such as grass- and tree pollen, excreta of insects and mites, and degraded plants products. In order to recognize these bioorganic particulates, airway epithelial cells, mast cells, and phagocytes (monocytes, macrophages, and dendritic cells) express groups of innate

receptor molecules. In vitro studies with different allergenic extracts have shown that epithelial cells can be activated by proteases present in different allergen extracts. However, activation with house dust mite extracts is also found under conditions that are heat- and protease inhibitor-resistant, indicating activation by additional pathways [35, 36]. Both protease-dependent and protease-independent activation of airway epithelial cells may facilitate transport of allergens over the epithelial cell layer, by opening of tight junctions and/or loosening of cell to cell contacts.

Enzymatic activities have been proposed as factors that may facilitate sensitization to environmental allergens [37]. In vitro studies have shown morphological changes of cells in culture and production of cytokines, reflecting loss of cellular contacts and activation of epithelial cells. Similarly, fungal extracts showed both morphological changes, e.g., shrinking and/or shedding of epithelial cells, that were dependent on the serine proteinase activity [38, 39], which was dependent on the activity and quantity of the proteinases present in the fungal extracts [40]. Proteinases in house-dust mite extracts induced the release of proinflammatory cytokines, changes in permeability, and damaging of epithelial cultures [37, 41]. Both serine proteinases (Der 3, Der p 6, Der p 9) and the cysteine proteinase (Der p 1) caused detachment and activation of epithelial cell cultures [42, 37, 43]. Der p 1 was shown to disrupt cellular contacts by degradation of tight junction molecules such as occludin and ZO-1 as well as adherens junction molecule E-cadherin, thereby augmenting mucosal permeability [44–46].

It maybe proposed that facilitation of allergen transport over the epithelium by proteolytic activity will result in enhanced IgE antibody formation to both the proteinase molecules as well as to the nonprotease components in the allergenic source. This facilitating mechanism may explain why nonenzymatic components such as Der p 2 also can behave as major allergens in house dust mite extracts.

Clearance of these allergenic particles is actively guided by innate recognition, similarly to the mechanisms used for antimicrobial responses. For detection of these nonself substances, the innate immune system uses a wide variety of receptors. The molecular structures detected by innate receptors were originally called pathogen-associated molecular structures (PAMPs) [47, 48]. Toll-like receptors (TLR) was firstly described as an important component against fungal infection [49], and described in humans 1 year later [48]. The TLRs 1, 2, 4–6 were shown to bind specific surface markers of microorganisms, e.g., lipopolysaccharide (LPS) and peptidoglycans, while TLR3, and TLR 7–9 detect viral RNA and hypomethylated CpG DNA motifs. Several other pattern recognition receptors were detected such as the nucleotide-binding oligomerization domain receptors (NOD1 and NOD2) scavenger receptors (SR-A, SR-B etc.) [50–52], C-type lectin receptors, e.g., the mannose receptor [53, 54], macrophage galactose-type lectin recognition receptors (MGL) [55], and dectin-type receptors [56–59]. Activation of TLRs generally results in the activation of intracellular signaling cascades that has been reviewed by different authors [60, 61]. Activation of these receptors by bacterial or fungal substances may result in a rapid antibacterial/antifungal responses and the induction of an inflammatory response by the release of different cytokines and chemokines. Recently it has even been shown that cadherins themselves may

become subject of attachment of different fungi and yeasts, thereby also inducing phagocytosis of fungal spores by airway epithelial cells [56]. Airway cells that bear TLRs and C-type lectin receptors are macrophages and monocytes, while also epithelial cells generally showed expression of TLRs, dectins, and MR [53, 56, 62–64].

The hygiene hypothesis suggested the necessity of bacterial exposure (infection) in the prevention of atopic asthma [65, 66]. Many bacterial substances (LPS, CpG, peptidoglycans) apparently promote Th1-type immune development, leading to the concept of Type 1 pathogens activating PAMPs on dendritic cells, resulting in the release of IL-12 in an adaptor protein (My88)-dependent way [67, 68]. The development of Th2-directed immune responses was seen either as a default pathway in the absence of bacterial substances (supporting the hygiene hypothesis) or to be specifically induced by Type 2 pathogens and allergens activating a group of unidentified Th2-type activating receptors [67]. The TLR molecule MyD88 apparently plays an important role in the activation of Th1 development [69], while absence of MyD88 augmented the Th2-type responses [70, 71]. In contrast to a LPS/TLR-driven Th1 response, recent studies show that LPS can induce both a Th2- and a Th1-type response in a TLR4/ My88-dependent way. The effect of LPS was dose-dependent, facilitating a Th2-type response at lower concentrations and a Th1-type response at higher LPS dosages [72–75]. The release of Th2-cell attracting chemokines, e.g., CCL17/thymus and activation-regulated chemokine (TARC) by dendritic cells and epithelial cells, may contribute to the allergen-induced Th2-mediated inflammatory response in the asthmatic airways [76–80]. CCL17/TARC interacts with CCR4 receptors that are predominantly expressed by Th2 lymphocytes. This induces a chemotactic response and may also lead to β_2-adrenergic unresponsiveness, leading a loss of negative control over Th2 cytokine production (e.g., IL-4, IL-5, and IL-13) [81, 82]. How and why these innate receptors on airway cells play a role in the development of asthma is not clear. Genetic studies show SNPs on different components of the innate recognition pathway that may in part explain susceptibility for atopy, BHR, and other determinants of atopic diseases. Polymorphism for CD14 was shown to be associated with sCD14, total IgE, and skin tests [83–85]. Similarly, a polymorphism in the TLR gene (TLR2/-16934) was a major susceptibility gene for children living on farms [86]. Especially, polymorphisms of the intracellular NOD1 protein, which bind cell wall peptidoglycans of gram-negative bacteria, were shown to be associated with atopic eczema and asthma [87, 88]. These polymorphisms has recently been reviewed [89–91].

Role of Protease-Activated Receptors in Asthmatic Reactions to Allergens

A recognition system that may play an important role in allergen-driven asthmatic reactions are receptors able to detect proteolytic activities, the protease-activated receptors (PARs), detecting proteolytic activities present in the vicinity of airway cells. Proteases are often present in inhaled substances, e.g., excretion products of

bacteria, fungi, grass pollen, etc. but also from airway tissue cells (mast cells, inflammatory cells). This receptor family, PAR 1–4, is expressed by most cell types involved in asthma, connective tissue, epithelial and endothelial cells, smooth muscle cells, monocytes and macrophages, mast cells, and inflammatory cells [92–95]. Primary cultures of epithelial cells also express all four PARs (PAR 1–4) [96, 97], epithelial cell lines may show different expression patterns, mainly PAR 1–3 with predominant expression of PAR-2 [98].

PARs are G-protein-coupled receptors (GPCR). Activation of the specific G proteins coupled to the PAR family can result in two major responses: (1) Induction of intracellular signaling pathways that are involved in production of proinflammatory cytokines, e.g., IL-8, MPC-1, IL-6 and growth factors [99–103] as well as production of the anti-inflammatory prostaglandin E2 (PGE2) [96, 104] with airway smooth muscle relaxation properties [95, 99, 105, 106]; (2) Transactivation of the EGF receptor through the activation of ADAMs [107, 108] and subsequent release of growth factors, thereby promoting airway remodeling and mucus hypersecretion. The proinflammatory role of PAR-2 has been supported by mouse, guinea pig, and human studies, showing eosinophilia and BHR with PAR-2 overexpression and lower levels of bronchial reactivity and IgE in the absence of PAR-2 [109, 110, 111, 112]. In asthmatic patients, increased expression of PAR-2 has been observed on the bronchial epithelium [93], suggesting that there maybe a disequilibrium in asthmatic patients between pro- and anti-inflammatory activities that will favor the proinflammatory actions. The possible role of PAR receptors in allergic respiratory diseases has recently been reviewed by Reed and Kita [94].

The knowledge on environmental proteolytic activities is still limited. Extracts that are used in vitro are often derived from preparations used for skin-testing purposes that have lost their proteolytic activities during allergen preparation. Some inhaled allergens contain stable proteolytic activities such as house dust mite- and fungal extracts that show activation of airway epithelial cells with corresponding release of proinflammatory cytokines [35, 36, 40]. The house dust mite serine proteases Der p3 and Der p 9 have been shown to activate PAR-2 receptors on airway epithelial cells [113], while Der p1 activated PAR-2 and inactivated the PAR-1 receptor [114]. Proteolytic activities of allergens may cause disruption of the epithelial cell layer either indirectly through activation of PARs and directly by destruction of adhesion molecules causing cell detachment [115] and/or opening of tight junctions [116–118], thereby facilitating allergens to pass the epithelial cell layer [46]. Recently, it has been described that activation of PAR-2 disrupted E-cadherin-mediated adhesion between cells and compromised the epithelial barrier [119] (Fig. 1).

In contrast to the stable proteases, pollen extracts contain more labile proteases that can only be detected in fresh pollen extracts, but these proteases show considerable activity [120]. In addition to mites and pollen, several other aeroallergens, including cockroaches, cat, and fungi have been documented to contain protease activity [121–125]. The significance of proteases in allergens for the inflammatory responses in asthma still needs further investigation.

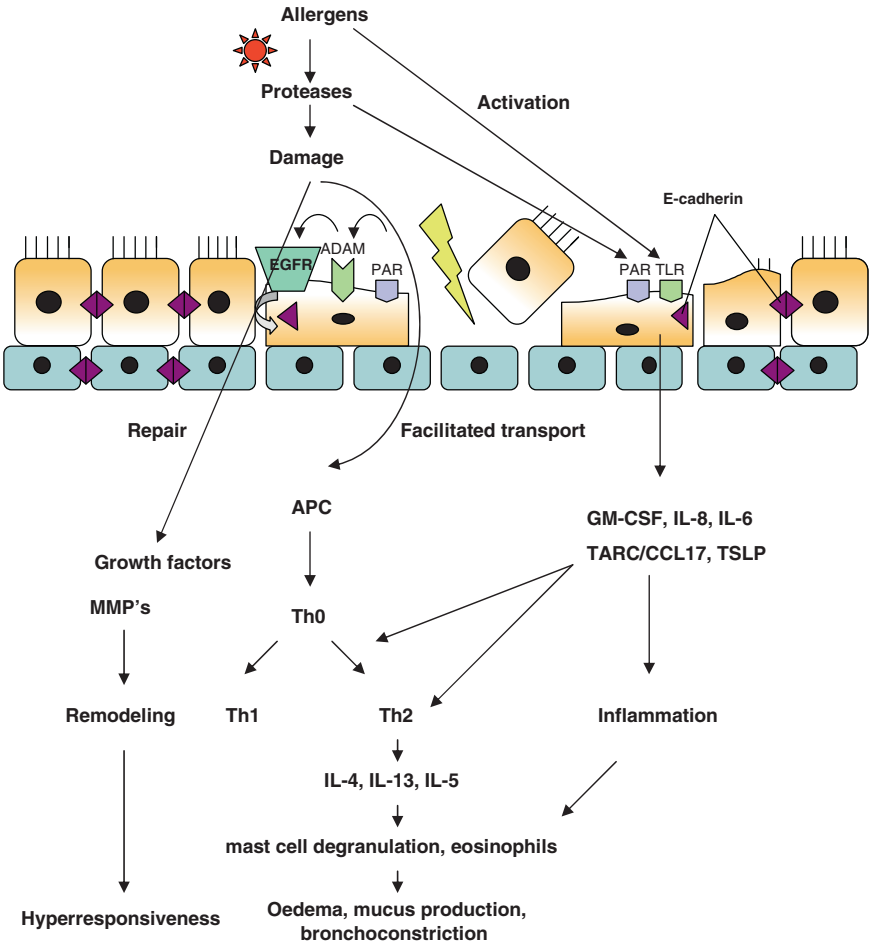

Fig. 1 Schematic representation of allergen-induced injury and the interaction between the epithelium and immune cells in asthmatic airway inflammation

Role of Epithelial Damage in the Immunogenicity of the Airway Epithelium

The proteolytic activity of allergens maybe an important factor in their allergenicity and has been demonstrated to be essential to overcome airway tolerance. Allergens that lack protease activity (such as ovalbumin, OVA) induce tolerance in mouse models when inhaled without prior parenteral immunization. When OVA is delivered through the airways in the absence of prior immunization, a tolerogenic state

is induced. Interestingly, this can be overcome by the addition of proteases to the OVA. In contrast, allergens that do contain active proteases can induce airway hyperresponsiveness and airway inflammation in the absence of further immunization protocols [126–129]. The mechanism of this protease-dependent enhancement of allergenicity is not fully understood. Since active proteases appeared not essential for allergen presentation [128], the mechanism maybe related to a more direct effect on the airway epithelium, including disruption of intercellular epithelial contacts and the possibly related increase in activity. The group of Jordana has shown that the allergic asthma manifestations after intranasal HDM administration in BALB/c mice were partially mediated by production of GM-CSF, an important maturation factor for dendritic cells [130]. Moreover, they previously showed that GM-CSF transgene expression in airway epithelial cells switched the induction of inhalation tolerance to OVA to an allergic inflammatory response [131]. The airway epithelium is a well-known source of GM-CSF, as well as additional proallergic factors. For instance, the airway epithelium is known to express thymic stromal lymphopoietin (TSLP) and CCL17/TARC, two cytokines which are upregulated in the asthmatic airways [132]. TSLP has emerged as potential key player in the sensitization phase toward environmental allergens and activates dendritic cells toward the induction of inflammatory T cells [133, 134, 135], while CCL17/TARC is a chemokine that preferentially attracts Th2-type cells. The exaggerated release of proinflammatory mediators by the airway epithelium in asthma maybe related to the loss of epithelial integrity induced by proteases. Proteases can disrupt epithelial integrity and concomitantly activate the airway epithelium to produce proinflammatory cytokines [96] through activation of the PARs, as described above. Indeed, in asthma patients, it has been demonstrated that increased permeability of the airway epithelium is accompanied by enhanced epithelial activity and increased expression of proinflammatory cytokines [3, 136, 137]. We have recently demonstrated that human bronchial epithelial cells express TARC in response to house dust mite extract (Der p). In this case, the ADAM-dependent activation of EGFR and the downstream MAPK signalling pathways appeared to play a crucial role [76]. As described above, Der p 1 and activation of PAR-2 can induce in the downregulation of E-cadherin-mediated intercellular contacts. In addition to regulation of the canonical Wnt/β-catenin signaling pathway, E-cadherin has been shown to negatively regulate multiple signaling pathways, e.g., activity of receptor tyrosine kinase EGFR in kidney cells and MEK/ERK-1/2 signaling in squamous carcinoma cells [138, 139]. To study the contribution of E-cadherin downregulation on proallergic activity of the bronchial epithelium, E-cadherin was downregulated by small-interfering RNA (siRNA). We found that the downregulation of E-cadherin expression is associated with increased EGFR activation and downstream signaling, with a subsequent increase in expression of Th2-attracting chemokine CCL17/TARC as well as TSLP [140]. Thus, disruption of the epithelial barrier by protease-containing allergens may contribute to the development of Th2-mediated airway inflammation in asthma. This may, at least in part, be mediated by the loss of E-cadherin-mediated intercellular contacts, rendering the epithelium more activated with respect to production of the proallergic factors, e.g., CCL17/TARC and TSLP (Fig.1).

Epithelial Cells and Innate Recognition to Fungi

Epithelial cells have been recognized as PPR-bearing cells with activation profiles similar to monocytes, showing a rapid antimicrobial response, release of proinflammatory cytokines, and antigen presentation to lymphocytes. The antimicrobial role of this innate recognition is suggested by the release of antimicrobial peptides by tracheobronchial epithelial cells after activation of the TLR2 [141]. Furthermore, the release of the antibacterial and antifungal agent ALP/SLPI was shown to effectively kill bacteria and spores and mycelium of *Aspergillus fumigatus* [28, 142, 143, 144, 30]. The role of innate recognition of fungal particulates by airway cells has been explored for just a limited number of fungi and yeasts. Both spores and mycelium of *A. fumigatus* and Candida have been studied for their interaction with airway epithelial cells. Epithelial cells bind spores of *A. fumigatus* followed by phagocytosis [145, 146]. This binding to epithelial cells was enhanced by factors released by spores of Aspergillus and inhibited by SP-D [147]. Binding to epithelial cells followed by phagocytosis is possibly mediated by so-called adhesions of the Als family, showing binding of Als-3 to E-cadherin, which also induced the phagocytosis of *Candida albicans* and *Saccharomyces cerevisiae* to epithelial cells [56]. Recently, also the firm binding of *A. fumigatus* mycelium fragments to airway epithelial cells (A549) has been demonstrated, indicating binding by innate receptors. Receptors involved in such binding of fungi may include TLRs, dectins, and or mannose-binding receptors. These receptors have been shown to be actively involved in fungal adherence and phagocytosis by macrophages [57, 148, 149]. mRNA expression for TLR 1–10 were demonstrated for airway epithelial cells (BEAS-2B and primary airway cells), while functional activity was shown for TLR2, TLR3, TLR4, and TLR5 [150–152]. Furthermore, expression of TLR3 protein was shown by FACS analyses [153, 150]. However, in contrast to the clear positive histological staining for TLR2 and TLR4 on alveolar macrophages, no such staining could be demonstrated for the A549 cells, suggesting lower expression of the TLRs on airway epithelial cells [154]. While binding of mycelium fragments to A549 epithelial cells did not show morphological changes, primary epithelial cells showed gap formation at the binding sites of mycelium, suggesting activation and detachment of epithelial cells [155].

In summary, the interaction of allergens with the airway epithelial cell layer has most clearly been demonstrated for those allergens that contain proteases, while interactions based on innate nonprotease-based interactions are also clearly present but the receptors involved not yet clearly defined.

The proteases may act in two different, but interdependent action profiles:

1. Attack on epithelial integrity and disruption of the epithelial barrier. This may occur directly through proteolytic destruction of junctional proteins and indirectly by activation of PAR-2, which may induce loss of E-cadherin-mediated cell to cell connectivity.
2. Induction of intracellular signaling pathways through PAR activation as well as loss of negative control by E-cadherin, which may result in increased activation

of the epithelium with respect to the expression of proallergic factors, e.g., GM-CSF, the Th2-attracting chemokine CCL17/TARC, and the Th2-differentiation promoting cytokine TSLP.

However, the importance of receptors that are involved in innate recognition is less clear. Studies of TLRs and epithelial cells indicate that a variety of receptors including PARs, TLRs, are in part expressed on epithelial cells and able to react functionally to allergens, bacterial and fungal stimuli. Expression of mRNA has been shown for most of the TLRs and functional activity for TLR2–5. However, only TLR2 and TLR3 have been demonstrated as a protein on the epithelial surface. The importance of the role of TLRs or other innate receptors in detection of allergens, the significance for the cognate immune response, and their role in allergen- and fungal-induced asthma is a subject of current research. However, studies for defining the specific role of epithelial cells and monocytes and dendritic cells in removing inhaled bioorganic materials and direction of the immune responses still need to be done.

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Sensitisation to Airborne Environmental Allergens: What Do We Know and What are the Problems?

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Sources of Allergens

The most widely distributed sources of allergens are the pyroglyphid *Dermato phagoides pteronyssinus* and *Dermatophagoides farinae* mites [1], temperate grass pollens [2] and cats [3]. Other important allergens with less global distributions are birch [4], olive [5], ragweed and mugwort pollens [6]. Cockroach allergy is important for inner-city dwellers in America [7]. Dog allergy has been more evident in regions with low exposure to other allergens but is also a frequent source of sensitisation elsewhere [8]. The glycyphagid mite *Blomia tropicalis* is important in highly populated tropical and subtropical regions [9]. The conifers Japanese cedar in Japan and mountain cedar in USA and to a lesser degree cypress are regionally important [10]. Allergens from Aspergillus, Alternaria, Cladosporium and Penicillium moulds sensitise 5–10% of most populations and are associated with severe asthma [11]. Emerging sources of sensitisation are domestic exposure to mice in inner city environments, and pollens from the weeds *Salsola kali* (Russian thistle or tumble weed) and *Chenopodium album* (lambs quarter or goosefoot) [5]. The pollens occur worldwide but have attracted interest in areas of desertification.

Allergens and Dominant Allergens

Quantitatively the IgE binding to most sources of allergen is directed to a small number of dominant allergens. Birch pollen has the most dominant allergen with 80% of allergic people in Scandanavia producing 90% of their antibodies to Bet v 1 [12].

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_18, © Springer 2009

Olive Ole e 1 has a similar dominance [5]. The group 1 and 5 (including the related group 6) grass pollen allergens collectively bind 80% of the IgE in 95% of sera [2]. Amb a 1 is the dominant allergen for ragweed accounting for 50% of IgE binding with a range of 25–85% [13]. The combination of the group 1 and 2 house dust mite allergens [14, 15] and Fel d 1 from the cat [16] constitute about 50% of the IgE binding to their corresponding extracts although the dander extract used for cat may not be the major source of all cat allergens [17]. Can f 1 constitutes 70% of the IgE binding to dog saliva and Can f 2 lesser binding but other important allergens may exist [18]. The cockroach group 2 and 5 allergens bind IgE with a prevalence of 70% in highly allergic patients [19], constituting 50% of the IgE binding. Mouse allergy is considered to be directed to the urinary Mus m 1 but little attention has been given to other allergens. Dominant fungal allergens have not been well defined. IgE antibodies to Alt a 1 associate well with the ImmunoCAP scores (Phadia AB Sweden) to Alternaria extract [20] and similarly IgE to Asp f 1 for Aspergillus [21]. The group 5 and 8 allergens have also been shown to be prominent for Alternaria and Cladosporium [22].

Spectrum of Anti-Allergen Responses

For Timothy grass, IgE binding to allergens other than Phl p 1 and Phl p 5/6 has a sporadic pattern. Binding to the EF-hand calcium-binding proteins (Phl p 7) and to profilin (Phl p 12) is of interest because antibodies to these proteins cross-react with homologues from disparate species. About 25% of sera had low-titre IgE to profilin while the EF calcium-binding allergens induced fewer but higher titres [23]. Multiple pollen sensitivity however was not associated with responses to these allergens but higher IgE titres to all the allergens [24]. The IgE binding to Bet v 1, 2 and 4 has been compared [12, 25]. An almost monoreactivity of the IgE to Bet v 1 was found in Scandinavian countries while people from central European and Mediterranean regions had more reactivity to the Bet v 2 and Bet v 4. In Italy, 11% of the patients did not have IgE binding to any of the allergens [25]. IgE binding to a panel of five cockroach allergens showed a broad and sporadic reactivity to allergens other than Bla g 2 and 5 but some could induce high-titre IgE responses [19].

A distinct hierarchy of IgE binding was found to a 9-allergen panel of house dust mite allergens regardless of the total size of binding [15]. IgE to Der p 1 and 2 made up about 50% of the binding while collectively binding to Der p 4, 5 and 7 accounted for 30%. Binding to Der p 3, 8, 10 and 20 were low. Comparison with other studies indicate that IgE antibody titres to Der p 6, 9, 13, 16 and 17 will also be very low [1]. Cross-reactive IgE binding might be expected from the conserved Der p 10 (tropomyosin) and Der p 20 (arginine kinase). Their low IgE binding indicates that this was insignificant in the study environment. It has however been demonstrated that a few people from environments that become sensitised to cockroach do make cross-reactive anti-Der p 10 antibodies [19]. Prevalent anti-group 10 antibodies have been found in Japan and Africa but only in people with antibody

to the dominant mite allergens. A different pattern of anti-house dust mite antibodies was found in tropical Australia. Sera from skin-test-positive aboriginals had antibodies to the amylase Der p 4 but not group 1 and 2 allergens [26].

Several cat allergens besides Fel d 1 bind IgE at high frequency and the salivary lipocalin Fel d 4, has been shown to bind IgE in 70% of cat allergic people and for half of these people the titres were higher than those to Fel d 1 [17]. The anti-Fel d 4 titres were low but this now needs to be viewed in the knowledge that the IgE anti-Fel d 1 titres also can be low for most cat-allergic people [27].

IgE Antibody and Allergic Responses

The IgE titres to the dominant allergens of birch, grass and mite are about 50 ng/ml, 20 ng/ml for Amb a 1 and 20 ng/ml for the cockroach Bla g 2 and 5. Many people have low levels around 5 ng/ml to Fel d 1 [17, 27] although some people have over 100 ng/ml [16]. IgE to Mus m 1 is only about 1 ng/ml [28].

Nasal provocation and skin tests showed that the high-IgE-binding Phl p 1 had low responses [29] and the minor IgE-binding Phl p 2 high responses. Structural studies have now shown that a small region of Phl p 1 binds IgE so this could restrict cross-linking of IgE on mast cells [30]. Der p 1 [31], [32], Der p 2 [33] and Bet v 1 [34], however, have multiple IgE-binding regions. There could still be some limitation since although the VH gene usage for IgE is not restricted or lacks mutation and VDJ diversification, the total size of the repertoire is small [35]. The formation of multimers could increase cross-linking. Many dominant allergens are multimeric including Der p 1 [36], Bet v 1 [37], Fel d 1 [38], Phl p 5 [39], Alt a 1 [40], Can f 1 and 2 [41] and Equ c 1 [42]. Combinations of allergens also induce more degranulation [43] but for mite [15, 44], cockroach [19] and grass pollen allergy [24], people sensitised to more allergens from the one source are not more symptomatic.

IgE antibody produced to carbohydrate determinants found on allergens of most is mostly directed to monovalent substitutions of N-linked glycans so cross-linking of IgE receptors would not be expected. Phl p 13, a grass pollen allergen that has multiple glycans, however induces mediator release [45]. Recently IgE antibodies to the carbohydrate on cat IgA and IgM were found in 40% of cat-allergic subjects but the ability to induce hypersensitivity reactions was not reported [46].

IgE in the Prediction of Allergic Disease

House dust mite allergic children with persistent asthma have high IgE antibody titres [47] but only about 20% of those with the high titres develop disease. Exacerbation of intermittent asthma is however a more frequent health problem, producing 75% of hospital admissions for asthma in children [48]. Many such

children with mite-allergy have quite low titres, less than 10 ng/ml [15]. An analysis that measured the asthma symptoms in relation to the summated anti-allergen IgE titres showed a 50% probability of current wheeze at 65 ng/ml [8] but reductions in lung function was a continuous variable down to 4.4 ng/ml. Even children with the highest titres only had a 60% probability of wheeze.

Mucosal Antibody

IgE is not only produced in the mucosa but this is a site for class switching to epsilon as demonstrated by recombinant switch circles. These can be induced by allergen challenge in pollen-sensitive people [49] and more switch circles are found in the pollen season. The initial sensitisation event probably occurs in the draining lymph nodes but the mucosal switching has a potential for local amplification. The VH5 bias for epsilon antibody transcripts in the nasal mucosa of allergic rhinitis patients suggests that this occurs [50]. Switch circles could be an important measure for monitoring allergic disease. Mucosal IgA antibodies are only found in allergic people. IgA2 is up-regulated by TGF-β so it may be a marker for the action of this regulatory cytokine, as shown in immunotherapy [51].

IgG Antibody

For grass [52], ragweed [53], mite [15] and birch [54], IgG antibodies are only found in sera with IgE. Fel d 1 has, however, been reported to induce IgG antibodies in most people exposed to the allergens [55] possibly because the amount of allergen in the inhalable air of homes with cats is 50–100-fold more than that found for mite and pollen and even ten-fold higher in homes without cats [56]. It has been proposed that this tolerises for IgE and while maintaining IgG production [55]. A recent study however found IgG antibodies to cat were ten-fold higher in people with IgE [57]. Dog allergens are also readily detected in undisturbed air [58] but although IgG antibodies to dog extract correlates with IgE antibody [59], IgG antibody production to the dominant allergen has not be measured. Mouse allergens have also been reported to induce IgG in non-allergic people in studies of occupational exposure [60] but data from domestic exposure showed a strong association with IgE. This may be related to the 50 times lower of amounts of Mus m 1 in the air compared to Fel d 1 [60].

The absence of IgG antibody to pollen and mite allergens in non-sensitised people shows that they either do not make immune responses to the allergens or that their responses do not lead to significant antibody titres. Not all allergic people produce IgG. Hales et al. found IgG in 70% of mite-allergic children but only in 40% of adults. Further, only 25% of children admitted to an emergency department

had IgG and these were low, indicating a relationship with susceptibility to exacerbation [15]. Jarvis et al. seemingly found the opposite for adults but the symptoms described were unlikely to require many visits to an emergency department and probably just reflects the higher IgG found in sensitised subjects [57].

The hierarchy of IgG binding to mite allergens was similar to IgE being directed to the dominant Der p 1 and 2 allergens with lesser and less consistent binding by the mid-potency allergens and little to the weak IgE-binding proteins [15]. Low IgE binding is therefore not a deviation to an IgG response. Cockroach IgE and IgG binding had a slightly different relationship [19]. The dominant Bla g 5 bound the most IgG antibody but the minor Bla 4 and Bla g 7 allergens also had high IgG titres even in sera without IgE. Grass pollen allergens induce lower IgG antibody titres than mite and cockroach. For Timothy, the highest binding was to Phl p 5 with antibodies to Phl p 1 being low [61].

T-Cell Responses

Few studies have examined in vivo responses to purified allergens. Challenge with the Der p 1 and 2 induced late reactions and increased serum IL-5 [62]. Doses of house dust mite extract that induced a similar degree of early bronchoconstriction as the allergens induced more serum IL-5 and larger late responses, possibly indicating the importance of other allergens.

The precursor frequencies of T cells responding to purified allergens have not been examined but pollen [63] and house dust mite extracts [64, 65] have. The reported frequencies were quite large, with 0.05–0.1% for allergic subjects and 0.01–0.02% for non-allergic subjects. By comparison, unvaccinated people have frequencies in the region of 0.001% [66] for other antigens, and this rises to about 0.02% after vaccination. Thus even non-allergic subjects show a considerable expansion. The allergen-responsive T cells of allergic subjects are mainly in the memory CD45RO + population [64] while non-allergic show both CD45RO + and CD45RA + cells [64].

T-cell responses to the dominant allergens from grass [67, 68], birch [69], weeds [70] mite [71] and cat [72] have been studied. Proliferative responses induced in the peripheral blood mononuclear cell (PBMC) from allergic people are generally better than those induced from non-allergic but with considerable overlap [71]. The induction of the Th2 cytokines IL-5 and IL-13 can be readily detected while measurement of IL-4 from primary cultures is best conducted with highly sensitive assays. The discovery that T cell lines cultured from PBMC of allergic people were Th2 biased and that the clones from non-allergic subjects were Th1-biased was a milestone in human immunology. It is likely that the investigators observed the polarising effects of culture milieu, especially the potent inhibitory activity of IL-4 on Th1 responses. T-cell responses measured without extended culture show that cells from allergic and non-allergic subjects produce similar [73] or even increased IFN-γ from cells of allergic subjects [74–76]. It appears that Th2 polarisation is, however,

best found in the lungs and not the PBMC [77] and studies on thymic stromal lymphopoietin (TSLP) clearly show the need to study in situ responses [78]. TSLP, an epithelial cell product induced by tissue damage, is powerful inducer of Th2 responses mediating both expansion and polarisation while maintaining the central T-cell memory. Recent studies have identified that TCR-activated T cells express the TSLP receptor, thus providing a marker for the allergy-mediating cells and evidence for a direct as well as a dendritic-cell-mediated effect [79].

The expansion of allergen-responsive T cells in vivo can be inferred from their chemokine receptors and chemokine production. Th2 cells preferentially express the receptors CCR3, CCR4 and CCR8, and migrate to their respective ligands, eotaxin (CCL11), monocyte-derived chemokine (MDC) (CCL22) and thymus- and activation-regulated chemokine (TARC) (CCL17). Bronchial lavages of unchallenged lungs of asthmatics show the accumulation of CCR4 + CD4 + cells and their ligands TARC and MDC [80]. This can be enhanced by allergen challenge where endobronchial biopsies showed that virtually all T cells expressed CCR4 with some co-expression of CCR8 and the epithelial cells produced the T-cell chemotactic MDC and TARC [81]. The Th1-type IP-10 chemokine can also be produced in asthma, as shown following lung challenges with ragweed, house dust mite and cat extracts [82, 83]. Patients with late phase reactions to allergen challenge produce more of both the Th1 and Th2 chemokine [82].

PBMC have also been studied for chemokine bias. Stimulation with grass extract has increased the proliferation of CCR4 T cells in cultures from allergic but not non-allergic subjects in keeping with the Th2 phenotype of allergy [63]. CCR4 could be detected on 40% of the responding T cells. T-cells from PBMC of allergic subjects also produce the Th2 chemoattractants TARC and MDC [84, 85] in greater quantity than PBMC from non-allergic people.

T-Cell Epitopes

For most allergens, T cells from both allergic and non-allergic people respond without preference for particular epitopes in keeping with the general absence of convincing MHC associations in allergy[1, 86]. There are nevertheless some interesting exceptions. Responses to mugwort allergen Art v 1 show strong linkage to HLA-DRB1 *01 and T cells from patients recognise an immunodominant peptide presented by this allele [70]. Heavy O-glycosylation of Art v 1 may limit antigen-processing and thus restrict the presentation. A region of Der p 1 is also immunodominant. Peptides in the central loop are the most stimulatory [87–89] and the responding cells have a bias to the T-cell receptor Vbeta18.1 [89, 90]. Their responses are however not directed to one epitope and can restricted by DR, DP and DQ alleles [87]. A concordance between IL-10 production to Fel d 1 and the HLA-DRB1 allele, *0701 has been reported. T cells responding to peptide 1–24 of chain 2 presented by this allele made strong IL-10 responses [72]. These epitope-specific cytokine responses need to corroborated.

T-Cell Regulatory Responses

Despite recent interest in regulatory T cells, the responses that regulate sensitisation are unknown. The evidence that IL-10 prevents allergy is yet to be convincing. Allergen-stimulated PBMC from healthy subjects have most frequently been found to produce less IL-10 than cells from allergic subjects as shown for house dust mite [76, 91, 92], cat and pollen [93, 94]. Increased IL-10 mRNA has also been found in bronchial and skin challenge sites [95] and non-allergic and asymptomatic sensitised people produce less IL-10 to stimulation with grass and birch pollen [63]. A possible regulatory role for IL-10 was however indicated in two independently conducted studies of house dust mite. Allergen extract induced more IL-10 from the T-cells of allergic subjects, but there was a negative correlation between the IL-10 and the skin test reaction [76, 91].

Evidence for IL-10 regulation has been obtained by showing the addition of anti-IL-10 receptor antibodies to PBMC cultures of healthy people enhanced proliferative responses to Der p 1 [96] and subsequent experiments by these investigators showed that allergen-stimulated cultures from healthy people had more IL-10 producing T-cells. Perhaps importantly the cells were examined 12 hours after allergen stimulation [73] and in the absence of other cytokines lacked proliferative activity. The lack of supporting cytokines may explain why other investigators find non-allergic subjects make less IL-10. The inhibitory effects of TGF-β are well documented and TGF-β is an absolute requirement for T regulatory cells. Allergic people however produce more TGF-β following challenge with allergen extracts [97] so its production does not appear to be a controlling factor.

Suppressive effects of CD4 + CD25 + T regulatory cells have been demonstrated on the proliferation responses of PBMC cultured with cat and pollen allergens. There was however no convincing difference in the activity of cells from allergic and non-allergic subjects [98, 99]. Indeed studies of atopic dermatitis showed that house dust mite extract stimulated more of the regulatory cell transcription factor FOXP3 from PBMC from HDM allergic subjects than PBMC from non-allergic subjects [100]. It is possible these effects are linked to increased IL-2 production by the higher responses of allergic subjects. The studies on induction of FOXP3 by allergen have only examined allergen extracts so it not known if allergens themselves induce the regulatory effects and how this relates to allergenicity. Recent studies have now shown that all T cells undergo a FOXP3 CD25 + differentiation phase and may not be permanently suppressive [101]. Better definition of the cells and the study of their action in an authentic environment is a research priority.

Allergen Exposure

Pollen exposure is required to induce allergy but the prevalence of sensitisation and disease are similar over a wide range of exposure even with a trend for reduced sensitisation with high exposure. Comparisons of different geographical regions [7, 102]

also demonstrate a positive association of mite allergy exposure. Studies of homes in a region with low exposure found a relationship with sensitisation [103] but this has not been apparent, for sensitisation or symptoms, in regions with higher exposure [7, 57, 104]. Attenuation of sensitisation with higher mite exposure has been found in some [105, 106] but not all studies [57]. When only sensitised subjects are analysed, however, the development of symptoms increases with exposure [107]. Exposure to cats in infancy has been observed to protect against cat allergy. This has been associated with the development of IgG4 antibodies without IgE [55] and when cat and mite allergy was examined in the same homes, the effect was specific for cat [108]. Other studies have not fully supported [109] these observations or have been contradictory [57], finding a strong association of IgE and IgG antibody that increased with exposure. A study of T-cell responses also failed to reveal a cytokine pattern that could be associated with selective IgG4 response [72]. Studies with cat allergies and cat ownership are complex with the need to consider factors such as exposure to microbial products associated with cat ownership and the attitudes of high-risk families to keeping cats.

Conclusions

Only 60% of children with high IgE anti-allergen titres develop disease so there is considerable scope for discovering the important determinants for symptomatic sensitisation. The dominant allergens of most sources of allergens are now well characterised but it has not been conclusively demonstrated that they are the driving force for sensitisation. Evidence for this could lead to a wider use of allergens instead of extracts and therefore studies will produce quantitative and reproducible results. Examining the spectrum of the responses can however be highly informative, showing for example, that the specificity of responses is affected by geography. This occurs for pollens in Europe but strikingly an Australian aboriginal populations classified as house dust mite allergic with extracts do not respond to the dominant allergens. The nature of their allergy would be expected to be quite different to that found in other populations. For cat, IgE antibody is thought to be mainly directed to Fel d 1 but studies showing that Fel d 1 titres are often low and lower than lipocalin suggest that re-evaluation is warranted. Allergy to moulds remains poorly characterised. In mite and pollen allergy non-sensitised people do not produce IgG antibodies and sensitised people do not produce IgG antibodies to poor allergens. The precursor frequency of allergen-responsive T cells is high even for non-allergic people so regulatory responses occur. Studies of T regulatory cells and IL-10 and TGF-β production have perhaps counter-intuitively usually found higher regulatory responses in allergic than healthy people so this is an unresolved area of investigation. Increased IL-10 mediated regulation has however been found studying responses early after allergen stimulation but this needs to be corroborated and the role of chemokines MDC, TARC and TSLP point to shortcomings in studying anti-allergen responses in simple tissue cultures systems. The preferential

partitioning of anti-allergen responses into those made by Th1 and Th2-type chemokine responsive cells provides an avenue for more meaningful *ex vivo* observations. The role of IFN-γ in allergy is uncertain with many studies showing similar or increased *ex vivo* release from cells from allergic subjects and that allergens induce high titres of the Th1-dependent IgG1 antibodies. The switching of IgE antibody production in the mucosa may provide a better measure of an active allergic response and quantitative measures of IgG antibody to defined allergens may be markers for protection, as shown for mite allergy in children.

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The Immunological Basis of the Hygiene Hypothesis

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Summary

The hygiene hypothesis has gained much attention as an explanatory model for increases in the incidence of allergic diseases. Since epidemiological evidence mainly comes from cross-sectional studies, which are not able to elucidate cause–effect relationships, this concept is still in conflict with opposite results. The role of microbial compounds as important exogenous triggers of immuno-programming is central to the hygiene hypothesis. Several prototypical components from both gram-positive and gram-negative bacteria have been investigated under experimental and clinical conditions. These approaches clearly demonstrate that the route of exposure, the time of exposure, and the dose are critical variables, which determine the outcome of downstream immune responses. The innate immune system plays a central role in the initiation of effector responses, by signaling through pattern recognition receptors, particularly toll-like receptors (TLRs) and balancing the type of T-cell effector response, including TH-1, TH-2, and regulatory T cells. Recent studies focus on the role of microbiota and the commensal gut and skin flora as immuno-modulators. Most recently, *Acinetobacter lwoffii* and *Lactococcus lactis* have been identified in the environment of traditional farms further supporting the concept that environmental components play a decisive role in programming early immune responses.

The Facets of the Hygiene Hypothesis

As many other chronic diseases, allergic disorders seem to have their origin in a misleading interaction among the environment, lifestyle habits, and the genetic background of individuals. Regarding allergic diseases, neither the exogenous factors

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_19, © Springer 2009

nor the basic genetic conditions are completely elucidated, and the switch from a well to a misbalanced character of this interplay is still not well understood. The dramatic increase in the "allergy epidemic" observed in affluent countries throughout the last decades has given an impetus to the research in allergy initiation [1]. In coincidence with a dramatic decrease in infectious diseases, this scenario gave rise to suppose that these inverse trends are driven by the same force [2]. Summing up these observations, Strachan initially formulated the so-called hygiene hypothesis postulating that public health policies and Westernized lifestyle led to germless environments in developed societies [3]. Higher personal hygiene and improved living standards combined with the trend to a nuclear family type seem to be associated with the increase of allergies as a result of a diminished exposure to bacterial components and a degradation of the natural commensal saprophyte flora, stimuli that may act as a defense against the development of allergic diseases. So far, the hygiene hypothesis was merely based on epidemiological associations but failed to explain how this stimuli protect from allergies. The integration of the immunological evidence that inflammatory allergic diseases are driven by a Th2-balanced immune response while inflammatory infectious processes are characterized by a Th1 cell response led Strachan et al. to an enhanced approach of the hypothesis: coming from a Th2-mediated prenatal environment, the naïve immune system of newborns needs to be stimulated by microbial compounds of a natural environment to boost Th1 responses. The lack of these exogenous stimuli results in a missing immune deviation from the Th2 to Th1 balance and shapes the immune system in a Th2-mediated direction with a higher tendency to develop allergic disorders [4] as shown in Fig. 1.

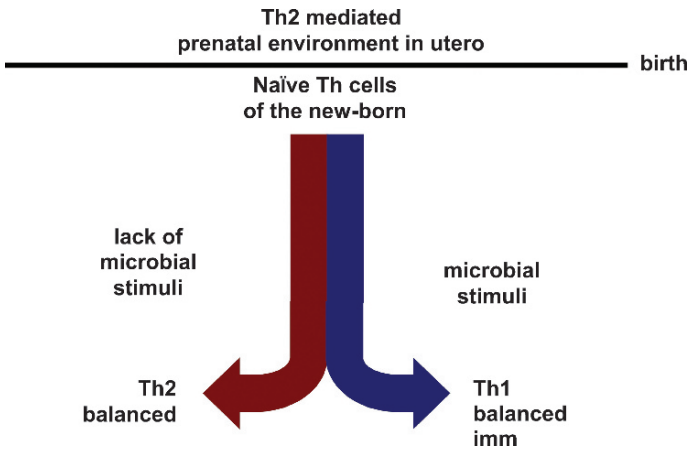

Fig. 1 Programming of the neonatal immune system by microbial agents of the environment. Coming from a Th2-mediated prenatal environment, the naïve immune system of newborns needs to be stimulated by microbial components of a natural environment to boost Th1 responses. The lack of these exogenous stimuli results in a missing immune deviation from the Th2 to Th1 balance and shapes the immune system in a Th2-mediated direction with a higher tendency to develop allergic disorders

This dichotomous approach was modified by Wills-Karp et al. [5] to harmonize the hypothesis with new epidemiological and immunological findings. Surveying world-wide epidemiological trends in infectious, allergic, and autoimmune diseases, Bach [6] concluded from his data, showing increasing prevalence rates in both allergic and autoimmune diseases, that Th2-driven diseases as well as Th1-mediated disorders burden the world's health increasingly. Recognizing the innate immune system as an effective sensor system at the first line to the microbial environment and identifying T-regulatory cell populations as a potent tuning tool in the balancing of tolerance and susceptibility, the hygiene hypothesis became more dynamic. Besides the dichotomous model of a missing immune deviation, a counter-regulatory model was designed postulating that the induction of an anti-inflammatory regulatory network by persistent immune stimuli may be necessary to induce tolerance against ubiquitous and common compounds and to establish defense against pathogens [7]. Support for this point of view came from research on parasitic infections. Th2-skewed worm infections, mainly caused by helminths, are not associated with allergy. More recently, elevations of anti-inflammatory and regulatory cytokines, such as interleukin-10, that occur during long-term helminth infections, have been shown to be inversely correlated with allergy [8, 9].

The loss of the natural microbial environment as could be observed in postmodern societies may threaten the regulatory ability of the human immune system, a network that has been adapted to a wide range of compounds in a long-lasting phylogenetic co-evolutionary process. Being catapulted from the "stone age to the space age", this system may not be able to adapt adequately to a changing scenario tumbling to the one or other extreme.

Evidence from Epidemiological Studies

One of the first observations leading to the hygiene hypothesis was reported by Strachan in 1989 [10]. Growing up in a large family with a number of siblings was inversely associated with hay fever. This sibling effect has contributed to a higher infection rate of children with several mainly older siblings. In accordance with these findings, day care attendance in the early childhood was found to be protective against asthma and recurrent wheezing [11–13]. Most recent studies provided divergent findings: the Glasgow Alumni Study, surveying students born before 1980 confirmed an inverse association between family sibship size and allergic diseases for this age group [14], while a study from the Netherlands performed on families with children born between 1988– and 1990 provides evidence that birth order, and not sibship size, appeared to be associated with allergies. With regard to asthma this association failed to be significant [15].

In line with these findings, studies conducted in East and West Germany in the 1990s comparing prevalence rates and potential risk factors of respiratory symptoms and allergies added further evidence to the hygiene hypothesis. The prevalence of asthma, wheezing, and allergic rhinitis was significantly lower in the East German

population when compared to those who had grown up in West Germany prior to the reunification in 1990 [16]. These differences disappeared in the following years. Studies conducted in children from East Germany born after 1990 reported increasing prevalence rates of asthma, hay fever, and atopic eczema. This changing scenario might have been caused by the anticipation of a Westernized lifestyle within the East German population, and consequently this might be the reason for the increasing prevalence rates of allergic diseases in the eastern part of Germany [17].

Another epidemiological observation indicates an association between the pet ownership and the development of allergic diseases. This "protective pet effect" has been suggested to result from a modified Th2-cell response, or alternatively caused by an increased microbial load in homes where pets are kept [18]. This assumption is supported by the results of the AIRALLERG study that aimed to determine and compare indoor exposures related to allergy in homes of three European countries [19]. The study results demonstrated significantly higher levels of endotoxin, a cell wall compound of gram-negative bacteria that acts as a stimulus on the Th1-immune response, in houses of cat owners than in homes where no cat is kept [20]. Several epidemiological surveys have shown that pet exposure in the first years of life is associated with lower prevalence rates of rhinitis and asthma. Additionally, it was shown that pet ownership may also act protective on pet-specific sensitization. At present, it must be stated that these associations are reported inconsistently with respect to the type of pet, the onset of exposure, and the atopic or allergic outcome [21–23]. A birth cohort study conducted by the Multicentre Allergy Study (MAS) group showed that the levels and the ratio of specific immunoglobulin E (IgE) and IgG released as a response to cat allergen exposure that influences the direction toward the development of an allergic reaction or a protection against it [24].

Three large surveys conducted in different European populations characterized by an "alternative lifestyle" revealed observations matching the major assumptions of the hygiene hypothesis. In families which are adapted to an anthroposophic lifestyle, characterized by the avoidance of antibiotics and the preference of fresh or fermented probiotic and vegetable food, lower prevalence rates for allergies and asthma could be observed [25–27].

These results pointed out that epidemiological study designs should focus on comparisons between populations living in a traditional way and those characterized by modern lifestyles to elucidate the role of Westernization in the development of allergies and asthma. Thus, substantial support came from epidemiological studies exploring the traditional farming environment with regard to the allergic outcomes in farming families [28]. In contrast to the urban lifestyle farming and particularly the traditional way to raise livestock and to handle agriculture is characterized by higher contact rates of all family members to the microflora of stable animals containing a typical spectrum and a high amount of microbes and microbial compounds different to those from other environments, e.g., urban dwellings or rural settings with conventional farm units [29, 30]. Being raised on a traditional farm involves an early and a frequent exposure to these farm-related compounds. A number of studies comparing farming and non-farming environments affirmed the so-called farming effect, conveying that the early exposure to

farming inhalants and products is associated with a decreased risk of developing an allergic disease. The SCARPOL study conducted in Switzerland was one of the first studies to confirm the farming effect showing that children born and raised on farms have a 50% reduced risk of developing allergic diseases in contrast to children from non-farming environments [31]. Subsequently, studies in other rural regions focusing farming environments tighten these results by adding more knowledge about farming exposures and their consequences on allergic diseases [32–35].

The protective effect of the exposure to livestock was underlined by a study conducted by von Ehrenstein et al. [36] in Bavarian rural regions. The cross-sectional ALEX study performed in Austria, Germany, and Switzerland gave new insights into the onset of allergy and asthma by pointing out that pre- and postnatal exposure to the farming environment is protective against allergic outcomes [37, 38]. These results may hint that a traditional farm environment is able to shape the immune system probably already *in utero*. These findings were supported by the results from the cross-sectional Europe-wide PARSIFAL study comparing farm children, scholars from anthroposophic Steiner Schools and their reference groups concerning pre- and post-

Table 1 Segments of population associated with protection from allergies and asthma.

Segments of population	Findings	Authors
Farm environment	Reduced development of allergic disorders in children from farm environment	von Ehrenstein et al. [36]
	Pre- and postnatal exposure to the farming environment is protective against allergic outcomes	Ege et al. [37], Riedler et al. [38]
	Not all farming but traditional farming environments are protective against allergy	Ege et al. [39]
East and West Germany	Low prevalence of asthma among East as compared to West German children	Nowak et al. [16]
	Increasing incidence of asthma in East Germany after reunification	Heinrich et al. [17]
Sibling effect–birth order, day care attendance	Inversed association between family sibship size and allergic diseases in students born before 1980	Kinra et al. [14]
	Birth order appeared to be associated with allergies	Bernsen et al. [13]
	Day-care attendance was associated with a decreased risk of asthma	Celedon et al. [12]
Pet ownership	High endotoxin levels in houses of pet owners	Giovannangelo et al. [20]
	Protective effects of cat or dog ownership on the sensitization and/or on allergic outcomes	Warmbolt et al. [21], Sandin et al. [22], de Marco et al. [23]
Anthroposophic lifestyle	Use of antibiotics in the first years of life preceded the manifestation of wheeze	Kummeling et al. [25]
	Dietary habits particularly the consumption of farm milk influence the risk of allergies	Waser et al. [41]

natal exposure to population-specific environments [39, 40]. Besides a protective *in utero* effect by working in an animal shed during pregnancy, children's risk to develop asthma was also significantly reduced by consuming unpasteurized unskimmed farm milk within the first year of life [41]. The results indicate that consumption of farm milk may offer protection against allergy and asthma. Selected studies on segments of population contributing evidence to the hygiene hypothesis are listed in Table 1.

Perkin and Strachan [42] provided data of pooled estimates in a meta-analysis based on a systematic review in MEDLINE (1966–2004) and EMBASE (1980–2004), revealing highly significant overall odds ratios <1 for farming factors (being raised on a farm, early and frequent contact with livestock, and consumption of farm milk) and different allergic diseases.

Taking these findings together early exposure (pre- and postnatally) of protective agents against allergies may open a "window of opportunity" to shape the immune system into a non-allergic direction.

Focusing the onset of allergic diseases and the early lifetime interval to shape the immune system the question arose how long these effects may continue in lifetime and how to maintain the protective effect. Three studies emphasized the question whether these protective impacts of farming environments continue into adulthood and which factors do support the maintenance of protection. A study from the Netherlands provided evidence that a farm childhood in combination with current livestock farming protects against allergic disorders [43]. Results from a cross-sectional survey nested in the European Community Respiratory Health Survey (ECRHS) indicate that environmental factors encountered in childhood may have a life-long protective effect against the development of allergies [44]. This result was confirmed by studies from Finland [45] and Germany [46].

So far, the majority of the epidemiological observations support the hygiene hypothesis, but a phenomenon called "inner-city asthma" that arose over the last decades in the USA seemed to contradict the hygiene hypothesis. Inner-city asthma is described as a high prevalence of asthma in inner-city children from Afro-American and Hispanic-American families, living under poor hygienic conditions and being characterized by a low socio-economic status [47]. According to the hygiene hypothesis, these determinants should indicate a low prevalence of asthma but contrary to this expectation these populations have a high rate of asthma [48]. Several studies reported associations between the elevated prevalence of asthma in inner-city districts and different indoor contaminations in households and schools, e.g., cockroach and mouse infestations combined with use of illegal pesticides, molds, and airborne fungi releasing natural antibiotics and environmental tobacco smoke [49, 50]. But these findings provided no rationale toward the hygiene hypothesis until now. Matricardi et al. [51] analyzed the pattern of the increase in asthma, hay fever, and atopic sensitization in Europe and the USA to explain inner-city asthma within the framework of the hygiene hypothesis. Therefore, they collected historical descriptions of hay fever and asthma as well as the currently available related literature. As a result, they described the underlying process of Westernization as a historical phenomenon that first affected the wealthy population at the end of the 1890s, expanded among the middle classes during the first half

of the twentieth century, and now cascaded down to affect the first-generation immigrants from Africa and Latin America. In conclusion, they defined inner-city asthma in line with the hygiene hypothesis as the final stage of a class-driven urbanization and Westernization in the USA.

The Role of Microbial Compounds in Allergy Protection

Recent results from the PARSIFAL study reported by Ege et al. [52] pointed out that the "farming effect" is based on a synergism of different farming activities and characteristics mainly frequent on traditional farms. The so-called farming effect includes the kind of livestock kept on the farm, the frequency, onset, and the time period of staying in the animal shed, involvement of children in haying, and the use of silage. Each of these factors attributing to the summing effect contributes to a wide spectrum of compounds derived from the commensal microbial flora of the livestock and crop and their processed products as well. One of these microbial compounds hypothesized to be involved in the interplay between farm environment and the human immune system is endotoxin (lipopolysaccharide, LPS) [53]. Endotoxins, cell wall components of gram-negative bacteria, can be detected in high amounts in farming environments. The ALEX study provided knowledge that endotoxin concentrations in stables and households of farmers are significantly higher than in houses of non-farmers [54]. Additionally, the tendency to develop an allergic disease is inversely associated with the exposed endotoxin concentration measured in the mattresses of farm children. Concurrent to these findings, blood cells of farm children protected from asthma and atopic sensitization are reported to produce high levels of CD14, the endotoxin/LPS receptor on human cells [55]. Furthermore, high endotoxin levels in the house dust were associated with higher levels of interferon (IFN)-γ after mitogenic stimulation of peripheral blood cells in 9- to 24-month-old children [56]. IFN-γ as a Th1-associated cytokine is believed to counteract directly Th2-driven allergic diseases. To clarify the role of microbial compounds such as endotoxins, results from epidemiological studies needed to be proven under experimental conditions. Animal models are helpful tools in the assessment of cause–effect relationships and in the elucidation of the underlying mechanisms. A number of animal models aimed to verify the protective effect of endotoxin exposure but displayed the Janus-faced nature of this compound [57, 58]. The local as well as systemic administration of LPS before allergen challenge led to protection against sensitization accompanied by a suppression of the IgE-production, a reduced airway eosinophilia and a suppressed Th2 response [59]. Delayre-Orthez et al. [60] could show that this effect is dose dependent: high-dose exposure led to an extended protection whereas low doses of LPS induced allergic inflammatory responses. A strong allergic inflammation could be observed when sensitized animals were treated with LPS after allergen challenge [61]. Blümer et al. [62] and Gerhold et al. [63] showed that LPS treatment of the mother has a reducing effect on allergic-airway inflammation of OVA-sensitized offspring in the

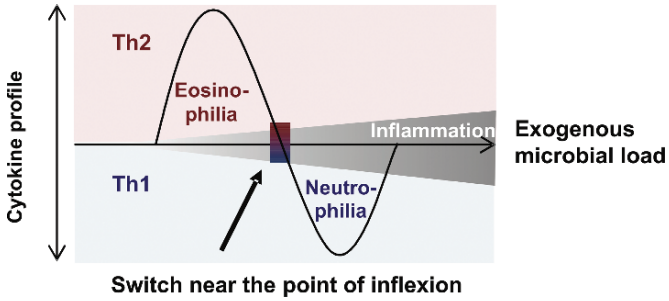

Fig. 2 The endotoxin switch (modified from Vercelli [64]). Low exposures of exogenous polarize Th-cell populations toward the Th2-direction. In the presence of a high exogenous microbial load Th-cells are primed toward a Th1-fate. Sensing the environmental conditions by pattern recognition receptors, cells at the interface to the environment recognize bacteria by binding bacterial components, e.g., endotoxins. In this model, endotoxins mediate between the environment and the immune system serving as a switch between the cytokine profiles at medium levels of the environmental microbial load near the point of inflexion

perinatal mouse model of asthma. These studies pointed out that a protective effect of endotoxins could be transmitted during pregnancy.

Summing up these results, Vercelli [64] proposed a functional model characterized by a bimodal course. Stimulated by low exposures of exogenous microbial agents the human immune system responses with a Th2-cytokine profile, while high bacterial concentrations provoke a Th1 bias. Sensing the environmental conditions by pattern recognition receptor cells of the innate immune system recognizes potential pathogens by binding microbial components, e.g., endotoxins. In this model, endotoxins mediate between the environment and the immune system serving as a switch between the cytokine profiles at medium levels of the environmental microbial load (Fig. 2).

Meanwhile a number of microbial compounds derived from gram-negative as well as from gram-positive bacteria were shown to exert allergy-protective effects. Peptidoglycans, components building up the matrix of bacterial cell walls, were shown to have protective activity in animal models [65].

Protective Effects of Bacteria Derived from Stable Dust and Probiotic Milieus

The evidence from epidemiological studies gave rise to analyses of the farming environment for the active principles causing these protective effects and to prove these effects in animal models of experimental allergy. In addition to the aforementioned bacterial compounds, the bacteria themselves may help to elucidate the character of their anti-allergic protection.

Peters et al. [66] observed anti-allergic effects of sodium chloride extracts from dust collected in stables of animal farms with a low prevalence of allergies and

asthma in animal model of allergic asthma. Treatment of mice by inhalation of this extract during sensitization with ovalbumin (OVA) inhibited the development of airway hyper-responsiveness and airway eosinophilia on allergen challenge. Additionally, the production of interleukin (IL)-5 by stimulated splenocytes and antigen-specific IgG1− and IgE-levels were suppressed leading to the conclusion that stable dust may contain immune-modulating substances that can interfere with the development of both cellular and humoral immunity against allergens. Similar results were obtained by Debarry et al. [67] in an acute model of allergic asthma by intranasal application of bacterial isolates obtained from stable dust. By screening for prominent bacterial strains in cowshed dusts, they could isolate two species identified as *A. lwoffii*, a commensal gram-negative microbe frequent on mammalian skin, and *L. lactis*, a probiotic gram-positive bacterium derived from fermentation processes of silage and milk. These bacteria were separately applied to mice before sensitization with OVA. Treated mice showed a strongly reduced allergic phenotype characterized by an improved airway hyper-responsiveness, decreased infiltration of eosinophils into the lung tissue, and reduced Th2-cytokine levels in bronchoalveolar lavage (BAL) as well as in stimulated splenocytes when compared to untreated OVA sensitized and challenged mice. *In vitro* experiments confirmed these findings underlining that both species induced Th1-directing features in dendritic cells (DCs, activation of IL-12 and upregulation of Th1 polarizing “notch-ligand Delta-4 expression). In addition, activation of HEK293-cells through nucleotide-binding oligomerization domain (NOD)2 and TLR2 by *L. lactis* and TLR4 as well as NOD1 and NOD2 by *A. lwoffii* could be observed (see Fig. 3). Romagnani [68] confirmed these findings by reporting similar results from his laboratory concerning the maturation of human DCs and the polarization of T-helper cell populations. Taking together these data indicate that the missing immune deviation appears to be more convincing than the concept of a decreased immune suppression to explain the high incidences of allergic diseases in affluent countries.

Probiotic bacteria, food-additive microorganisms providing a benefit to the human health, were described to be helpful in the prevention of allergic diseases. Being a substantial part of a healthy gut microflora (microbiota), these bacteria are able to modulate intestinal immune functions and may also act as protective factors against atopy and asthma [69]. Thus, features of probiotic bacteria contribute to the underlying mechanisms of the hygiene hypothesis. The colonization of the neonatal gut by microbes from the neonate environment leads to a microbial stimulation of intestinal immune system toward a Th1-phenotype to compensate the Th2-bias established *in utero* to prevent rejection of the fetus [70]. Beside shaping the intestinal immune system by modulation of human colonic dendrite cells toward Th1-promoting activities, probiotic bacteria are involved in the stimulation of transforming growth factor (TGF)-β producing Th-cells. This cytokine acts on B cells inducing them to switch to IgA-production, a mode to reduce inflammation and to establish clinical tolerance against common antigens. The lack of the microbial stimulus may lead to an elevated IgE-production by B cells with an increased risk of allergic reactions due to the subsequent activation of mast cells [71, 72].

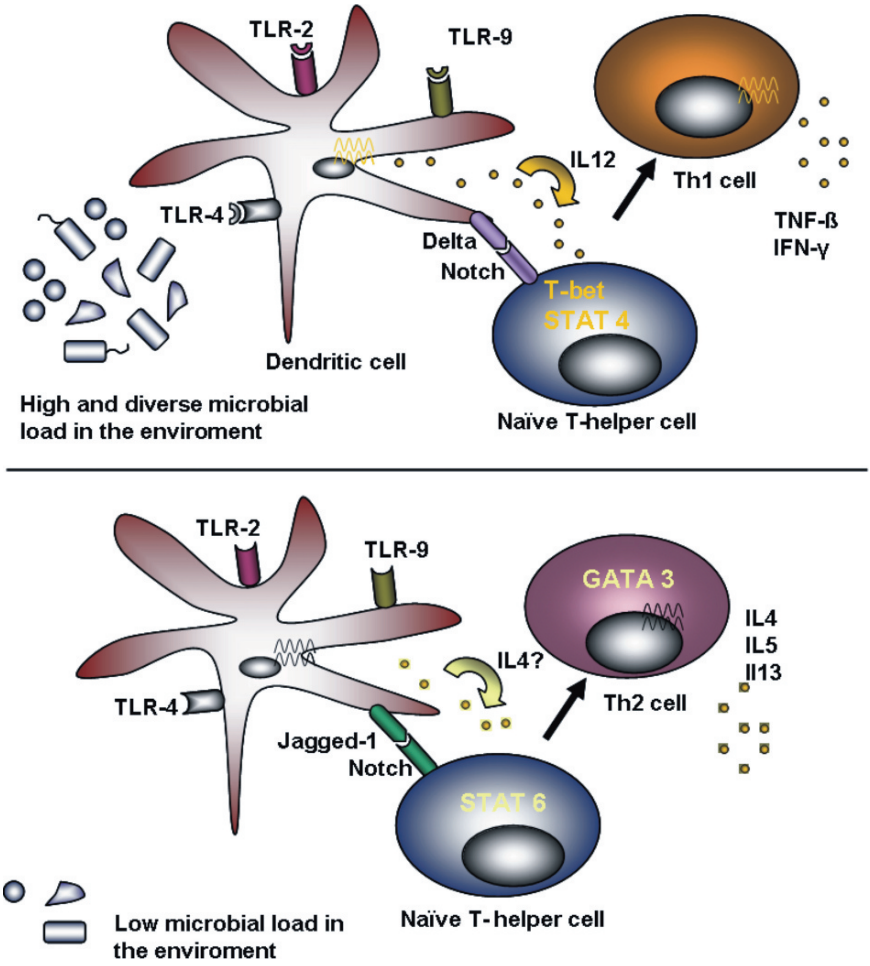

Fig. 3 The role of dendritic cells in the programming of Th-cells by environmental stimuli according to *in vitro* results from Debarry et al. [67] and Romagnani [68]. Strong activation of TLRs by PAMPs (pathogen-associated molecular pattern) leads to upregulation of Delta-4-ligand as well as IL-12 expression, thereby promoting Th1 response. As a result of missing TLR activation, Th2 maturation is mediated by a Jagged-1/notch interaction and probable involvement of Il-4

In line with these observations atopic infants, suffering from (mainly food-) allergies, show different patterns of colonization with intestinal microbiota when compared with healthy children [73]. In particular, the lack of the typical infant *Bifidobacterium* microbiota and the high number of *Clostridia* is obvious in atopic children [74]. Some authors reported a divergent composition pattern of the infant *Bifidobacterium* microbiota principally formed of *Bifidobacterium adolescentis* frequently found in adults [75]. Results of a case–control study reported by Murray

et al. [76] failed to confirm this observation in infants with allergic respiratory symptoms when compared to healthy children.

Another group of bacteria with probiotic properties is the genus *Lactobacillus*. These bacteria are able to colonize the human gastro-intestinal tract in the very early stages of life and to a large extent. When compared to *Bifidobacteria* these microorganisms are easy to grow and store in culture. Hence, *Lactobacilli* are the main probiotic bacteria of choice in studies of allergy therapy and prevention. In various studies and experimental approaches, *Lactobacilli* were shown to have immune-modulating capabilities by modifying the cytokine pattern of the host. Depending on the applied *Lactobacillus* species, the pre-conditions of the host as well as the time point of application modulate results in an up-regulation of either anti-inflammatory or inflammatory cytokines. Focusing on the early stages of life and the capacity of these species to prevent atopic disorders, a variety of epidemiological and experimental data are listed in the literature [77]. However, the strongest evidence for the effectiveness of a bioactive agent in humans comes from a clinical trial. Reviewing clinical trials of the last decade with regard to preventive properties of *Lactobacilli* two clinical research groups provided contradictory results (see Table 2). While the group of Kalliomäki and Isolauri from Finland reported protective effects (RR < 1) on the development of atopic eczema in pre- and postnatally treated high-risk infants with maintained effects in follow-ups after 4 and 7 years [78–80], the group of Taylor et al. [81–83] from Australia did not verify any significant findings, partially confirmed by Kukkonen et al. [84]. Another trial in which prebiotic substances were applied to high-risk neonates showed protective effects on atopic eczema [85].

This issue led to a controversy and a discussion on the body of clinical evidence and the lack of convincing and consistent results is still going on.

Experiments in acute and perinatal animal models of experimental allergic asthma revealed more consistent results [86]. In majority of the experiments, the treatment with *Lactobacilli* led to a shift toward non-allergic immune responses in most of the experiments mainly by decreasing Th2-cytokines, airway eosinophilia, and a reduction of IgE-levels when compared with the control group [87–91] (see Table 3). The feasible beneficial effects of pro- and prebiotics on the development of allergies mediated by the immuno-modulatory capability of the gut microbiota were summed up in the “microflora hypothesis” postulated by Noverr and Huffnagle [92], allocating this group of microorganisms and their products as potential protectives against allergies.

The Role of the Innate and the Adaptive Immune Response

Searching for a link at the interface between environment and the human immune system, cells of the innate immune system are in the focus to play a major role in the discrimination between potential pathogens and harmless components from the environment. Jeopardized by a range of pathogens a rapid detection of potential

Table 2 Double-blind, randomized placebo-controlled trials and follow-ups to study preventive effects of *Lactobacilli* in allergic diseases.

Organism	Application	Disease and immunological outcomes	Authors
Lactobacillus rhamnosus GG (LGG) ATCC 53 103	Prenatally to the mother and 6 months to the child	Significantly reduced relative risk for atopic eczema; number needed to treat = 4.5 (95% confidence interval 2.6–15.6)	Kalliomäki et al. [78]
	See above: 4-year follow-up of the aforementioned trial	Significantly reduced relative risk for atopic eczema	Kalliomäki et al. [79]
	See above: 7-year follow-up of the aforementioned trial	Significantly reduced relative risk for atopic eczema	Kalliomäki et al. [80]
Lactobacillus acidophilus LAVRI-A1	Newborns of women with allergy daily application for the first 6 months of life	*L. acidophilus* did not reduce the risk of atopic dermatitis and was associated with increased allergen sensitization in infants receiving supplements	Taylor et al. [81]
		FOXP3 mRNA expression at 6 months of age is higher in infants who develop atopic dermatitis, but is not affected by giving probiotics from birth and did nit diminish the risk of developing atopic dermatitis	Taylor et al. [82]
		Probiotic supplementation did not alter early innate immune responses in this population at high risk of developing allergic disease	Taylor et al. [83]
Mixture of 4 probiotic bacteria with prebiotic galacto-oligosaccharides	Mothers 2–4 weeks before delivery, offspring for 6 month after birth	No effect on the clinical outcomes of allergic diseases but tended to reduce IgE-associated (atopic) diseases in 2-year-old infants	Kukkonen et al. [84]
Prebiotic mixture of galacto- and long chain fructo-oligosaccharides	Neonates first 6 months of life in formula fed infants at high risk of atopy	Beneficial effect of prebiotics on the development of atopic dermatitis in a high-risk infants	Moro et al. [85]

Table 3 Animal models aiming to study preventive effects of *Lactobacilli* in allergic diseases.

Organism	Type of animal model/application of the organism	Findings	Authors
L. rhamnosus GG *Bifidobacterium lactis* (Bb-12)	Sensitization/challenge: OVA-sensitized asthma model Application: oral treatment 8 weeks after birth, during sensitization and airway challenge	Bb-12 or LGG suppressed all aspects of the asthmatic phenotype Inhibition of subsequent allergic sensitization and airway disease by induction of T regulatory cells associated with increased TGF-beta production	Feleszko et al. [87]
Lactobacillus reuteri *Lactobacillus salivarius*	Sensitization/challenge: OVA-sensitized asthma model Application: oral treatment before antigen challenge	Oral treatment with live *L. reuteri* can attenuate major characteristics of an asthmatic response in a mouse model of allergic airway inflammation	Forsythe et al. [88]
Lactobacillus casei subsp. *casei* with dextran	Atopic dermatitis-like skin lesions in NC/Nga mice Application: oral treatment	The combination of *L. casei* subsp. *casei* and dextran significantly decreased clinical skin severity scores and total immunoglobulin E levels in sera of NC/Nga mice that had developed picryl chloride-induced and *Dermatophagoides pteronyssinus* crude extract-swabbed atopic dermatitis-like skin lesions	Ogawa et al. [89]
Recombinant Bet v 1-producing *Lactobacillus plantarum* and *Lactococcus lactis* sp.	Sensitization: purified Bet v 1 Application: intranasal pretreatment with live recombinant strains	Mucosal vaccination with live recombinant *Lactobacilli* led to a shift toward non-allergic immune responses along with enhanced allergen-specific mucosal IgA levels	Daniel et al. [90]
Lactobacillus plantarum and *Lactobacillus gasseri*	Sensitization/challenge: OVA-sensitized asthma model Application: oral administration of the heat-killed strains after sensitization	Significant reduction of the antigen-specific IgE Strain-dependent stimulatory activity for IL-12 (p70) production	Sashihara et al. [91]

pathogens at the first line of defense enables the immune system to activate subsequent and suitable steps to eliminate these agents.

The first response of an organism to a pathogen is mediated by antigen-presenting cells (APC) of the innate immune system. DCs, as the main APC population, have been shown to be critical for Th cell fate and subsequently for the development of asthma [93]. Depletion of $CD11^+$–DCs leads to significant abrogation of characteristic features of experimental asthma indicating that these cells are necessary and sufficient for the induction of a Th2-driven inflammatory allergic response [94]. This effect is mostly attributed to myeloid DCs since allergen-presenting plasmacytoid DCs exert rather allergy-preventing effects [95], and other subsets of DCs, so-called regulatory DCs, may even induce the activation of regulatory T cells (Treg) [96].

In addition to the kind of origin particularly the activation status of DC plays an important role. Whereas IL-4 and IL-10 production by DCs is crucial for Th2 cell development, IL-12 and/or type-1 interferons shape naïve Th-cells toward a Th1-direction. The expression profile of DCs is affected by the interaction of their so-called pattern recognition receptors (PRR) with pathogen-associated molecular patterns (PAMPs) of viruses, bacteria, and fungi. PRRs, mainly the TLR family, are sensors situated at the outer membrane. The interaction between PAMPs and PRRs is in contrast to the high-specific recognition of antigens by T cell receptors (TCR) pathogen-unspecific and initiates different effector cascades, e.g., the release of antimicrobial defensins and the signaling to the adaptive immune response [97, 98].

The family of the TLRs represents the best-characterized class of PRRs. So far, 10 human (TLR-1–TLR-10) and 12 distinct murine (TLR-1–TLR-13, except TLR10) TLRs have been identified. TLRs are transmembrane spanning proteins with an "extracellular" domain containing leucine-rich repeats and a cytoplasmic toll/IL-1 receptor homology domain (TIR). They are principally specialized in the detection of several prototypic components of extracellular or intracellular PAMPs such as bacterial lipoteichoic acids (LTA) (TLR2), LPS (TLR4), flagellin from flagellate bacteria (TLR5), CpG DNA (TLR9), dsRNA (TLR3), or ssRNA (TLR7/8) [99]. Ligand binding on TLRs leads to an activation of several intracellular signaling pathways including the NF-κB pathway by direct or indirect interaction of TLR with MyD88 (except TLR3), TIR-containing adaptor protein (TIRAP), TIR-containing adaptor inducing IFN-β (TRIF), or TRIF-related adaptor molecule (TRAM) [100]. As a result of successful TLR-mediated activation, expression of costimulatory molecules (CD80 and CD86) and proinflammatory cytokines (TNF-α, type-1 interferons, IL-1, IL-6, IL-10, and IL-12) is induced [101]. Most of these cytokines favor Th1 differentiation [102] whereas mice lacking the master adapter protein MyD88 display an increased Th2 response [103].

Besides their function in the defense of infectious agents TLR responses are involved in the onset of immuno-modulated diseases such as allergies and asthma acting as a mediator toward the Th-cell response [104]. According to the hygiene hypothesis, TLRs need to be stimulated in the early period of life to balance the Th-cell populations from the *in utero*-shaped Th2-bias into a Th1-direction [105]. A number of studies and experiments on TLRs and their ligands concerning the initiation of allergic diseases highlight the role of TLR4 and its ligand LPS. On the

basis of epidemiological findings that an early exposure to LPS may protect against allergic disorders by stimulating the Th1-cell activity, animal models should provide more evidence regarding the role of TLR-signaling in the prevention of allergies and asthma. Surprisingly, some experiments, mainly animal models of experimental asthma, showed pronounced Th2 responses and elevated allergic parameters as a reaction on LPS application [106, 107] whereas the others reported reduced asthma phenotypes after LPS application [108, 109]. Delayre-Orthez et al. as well as Eisenbarth et al. [110, 111] demonstrated that these divergent results could be explained by dose-dependent effects as described earlier. While up-regulation of allergic inflammation seemed to be controlled by mast cells after their activation and modulation through TLR4-mediated induction of GATA1 and subsequent increase in Th2-cytokine production [105], the down-regulation of allergic inflammations is in part mediated by nitric oxide synthase 2-activity [106]. Experiments with LPS preparations derived from different bacteria pointed out that the other factors than dose dependency may influence airway reactions. Pulendran et al. [112] administered different LPS to OVA-sensitized mice observing that these LPS preparations from different bacteria activate DC subsets to produce different cytokines, and induce distinct types of adaptive immunity in vivo.

In addition to TLR4, other toll-like receptors were shown to possess immunomodulatory features and could prevent allergic inflammation. Lauener et al. [113] reported from the ALEX study population that blood cells from farmers' children expressed significantly higher amounts of toll-like receptor 2 than those from non-farmers' children, indicating that these TLR might also be involved in the "farming effect". TLR2 acts as a PRR mainly specific for gram-positive bacteria finding natural ligands in LTA, a cell wall component of gram-positive bacteria. Kitagaki et al. [114] demonstrated that application of TLR9 ligand CpG DNA prior to sensitization prevents Th2 inflammatory responses and effectively interferes with the development of atopic airway diseases in a murine model of experimental asthma. Moreover, when administered in combination with an experimental allergen, CpG promotes the reversal of established eosinophilic inflammation. Recently, we found that TLR3 or TLR7 activation by viral TLR ligands has both preventive as well as suppressive effects on experimental asthma which is mediated by the additive effects of IL-12 and IL-10 [115].

To sum up, TLRs may play a key role in the prevention in allergic disorders. Further evidence for this concept comes from epidemiological association studies pointing out that certain TLR gene polymorphisms are associated with an increased prevalence of allergic diseases [116]. For example, allergic diseases in farmer's children could be contributed to a significantly elevated prevalence of a polymorphism found in the TLR2-coding gene [117]. Another TLR2 polymorphism seems to be associated with severe phenotype of atopic eczema [118]. Results from a Swedish study indicated that a polymorphism in the TLR4-gene is associated with asthma characterized by a decreased IL-12 production by APCs after LPS stimulation [119].

T-helper cells are essential components of the adaptive immune response, and meanwhile it is well established that Th2-skewing plays an important role in allergic

diseases with their characteristic cytokines IL-4, IL-5, IL-9, and IL-13. These characteristic cytokines are responsible for major features of allergy such as, isotype switching toward IgE in B-cells, proliferation, differentiation, and survival of eosinophils, and increased mucus production in airways or gut [120, 121]. Several types of T lymphocytes control these processes to balance the immune system adequately and to avoid Th2-driven responses to potentially allergenic agents. Th1 cells are the natural antagonists to Th2 cells producing IFN-γ, a cytokine that promotes cell mediated immune responses [122]. A recently published experiment concerning the role of the Th1-cell surface protein mucin domain-containing molecule (Tim-3) in the development of Th2-associated responses underlined the control function of Th1 on Th2 cells. By application of an anti-Tim-3 antibody in a mouse model of allergic asthma before each airway challenge with OVA a significantly reduced airway hyper-reactivity (AHR) and a decrease in eosinophils and Th2 cells in the lung could be observed. Additionally, IL-5 was significantly reduced in the BAL, whereas IFN-gamma levels were significantly increased by anti-Tim-3 antibody treatment [123].

Regulatory T cells mediate the balance between Th1 and Th2 and are crucial for the maintenance of (self)-tolerance. Within the Treg population, the $CD4^+CD25^+$ T-cell subset, which exhibits about 5–10% of all peripheral $CD4^+$ cells, is one of the most emphasized topics in the research on allergic and autoimmune disorders [124]. The development and function of the $CD4^+CD25^+$ Treg subset is regulated by the expression of Foxp3, whereas deletion of this transcription factor leads to a loss of suppressive activity [125]. The immunosuppressive function of $CD4^+CD25^+$T-cells is mediated by the release of inhibitory cytokines such as IL-10, TGF-β, and the expression of extracellular negative costimulatory molecules like CTLA4 and glucocorticoid-induced TNF receptor [126]. Due to their important role in the regulation of Th2 responses, it seems to be obvious that a deficiency or the failure of Treg-mediated suppression of Th2 activation is one main reason for the development of allergic diseases as postulated by the "regulatory approach" of the hygiene hypothesis [127, 128]. Indeed, it could be shown that allergen-specific $CD4^+CD25^+$ regulatory T cells suppress *in vitro* T cell proliferation as well as in vivo leukocyte and eosinophile recruitment and AHR. Transfer of OVA-specific Tregs into OVA-sensitized mice further leads to a suppression of Th2 response. This effect is strongly IL-10 dependent whereas it is, interestingly, not produced by allergen-specific $CD4^+CD25^+$ Tregs themselves [129].

Taking these results together immune deviating as well as regulatory mechanisms might be involved in the programming and balancing of the immune response and the development of allergic disorders.

Gene–Environment Interactions and the Hygiene Hypothesis

A strongly discussed issue is the impact of the CD14/-159-promotor polymorphism (C → T) on the development of allergies. CD14 as crucial part of the LPS-binding TLR4-receptor complex plays an important role in the interaction between pathogens,

including endotoxin and the immune system [130]. The single nucleotide polymorphism (SNP) in the −159 locus of the promotor region next to the CD14 gene seems to be associated with decreased levels of total serum IgE in homozygous carriers as reported by Baldini et al. [131] and later on by Koppelman et al. [132]. Another association study conducted by Ober et al. [133] came to reversed findings, namely, higher IgE levels in the total serum and higher rates of allergies in association with the T-allele. Results reported from a large cross-sectional study by Kabesch et al. [134] showed no associations between the presence of the T-allele and the serum IgE or the prevalence of atopic diseases. Coming back to the "endotoxin switch hypothesis" as suggested by Vercelli in 2003 (as mentioned earlier) this conspicuous divergence may be explained. Postulating that the amount of the environmental microbial load, sensed by the innate immune system via endotoxin-TLR4-signaling, influences the programming of T-helper cells toward a Th1 or Th2-direction in a bimodal or multimodal course, the presence of the T-allele may shift these course from Th2 toward Th1 at a given microbial load. Thus, even low levels might be protective against a Th2 bias. In settings with a higher microbial burden, a second switch from Th1 to Th2 could explain the associations of the allele with the high-burdened environment as found in the community of the Hutterites characterized by traditional farming lifestyle and a high number of individuals carrying the T-allele but suffering from allergies [131].

It has to be stated that the "endotoxin switch hypothesis" cannot explain the underlying immunological mechanisms and it cannot be answered how the polymorphism is linked to IgE (Fig. 4).

Further studies on gene by environment interactions may answer further unsolved questions concerning discrepancies coming up with hygiene hypothesis.

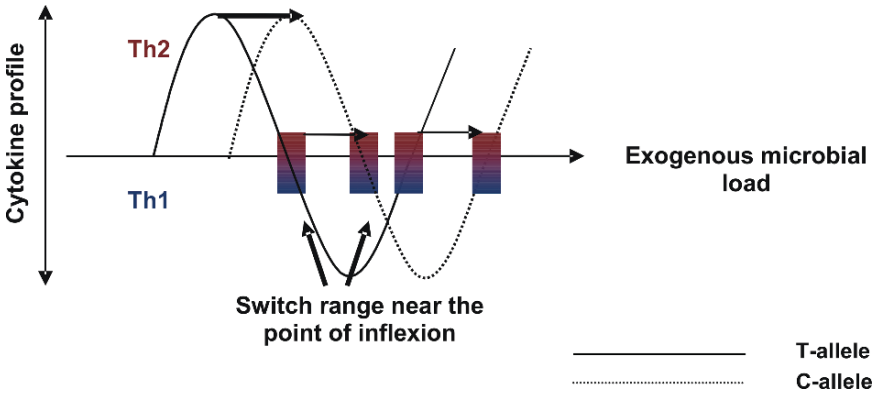

Fig. 4 Influence of CD14/-159-promotor polymorphism (C → T) on the endotoxin switch (modified from Vercelli [64]). Shift of the bimodal course of Th-cell maturation in the presence of T-allele toward lower levels of environmental bacterial load. To explain higher prevalence of allergies in populations living in high loaded environments a second switch (Th1 → Th2) has to be added

Opening the "Window of Opportunity" Will Promote Allergy Prevention

The hygiene hypothesis was originally postulated to explain the outbreak of the "allergy epidemic". Epidemiological as well as experimental approaches aimed to verify or to rule out this hypothesis provide new insights into the underlying immunological mechanisms that determine the onset of allergies. Causative factors coming from the genetic constitution, the environment and from lifestyle attitudes as well could be elucidated. Although critics of the hypothesis would not run dry, still there is no further idea to alternate the hygiene hypothesis. Moreover, the many scientific attempts clearly illustrate the importance of the pre- and early postnatal period of life and environmental effects on the development of allergies. The enlargement of knowledge with regard to this very early time of life may open the window of opportunity to develop new strategies in the prevention of allergy and asthma. As mentioned earlier, we found very promising results by application of stable-derived bacteria that reduces symptoms and immunological parameters of allergic asthma in an acute mouse model [67]. These bacteria might be the key to a new vaccine-like intervention strategy that may open the perinatal window to establish an effective management in allergy and asthma prevention. Experimental data obtained in our laboratory from a mother-to-child model using the probiotic *Lactobacillus rhamnosus* GG promised beneficial and preventive effects from oral maternal therapy [135]. Despite these therapeutic strategies, the research with regard to the hygiene hypothesis provides additional evidence to reconsider hygiene recommendations for the individual and homely environment particularly in view of high-risk groups.

New insights into the epidemiological, genetic, and immunological dimensions of the hygiene hypothesis and the protection against allergies may come from two presently running studies: the GABRIEL and the PASTURE/EFRAIM study [136], two Europe-wide studies emphasizing genetic and environmental causes of allergies, and asthma.

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Early Sensitization and Development of Allergic Airway Disease—Risk Factors and Predictors: Is the Adult Responder Phenotype Determined during Early Childhood?

Susanne Halken and Arne Høst

Introduction

Allergic airway diseases, i.e., allergic asthma and allergic rhinoconjunctivitis represent a heavy burden in childhood. In a recent prospective population-based study [1], the prevalence of current asthma and rhinitis was 14.4% and 15.1%, respectively, in 10-year-old unselected children, with a high degree of comorbidity as the prevalence of rhinitis was 42.5% in children with current asthma.

The allergens associated with allergic airway disease depend on the age, climatic, seasonal, and social factors, and housing conditions. In tempered and humid regions, allergy to house dust mites most often is associated with asthma followed by allergy to furred pets, whereas allergy to the fungus *Alternaria* spp. is important in arid climates in the USA and allergy to cockroach is important especially in urban communities in inner cities [2].

The prevalence and degree of sensitization to inhalant allergens has been shown to peak in young adults regardless of the allergen, and to diminish with adult age [3]. A strong association between indoor allergen sensitization and asthma has been confirmed, while exclusive sensitization to pollens is associated primarily with rhinitis [3].

The development and phenotypic expression of allergic disease depend on a complex interaction between genetic and several environmental factors such as environmental exposure to allergens, and nonspecific adjuvant factors (e.g., tobacco smoke). It is evident that environmental factors play a major role in the development of sensitization and allergic airway disease. It is also recognized that different phenotypes of, for example, asthma exists and that these may represent different long-term outcomes.

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_20, © Springer 2009

Identification of factors that might predict or influence the risk for development of allergic diseases and its prognosis is important for initiating possible preventive measures. Due to recall bias and selection bias, retrospective studies or cross-sectional studies are not very useful for evaluation of predictive/risk factors. Only prospective studies including accepted well-defined diagnostic criteria/outcome measures, a sufficient duration of follow-up, a sufficient follow-up rate, control for confounders, and a proper sample size for adequate statistical evaluation are useful for this purpose [4]. Regarding the few long-term follow-up studies from childhood to adulthood, changes over time of environmental factors, treatment modalities and diagnostic measures (e.g., for identification of sensitization) also may influence the results and conclusions.

The aim of this review is to evaluate possible predictors and risk factors as regards sensitization and development of allergic airway disease from childhood into adulthood focusing on asthma.

Definitions

There has been some controversy on definition of allergic diseases and use of different terms such as atopy/allergy and atopic/allergic. According to a recent, revised nomenclature [5, 6], allergic airway disease is defined as asthma and/rhinoconjunctivitis initiated by immunologic mechanisms (defined or strongly suspected), whereas the term nonallergic has been proposed when immunologic mechanisms cannot be proven. Allergy can be antibody- or cell-mediated. In most patients, the antibody responsible for an allergic reaction belongs to the IgE isotype and these are defined as IgE-mediated allergy. In non-IgE-mediated allergy, different mechanisms may be responsible (IgG, immune complexes, cell-mediated).

Sensitization is defined as the presence of a specific immune mechanism, most often as a positive skin prick test or detectable IgE antibodies directed towards a specific antigen. Sensitization does not necessarily imply an allergic inflammation, and especially a low degree sensitization [4] may be a normal and often transitory phenomenon especially in early childhood. The results of investigations of the prevalence of sensitization are highly influenced by methodological factors, such as the quality of extracts for skin prick test, assay for determination of specific IgE and the chosen cutoff level for positivity [4].

Natural Course of Allergic Diseases in Childhood

The expression of allergic diseases varies with age, and symptoms may disappear and be replaced by other symptoms. In infancy, the main atopic symptoms are atopic dermatitis, gastrointestinal symptoms and recurrent wheezing, whereas bronchial asthma and allergic rhinoconjunctivitis are the main problems later in childhood. Likewise, allergic reactions to foods, mainly cow's milk proteins are most common in the first years of life, whereas allergy to inhalant allergens mostly occurs later in childhood [7, 8].

Characteristically, sensitization evolves in the order of exposure: food, indoor allergens, and outdoor allergens. Sensitization to milk and egg most frequently occurs during the first 2–3 years of life, while sensitization to inhalant allergens occurs later in childhood with increasing prevalence with age [7–9]. Sensitization to indoor airborne allergens (house dust mites and pets) often occurs at a lower age than sensitization to pollen (birch and grass) [3, 7].

Different Asthma Phenotypes in Childhood

Different wheezing phenotypes exist within the "asthma syndrome." At least two different asthma phenotypes have been defined: one group of asthma children with a triggering or inducing of asthma and asthma symptoms through repeated early childhood infections ("infectious asthma") and another group with "allergic asthma" [10, 11]. Nonallergic asthma seems to have a better prognosis than allergic asthma [10,11].

Some large prospective population-based cohort studies that followed children from birth into adulthood have identified at least three different phenotypes in children with asthma:

- Transient infant wheezing
- Nonatopic wheezing
- IgE-mediated wheezing

as described by Stein and Martinez [12]. Recently, a fourth phenotype, late-onset childhood asthma has been added to this list [13].

Table 1 shows the most important characteristics of these different phenotypes. Most children with virus-associated wheeze during infancy do not wheeze after the

Table 1 Characterization of some different wheezing phenotypes in childhood

		Persistent		
	Transient early	Nonatopic (40%)	Atopic (60%)	Late onset
Symptoms 0–3 (5) years	+	+	+	no
Symptoms 3 – 6 years	No	+	+	no
Symptoms 6–11 years	No	Decreasing	+	no
Symptoms in adolescence/adulthood	No	(+)	+	+
IgE-sensitization	No	No	+	(+)
Family history of atopy	No	No	+	?
Lung function in beginning of life	Lower level	Normal	Normal	?
Lung function at 11 years	Lower level	normal/lower level	Impaired	?
BHR	No	(+)	+	+
Remission rate	—	High	Low	Low

BHR bronchial hyperresponsiveness

age of 3–5 years. A second group of children continues to wheeze beyond the third year of life; approximately 40% of these children are nonatopic and are more likely to develop airway obstruction in relation to viral infections. Meanwhile, studies have shown that up to 37% of early life wheezers still wheezed at the age of 10 years [14, 15]. This persistent wheezing phenotype was associated with high levels of atopy [14, 15]. In adults, asthma persisting from childhood into adulthood should be distinguished from asthma starting in adulthood [13]. The phenotypes of adult-onset asthma are still poorly defined [13]. Apart from the above proposals for asthma phenotypes, asthma may also be characterized by severity, lung function and bronchial hyperresponsiveness.

Development of Allergic Airway Disease: Predictors

In the evaluation of different predictive factors and risk factors, it is important to be aware of the different phenotypes. Especially, in infants and young children, the asthma diagnosis may be difficult, and in this age group, many children will have transient symptoms with wheezing episodes only associated with airway infections and a very good long-term prognosis. Thus, in evaluating the significance of sensitization and possible effect of exposure to allergens. it seems important to include allergic asthma, e.g., the so-called IgE-mediated wheezing, as an outcome measure.

Heredity

Although it is well-documented that atopic heredity is associated with an increased risk for development of allergic diseases [16–18], it has also been demonstrated that most children who develop possible "atopic symptoms," often including viral induced recurrent wheezing, during the first years of life come from families without an atopic heredity. Thus, the majority of young children with recurrent wheezing/asthma do not belong to high-risk groups for development of atopic disease [16], whereas a higher proportion of children with allergic airway disease, will have atopic heredity [19, 20]. In a large population birth cohort study, it was concluded that inheritance seems to be of prime significance of persistent childhood wheeze [21]. In another recent large prospective birth cohort study, it was found that infants born to atopic parents with a positive skin prick test to aeroallergens are at increased risk for aeroallergen sensitization during infancy, which persist to 2 years of age [22].

Infants with a pronounced atopic predisposition may have a primary immunoregulatory defect, which can be identified by various methods, e.g., elevated cord-blood IgE, low numbers of T cells, disturbed ratio of T-helper/T-suppressor cells and decreased function of T-suppressor cells [23]. Unfortunately, none of the tests are suitable for general allergy risk screening. However, elevated cord-blood IgE has been shown to be a better predictor of specific sensitization especially to inhalant allergens later in childhood than parental history [17, 24]. At present, the combination

of atopic heredity and elevated cord-blood IgE seems to result in the best predictive discrimination as regards development of allergic disease [16, 17, 24, 25].

Early Sensitization

Though transient sensation may be a normal phenomenon not necessarily associated with disease, many studies have demonstrated an association between sensitization in infancy and young childhood and development of allergic disease. These studies include unselected children, children with atopic heredity and children with early symptoms of a possible allergic disease, e.g., atopic eczema and recurrent wheeze.

Prospective studies have reported elevated serum IgE antibodies to hen's egg proteins in asymptomatic infants predictive for subsequent sensitization to aeroallergens and to the development of allergic airway symptoms [26, 27]. Sensitization to house dust mite allergens is found associated with an increased risk for early and late onset of asthma and persistence of asthmatic signs in children [28].

In a large prospective birth cohort study, persistent sensitization to any allergen from 1 to 7 years of age is found to be related to asthma at 7 years in children with a positive parental history of asthma [9]. In two other studies, atopic predisposition combined with sensitization to food allergens at 1 year of age resulted in the best prediction of sensitization to inhalant allergens at the age of 5 years [29]. In high-risk children, food-specific IgE in children before 24 months of age is found to be significantly associated with atopic disease (atopic eczema, food allergy and upper-airway allergy) at 24 months of age, and inhalant-specific IgE before 24 months of age significantly associated with upper-airway allergy [30]. Also, a positive skin prick test to hen's egg and/or cow's milk in the first year of life was independently predictive of adult asthma in atopy-prone infants [31].

Likewise, in infants with atopic dermatitis and wheezing, early sensitization to house dust mites, egg or cow's milk at 6 months of age was highly predictive of sensitization to house dust mites and persistent symptoms of asthma/atopic dermatitis at 5 years of age [32].

In large prospective observational whole-population birth cohort studies, atopic sensitization emerged as highly significant for wheeze, asthma and bronchial hyperresponsiveness at 10 years of age [20, 33].

Most studies on sensitization and development of asthma includes sensitization only as a dichotomous variable, i.e., individuals assigned as either sensitized or not, most often on basis of differing cutoff points. Often these, cutoff points have been low values, and probably the detection limit for the method used. Meanwhile, recent studies indicate that IgE quantification may be more useful for prediction of allergic disease. In a large Swedish prospective, population-based cohort study, positive IgE antibodies (ab) towards 14 common food and airborne allergens were found in 38% of the children with any allergic disease, whereas it was 17% among those without any allergic disease at 4 years of age [34]. Moreover, it was found that when the sum of IgE-ab levels was at least 34 kU_A/l, or more than four tests were positive, there was a 75% likelihood of identifying the individuals with any

allergic disease at 4 years of age. To identify those with asthma, as well as those with suspected allergic rhinitis, a significant interaction was found for the combination of the sum of IgE-ab levels and the number of allergens positive at tests [34]. Another recent population-based birth cohort study showed that the risk of current wheeze at the age of 5 years increased significantly with increasing IgE to mite, cat, and dog [35]. When IgE levels to these three allergens were summed, the probability of current wheeze increased 1.33-fold (95% CI, 1.21–1.47) per logarithmic unit increase, corresponding to an odds ratio of 3.1 at 10 and 4.25 at 30 kU_A/l. Similarly, increasing sum of mite, cat, and dog IgE was associated with reduced lung function at 5 years. Among sensitized children the sum of mite, cat, and dog IgE was the strongest associate of current wheeze at 5 years of age [35]. In another birth cohort study including 131 children with atopic parents, airway hyperresponsiveness at age 7 years significantly correlated with sensitization to cat, house dust mite, cockroach, and ragweed. Also in this study, there seemed to be a positive dose–response relationship, as children with the greatest airway responsiveness were much more likely to be sensitized to four or more allergens [36].

Many prospective studies have demonstrated that infants with atopic eczema and/or cow's milk allergy, especially in case of IgE-mediated reactions have an increased risk for the development of allergic airway disease during childhood [30, 37–39].

Development of Allergic Airway Disease: Risk Factors

Dietary Factors

In prospective observational birth cohort studies, it has been demonstrated that exposure to cow's milk proteins and introduction of solid foods before the age of 4 months of age is associated with an increased risk of recurrent wheeze/asthma up to 6 years [40–43]. Meanwhile, contradictory results are available and in a recent large prospective study from three to 21 years of age, it is concluded that breastfeeding for at least 4 weeks does not protect children against atopy and asthma and may even increase the risk [44]. Though interesting, the methods and data presented in that study do not seem to confirm the conclusion.

Because it is not possible or ethically acceptable to randomize to breastfeeding, the conclusions on the effect of breastfeeding are based on high-quality prospective birth cohort studies. Recent studies have shown that parent's choice of breastfeeding and the duration of breastfeeding are highly influenced by atopic heredity and early possible atopic symptoms, which may give rise to "disease-related modification of exposure" and reverse causation [45–47].

A possible effect of lack of or short-term breastfeeding on the development of atopic diseases may be due to either lack of a protective effect of human milk or exposure to cow's milk proteins. However, many other factors may contribute to

this effect. Children with atopic predisposition are breastfed for a longer period and have solid foods introduced at a later age, as well as breastfed infants are less exposed to other environmental factors such as tobacco smoke and pets, belong to a higher socioeconomic group and attend to daycare at a later age than formula-fed infants [48].

Allergen Exposure

Airway hyperresponsiveness in children is often associated with allergic sensitization [28, 49]. There seems to be a dose-dependent relationship between exposure and sensitization [2, 50–53], as well as an association between sensitization and development of asthma [2, 32, 50, 51, 54, 55]. A dose–response relationship between exposure to house dust mites and development and severity of asthma has been demonstrated [2, 54, 56]. In a recent longitudinal prospective study, cat allergen exposure in infancy was positively associated with sensitization at 2 years, but not at 6 years, as well as there was no association with allergic symptoms or disease at 6 years [53]. Meanwhile, cumulative allergen exposure from cat ownership and regular cat contact increased the risk of cat sensitization up to age 6 years [53]. Other longitudinal studies have shown that sensitization to house dust mites and animal dander antedate are predictors/risk factors for development of asthma in children [9, 28, 32]. However, in a recent prospective observational study [55], no direct significant association between early exposure to indoor allergens (house dust mite and cat allergens) and asthma up to the age of 7 years was found. Although no direct association has been found between early exposure to indoor allergens and development of allergic/nonallergic asthma [55], an association between exposure to indoor allergens and development of allergic asthma cannot be ruled out [2, 32, 50, 54, 56]. An association between allergen exposure and asthma should only be expected regarding allergic asthma.

Data from recent cross-sectional and retrospective studies have suggested that early exposure to pets might provide an asthma-protective effect for children [51]. The association between exposure to pets and the risk of asthma has been difficult to evaluate because of different study design and selection bias, e.g., parents of atopic predisposed or asthmatic children are more likely to remove pets from the home, may explain this controversy [50, 51, 57]. Also the influence of community exposure should be taken into account, as it is well documented that allergens from pets, especially cats, can be measured also in homes without pets in concentrations sufficient for inducing sensitization [51, 58]. Recent prospective observational studies suggest that early pet exposure is associated with a reduced risk of developing asthma until 4 years of age [59, 60] but not allergic sensitization until 4 years of age [60], and exposure to two or more cats in the first year of life may reduce subsequent risk of allergic sensitization against a panel of allergens, though not to cats, until 7 years of age [61]. Meanwhile, in none of these studies selection bias can be excluded, most of the information is collected by

means of questionnaires and the analyses did not include sibling asthma/allergy, which might heavily influence the families' choice of having pets. In one study, the follow-up rate was very low (57%), a very high percentage of parents had asthma (21%) and atopic heredity was not clearly defined [61]. Though two of these studies [59, 61] included allergen measurements in the methods, these values were not included in the analyses. A systematic review [50] concludes that exposure to pets appears to increase the risk of asthma and wheezing in children. Epidemiological studies suggest that in areas with low levels of allergens in homes, the prevalence of sensitization is low [51].

The apparent protective effect of exposure to pets may be due to avoidance behavior and "healthy pet keeper effect" [57, 62, 63]. A large cross-sectional study investigated the association between pet-keeping at time of birth and allergic symptoms in airways, nose, and skin among 14,077 young children (1–6 years) in Sweden [63]. They found that almost one-tenth of the population had got rid of pets because of allergy in the family, and 27.3% reported "avoidance" behavior toward pets. In a cross-sectional analysis, current pet-keeping was "protective." possible due to the fact that people avoid exposing their child to something that they believe is a risk factor for allergies. Pet-keeping at the time of birth was associated with wheezing, asthma, and rhinitis on pet-exposure later in life for children from families with an avoidance behavior, and was not "protective" for other children. There was also an indication of a dose–response relationship between the number of types of furred pets at time of birth and later symptoms in analyses adjusted for avoidance behavior or current pet-keeping. Thus, the distribution of pet-keeping in the population is largely explained by avoidance behavior, meaning that those who have pets mainly are those who can stand them, indicating a healthy pet-keeping effect [63].

The effect of exposure to indoor allergens seems to be most pronounced in children with atopic predisposition and with exposure during the first months/year of life [33].The German MAS (Multicentre Allergy Study) study showed that the dose–response relationship between early exposure to house dust mite allergen and cat allergen was most pronounced in children with atopic heredity [52, 55]. Another longitudinal study up to age 5 years demonstrated an association between early cat exposure and an increased risk of wheezing at or after the age of 3 years among children whose mothers had a history of asthma [64].

So far, a few prospective, randomized studies have investigated the efficacy of the avoidance of indoor allergens (house dust mites) [65–70] in infants with atopic predisposition or early atopic manifestations. Two of these studies with a follow-up to 7 [70] and 8 [69] years of age, respectively, have produced the first indication that a reduction in house-dust mite allergen levels in homes of high-risk infants may reduce the prevalence of sensitization to house dust mites and recurrent wheezing [69, 70].

There seems to be a synergistic effect of several coexisting environmental factors [71]. In countries with a so-called Western life style, most people spend more than 95% of their time in well-insulated modern buildings with reduced ventilation. The indoor environment of homes has changed over the last decades and there is

evidence of an increase in concentration of indoor allergens (house dust mites and pets) [72] and air pollutants [73].

Allergic rhinitis is mostly associated with allergy against outdoor allergens, e.g., birch and grass, but in case of perennial symptoms also, indoor allergens is relevant [3]. Recent studies demonstrated that pollen exposure in the first months of life is a risk factor for development of seasonal allergic rhinitis and a positive dose–response was indicated [74–76].

It has been hypothesized that the airway inflammation in asthmatics might precede the development of sensitization to environmental allergens. This does not seem to be the normal course in development of atopy and asthma considering the course of the allergy march in high-risk infants and the documented predictive capacity of early sensitization to food and airborne allergens as regards development of allergic asthma [26, 28, 32, 37, 38, 43, 74, 75, 77]. Neither is there any convincing evidence of such a hypothesis in the group of so-called nonatopic asthmatics with asthma symptoms caused by viral infections as elucidated in recent studies [10]. Therefore, in future studies on asthma in childhood the clinical, immunological and inflammatory type of asthma as well as the genotype should be described when possible.

Tobacco Smoke

Several studies have shown a significant association between parental (particularly maternal) smoking and increased wheezing and asthma in children [15, 20, 78–80]. This association is strongest up to 6 years of age. The severity and frequency of symptoms were related to the extent of exposure in the home [78].

Furthermore, passive smoking has been associated with sensitization to indoor allergens in some studies [71, 81], but not in others [82]. Importantly, maternal smoking during pregnancy is significantly associated with reduced respiratory function in early infancy and recurrent wheezing during infancy and early childhood [80, 83].

Recent studies indicate that some individuals with the genetically determined deficiency of gluthathione-*S*-transferase GST M1 and GST T1 enzymes are more susceptible to exposure to tobacco smoking and development of asthma [84, 85]. *In utero* smoke exposure in GST T1-deficient children was associated with development of recurrent wheeze/asthma and decrements in lung function [85].

Outdoor Pollution

There is convincing evidence of a cause–effect relationship between exposure to outdoor pollution and induction of atopic respiratory symptoms. As regards the possible relationship between outdoor air pollution and development of asthma/atopic airway disease, the findings of many studies are weak or contradictory [23, 86].

Immune Modulation

Although viral respiratory infections frequently trigger acute exacerbations of asthma, the relationship between such infections and asthma is not clear, in part because of the difficulty in defining asthma in young children. There is evidence supporting two different but not mutually exclusive hypotheses: (a) predisposed children are susceptible to asthma and severe respiratory tract infections or (b) severe viral infections may have a long-lasting influence on the subsequent development of asthma.

Recent studies indicate that early viral infections primarily are associated with so-called infectious type of asthma with a more favorable prognosis as regards recovery before 10–11 years of age [10, 11]. However, early viral infections do not seem to increase the risk of later atopic asthma [11].

Family size (number of siblings) has been hypothesized to be inversely related to the risk of atopy, but prospective studies have not been able to confirm this finding [49, 87–89]. The possible influence of different vaccines, e.g., tuberculosis, BCG (Bacille Calmette-Guerin) and pertussis vaccination on the development of atopic responses in children has been investigated; but at present, there is no evidence of a causal relationship [87, 90, 91]. It has been hypothesized that the intestinal microbial flora may influence the development of sensitization but no convincing evidence for this theory has been published [87, 92].

Persistence of Allergic Airway Disease: Predictors and Risk Factors

Atopic Heredity

Apart from being a predictor/risk factor for development of asthma, atopic heredity (first degree relative) is also shown to be associated with an increased risk for persistent asthma disease in children with diagnosed asthma [18, 20, 93, 94].

Sensitization

Chronic asthma is associated with sensitization to indoor allergens, which are more important than the outdoor allergens, probably because of time spent indoors [2]. Many studies indicate that sensitization in children with a wheezing syndrome are predictive of later childhood asthma [55, 93–96]. In a large population-based prospective birth cohort study with follow-up from birth to 10 years of age, chronic childhood sensitization (measured at 4 and 10 years) was significantly associated with significantly higher cord blood IgE, increased prevalence of aeroallergen

sensitization, persistent wheeze, eczema, rhinitis, and bronchial hyperresponsiveness at 10 years [97]. Sensitization to perennial allergens (e.g. house dust mites, cat, and dog) developing in the first 3 years of life was associated with chronic asthma and a loss of lung function at school age (13 years) [98]. Thus the remission rate of symptoms at 13 years was 90.2% among nonatopic wheezers versus 56.2% for atopic wheezers [98]. Moreover, it was found that concomitant exposure to high levels of perennial allergens early in life aggravated this process with loss of lung function and enhancing the development of bronchial hyperresponsiveness for those children who were sensitized [98]. Correspondingly, in the Manchester birth cohort, the combination of sensitization to indoor allergens and exposure to the sensitizing allergen also determined the level of lung function at 3 years [99]. Current sensitization to indoor allergens and also elevated cord blood IgE have been found to be determinants of impaired lung function in children with current wheeze at 7 years [93].

A recent population-based birth cohort study showed that, the sum of IgE to mite, cat and dog at age 3 years increased the risk of persistent wheeze by age 5 years (2.15-fold/logarithmic unit increase in the specific IgE) in a dose-dependent manner [35]. Data from the Tucson Children's Respiratory study with follow-up from birth to 16 years [96] showed that children with persistent or late onset wheeze were more atopic at age 6 years and they continued to be more atopic at age 11 and 16 years than never and transient early wheezers [96]. The results from this study suggests that wheezing at age 6 years, regardless of whether the children wheezed previously, is associated with continued symptoms through age 16 years and that the increased prevalence of atopy, present in persistent and late-onset wheezers at age 6 years, continues though adolescence [96].

Also, long-term follow-up studies from childhood to middle adulthood indicate a significant association between sensitization and persistent asthma in adulthood. In one study, a significant association between a positive skin prick test and severe childhood asthma and severe asthma later on in adolescence and adulthood until 28 years of age was found [100]. In another study from age 9 to 26 years, sensitization to house dust mites and cat allergens were strongly predictive for persistent or relapsing wheezing at age 26 years [101].

Severity, Lung Function, and Bronchial Hyperresponsiveness

Several studies indicate that severity of the asthma disease, lung function, and bronchial hyperresponsiveness may predict the persistence of the disease later in childhood and even into adolescence and adulthood [96, 100–102]. It has been shown that children with the early transient phenotype is not associated with impaired lung function later in childhood [12, 93, 102]. In a long-term follow-up study of a cohort of 378 asthmatic children from childhood (age 7 years) to middle adulthood (age 35 years), the presence of an atopic condition in childhood was found to increase the odds of more severe asthma in later life on in adolescence

and adulthood until 28 years of age [100]. Additionally, the odds of eczema and hay fever also increased with severity of asthma in childhood [100]. Another long-term prospective study of a birth cohort with atopic predisposition with follow-up from birth to the age 22 years showed that remission of wheeze was common in children younger than 5 years of age and likely if wheezing occurred on less than two occasions, whereas wheeze at 11 years of age was likely to persist [103]. Thus, frequent wheezing before age 5 years was associated with adult asthma and bronchial hyperresponsiveness [103]. Also, a recent prospective study from New Zealand from age 9 to 26 years found that airway hyperresponsiveness and low lung function in childhood were strongly predictive for persistent or relapsing wheezing at age 26 years [101].

In a community-based cohort study 575 children aged 8–10 years were reassessed 15–17 years later, it was shown that childhood characteristics that independently predicted asthma symptoms in adulthood were obstructive spirometry, airway responsiveness, atopy, recent wheeze and being a female. Children with all five characteristics had a likelihood ratio of 36.9 for asthma symptoms in adulthood [104].

Conclusion

Atopic airway diseases in children are mostly associated with allergic sensitization. Chronic asthma mostly is associated with sensitization to indoor allergens, whereas allergic rhinitis most often is associated with outdoor allergens. It is unlikely that one or few factors are responsible for an increasing prevalence of atopic diseases. Atopic heredity, elevated cord-blood IgE and early sensitization are well-documented predictors as regards allergic airway disease.

A clear association between exposure to indoor allergens (e.g. house dust mites, cats) and sensitization as well as a clear association between sensitization and development of asthma has been documented and several studies have shown that sensitization precedes and antedates development of allergic airway disease. Allergen exposure is a risk factor for sensitization and development of asthma later in childhood in high-risk infants and infants with early atopic manifestations (Fig. 1).

Different asthma phenotypes have been described such as early transient, early persistent and late onset as well as atopic versus nonatopic. It is now evident that early transient and nonatopic asthma is associated with a good long-term prognosis and a normal lung function. In contrast, early-onset persistent childhood asthma often is associated with persistent sensitization, loss of lung function and bronchial hyperresponsiveness. In children with allergic asthma, exposure to the relevant allergen is associated with an aggravation of this process and worsening of the prognosis. Also exposure to tobacco smoke is associated with persistence of symptoms. As shown in Table 2, predictive factors for persistence of asthma into adulthood are atopic heredity, sensitization to perennial allergens, severe disease, impaired lung function (obstructive), bronchial hyperresponsiveness and concomitant presence of other allergic manifestations, e.g., rhinitis.

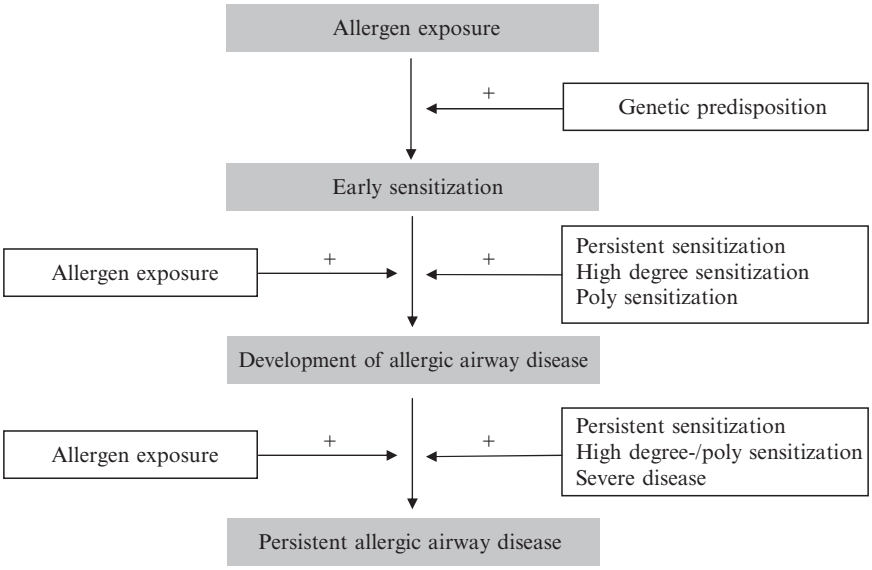

Fig. 1 Development of persistent allergic airway disease. Risk factors and predictors

Table 2 Predictors for development and persistence of allergic airway disease

Predictors for early sensitization and development of allergic airway disease
• Atopic heredity predicts atopic disease
• Elevated cord blood IgE predicts early sensitization
• Early sensitization to egg/CMP/HDM predicts later sensitization to aeroallergens and atopic disease, especially in children with atopic heredity or early atopic symptoms
• Persistent sensitization to any allergen predicts asthma in children with atopic heredity
Predictors for persistent allergic airway disease into adolescence / adulthood?
• Persistent sensitization
• High degree sensitization and polysensitization
• Early onset of persistent asthma
• Severe asthma disease
• Reduced lung function / bronchial hyperresponsiveness
• Presence of another atopic condition

Many children do not remit from their asthma; the more severe their asthma is, the less likely they are to remit. Data supports the tracking concept of the disease, mild disease remit or continue to be mild whereas severe disease more likely persist being severe into adulthood.

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T Cell Responses to the Allergens and Association with Different Wheezing Phenotypes in Children

Peter N. Le Souëf

Introduction

In determining relationships between T cell responses to antigens and wheezing in infants and young children, the patterns of development of Th1 and Th2 responses in early life need to be considered as these are closely related to clinical wheezing phenotypes. In addition, factors affecting T cell responses are relevant, as in general, these factors are also associated with wheezing patterns in this age group.

The role of Th2 immune responses in nature is also important. Indeed, the need for these responses at any time of life is still not well understood. Evidence suggests that they are needed for protection from parasitic infections, particularly those due to helminths [1], but this is still not well established [2]. In contrast, much more is known about the role of the immune system in protection from viruses and bacteria. Over the last few years, an individual's Th2 responses have been shown to be directly linked to their Th1 responses. Hence, the role of Th2 responses in nature is likely to be much more complex than is currently understood. Until there is a much better understanding of mechanisms, roles, and development of the various T cell responses, how these relate to wheezing in children may remain relatively obscure.

Ontogeny of T-Cell Responses to Allergens

Over the last decade, the pattern of T-cell responses in early life in humans has been elucidated. Although much knowledge has been gained about the pattern of observed changes in Th1 and Th2 immune responses, why these occur at the various stages of development has remained unclear.

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_21, © Springer 2009

Effect of *In Utero* Environment on T-Cell Responses

The *in utero* environment appears to be important in influencing later T-cell allergen responses since these are already different between infants at birth [3]. The initial environment the fetus experiences is intra-uterine and this is Th2 skewed [4]. However, cord blood mononuclear cells (CBMC) Th2 responses are reduced in infants of atopic mothers [5–7], reflecting delayed maturation of Th2 responses in infants destined to develop atopy [8]. Whether the degree of delay in maturation can predict later atopy is still not clear [4], although both Th1 and Th2 cytokine levels were reduced in cord blood in infants who later developed doctor-diagnosed asthma [9]. Maternal regulation of infants' antigen responses is unlikely to be due to transplacental transfer of allergen-specific IgG; a maternal influence on infants' T cell production of interferon (IFN)-γ is suggested as a more likely mechanism [10].

Determining the relative contribution of maternal or infants' genotypes to the development of T cell responses would be complex as both would need to accurately documented and accounted for since 50% of the infant's genetic makeup is maternal. Current epidemiological data suggest that the fetal environment, as determined by the mother's genetic makeup, could contribute to the infant's immune system's pattern of development. Development atopy in the offspring is more strongly related to maternal than paternal influence [11], but mechanisms for this are still unclear.

The *in utero* environment is also important for the first exposure of the fetus to allergens, as relatively large molecules can cross the placental from the mother to the infant [12]. Higher exposure to house dust mites (HDM) during pregnancy was associated with a lower percentage of IFN-γ producing stimulated CD4(+) CBMC [13]. The extent to which transplacental antigens can prime the early immune system response is yet to be determined.

Delay in Maturation of T-cell Responses in High-Risk Neonates

As noted above, at birth, infants' CBMC show variations in responses to stimulation and individuals who will later develop atopy and asthma show a reduction in the levels of both Th1 and Th2 cytokines [14]. In infancy, subjects who do not go on to develop atopy show selective downregulation of their Th2 allergen-specific responses, whereas those who develop atopy upregulate their Th2 responses [14]. The probable time course of these events has been suggested by studies of vaccine responses, as the exposure to antigen in such studies is tightly controlled in both dose and timing. In a such a study, delay in IFN-γ production accompanied the reduction Th2 cytokine responses to specific vaccine allergens [15]. The IFN responses recovered spontaneously around 12 to 18 months of age at the same time that Th2 cytokine responses became more prominent in at-risk infants [16]. By 5–6 years of age, Th2 responses to common allergens are enhanced in atopics [17].

The reason for the marked delay in T cell responses in atopic children is obscure, as there would be little advantage with this, although a postponement in the onset of atopic symptoms from inhaled aeroallergens may have a minor survival advantage. The relative impairment in Th1 immune responses could be of much greater importance and the possible contribution this makes to the spectrum of wheezing in children through impaired protection from viruses will be discussed later in this chapter. However, with respect to survival advantage, this problem with Th1 responses and hence protection from viruses points to a possible reason for the observed situation. Future atopic individuals with impaired early Th1 responses could be expected to be at a disadvantage compared with nonatopic individuals and this could be the reason that pro-Th2 genotypes are less frequent particularly in Europeans compared with Africans [2, 18]. The rationale of this is that modern humans had their origins in Africa [19] where the harsh, tropical environment required strong Th2 defensive responses to protect from endemic helminthic infections [2]. When modern humans left Africa around 50,000 to 100,000 years ago [20], those moving to Europe would have encountered a much cooler climate that would have been less conducive to helminthic disease, since helminths thrive in a hot, wet environment [21]. The high frequency of pro-Th2 genotypes found in people from tropical Africa [21] would not have been needed so much in a temperate climate and could be expected to have compromised Th1 responses early in life, as described above. With the rise of agriculture, throughout western Asia and Europe, abundant food resources supported larger populations that no longer needed to live nomadically [22]. The move from small groups of individuals who moved frequently to follow food sources to large communities that did not move would have been ideal for the transmission of viruses. Additionally, the formation of stationary communities and domestication of several animal species would have allowed animal viruses that were new to humans to infect large numbers. Viruses would have become a major health issue in large communities as the viruses themselves could now survive and continue to circulate within a group. Since many viruses are eliminated within a few weeks [23, 24], jumping between nomadic groups would rarely have been possible so that viruses would not have been a major problem in small, mobile groups. One could therefore speculate that the reason that African populations have a high prevalence of Th2 responses [18] with their associated lack of adequate Th1 responses is that helminth infections were a major problem whereas viruses were not. We speculated that those who left Africa ended up in cooler climates with more densely populated communities. Viruses and other infectious diseases were encountered and survival favored those with stronger Th1 responses. However, the extent to which these factors might have operated has still to be established.

The delay in Th1 responses that accompanies the delay in Th2 responses in those at-risk of developing atopy has several important implications. Principally, those with impaired Th1 responses are likely to have an increase in prevalence and severity of respiratory viral infections. If viral infections themselves modulate the programming of Th2 polarization, as has been suggested [25], the increase in viral infections could act to enhance immunologically mediated inflammation of the airways. However, a simpler and increasingly more plausible explanation is that the real problem in early life with respect to T cell function and wheezing is that atopy

is marker of a range of underlying immune system dysfunctional responses affecting both Th1 and Th2 responses but that the relative deficiency of Th1 responses is the main problem. The possibility that the impaired T cell responses, as characterized by the significantly lower IFN-γ responses to T cell stimulation, could lead to problems with infection has been raised on several occasions [26], but was seen more in the context of the role of respiratory viruses as triggers of asthma or enhancers of Th2 responses rather than a primary problem in itself. How well the possibility that Th1 impairment is the main problem fits the available data will be discussed later in this chapter when genotypic and other associations are considered.

Environmental Influences on T Cell Responses to Allergens: Smoke Exposure

External environmental influences also affect the development of Th2 responses while the fetus while still *in utero*. The most powerful known influence is maternal smoking. Unlike passive environmental tobacco smoke (ETS) exposure at other ages, the fetus is subjected to the same full systemic levels of soluble toxic agents in cigarette smoke as an active smoker [27, 28]. This level of exposure may be why maternal smoking during pregnancy has a much greater effect than postnatal exposure on subsequent respiratory morbidity [29–32]. ETS exposure via the air during infancy is likely to be greater than at any other age [33] and may contribute to the *in utero* exposure. ETS has been associated with the risk of an offspring experiencing lower respiratory infections [29, 34], episodes of wheeze in infancy and the preschool years [31], reduced lung function in childhood [30, 32], increased airway responsiveness (AR) [35], and the prevalence of asthma [30, 36]. The other possibility for the strong *in utero* effect of smoking is the timing of exposure and the susceptibility of the fetal immune system to the toxic products of tobacco prior to birth. Effects of maternal smoking on the fetus include increased fetal death [37], lower birth weight [28], reduced lung function, [38] and increased AR at birth [39].

Several recent studies have begun to outline the extent of the problem of the effect of parental smoking on the developing immune system. Strong evidence exists for the significance of this relationship. One important study used a genome-wide screen to demonstrate that the cytokine-rich area of chromosome 5 was susceptible to parental smoking, as in infants of smoking parents, linkage was demonstrated for asthma but was not present in those with nonsmoking parents [40]. This chromosomal region contains several of the most important genes that determine Th2 responses to allergens. Although the timing of exposure was not assessed [40], exposure during pregnancy would seem most probable due to the epidemiological studies as noted above. Another important study examined innate immune responses to a variety of toll-like receptor (TLR) ligands, including TLR2, TLR3, TLR4, and TLR9, in CBMC in newborns of smoking versus nonsmoking mothers. In this study, stimulated cells from infants of

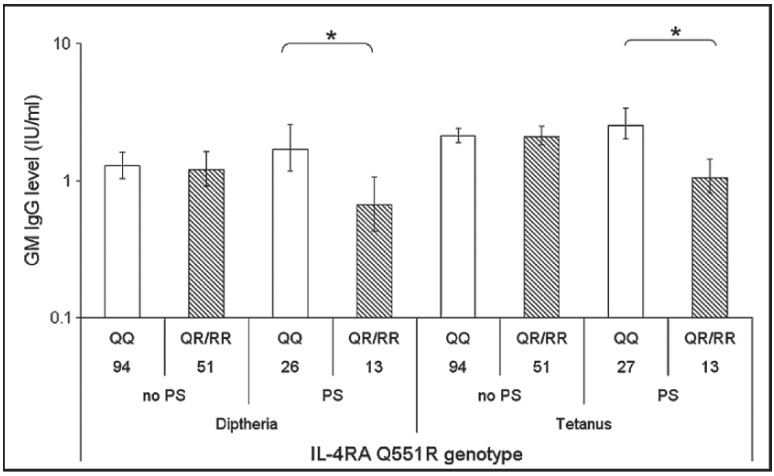
smoking mothers produced reduced levels of several cytokines including tumor necrosis factor (TNF) α, interleukin-6 (IL-6), and IL-10 [41].

Environmental Influences on T Cell Responses to Allergens: Inhalant Allergens

In atopic children, the degree of responsiveness to allergens is related to the strength of the exposure to that allergen. In children from warm, humid areas, such as Belmont in New South Wales [42], HDM are ubiquitous and present in high numbers and strong skin prick test (SPT) to HDM are seen and serum-specific HDM IgE levels are high in atopic children. In hot dry locations, as found in inland New South Wales, HDM are much less common as the environment does not suit them and positive SPT to them are much less common, but SPT are positive to other antigens, such as alternaria [43]. However, the prevalence of wheezing and asthma has not been shown to be different in such situations [43], suggesting that the reactions exhibited to antigens reflect the antigens in the environment and not any causal relationship between allergens and disease. Cat allergens are more difficult to understand. In an environment where both cat and HDM allergens were common, IgE levels to both mite and cat were strongly associated with wheezing (odds ratios, 5.2 and 6.5, respectively), but children who lived with a cat were less likely to show evidence of increased specific IgE to cat than those without a cat [44]. In contrast, cat ownership was not related to mite sensitization and those living with a cat had a lower prevalence of specific IgE to cat (28% vs. 66%, $p < 0.001$) than children not living with a cat [44]. How these interrelationships work is still not known.

Effect of Smoke Exposure on T Cell Responses: Genotype Specificity

Genetic studies have also begun to unravel the complexities of the early T cell immune responses related to smoking. In "at risk" (parental atopy) children recruited at birth, specific antibody responses to diphtheria and tetanus vaccine antigens were reduced with respect to levels of vaccine antigen-specific IgG levels and PBMC cytokine responses when the cells were stimulated by the vaccine antigen [45]. Positive results were detected for polymorphisms in IL-4, IL-4 receptor (IL-4R), and IL13, but only in those with a smoking parental [45]. For example, for the IL-4Rα 551 QR/QQ genotypes, reductions were found in vaccine-specific responses with respect to both antigen-specific IgG levels (Fig. 1a) and PBMC responses (IFNγ, $p = 0.002$; IL-10, $p = 0.01$; 1L-13, $p = 0.01$; IL-5, $p = 0.06$) to tetanus toxoid (Fig. 1b) and parallel reductions in polyclonal T-cell responses and innate immune responses in smoke-exposed infants [45]. The relevance of these data is that they suggest that exposure



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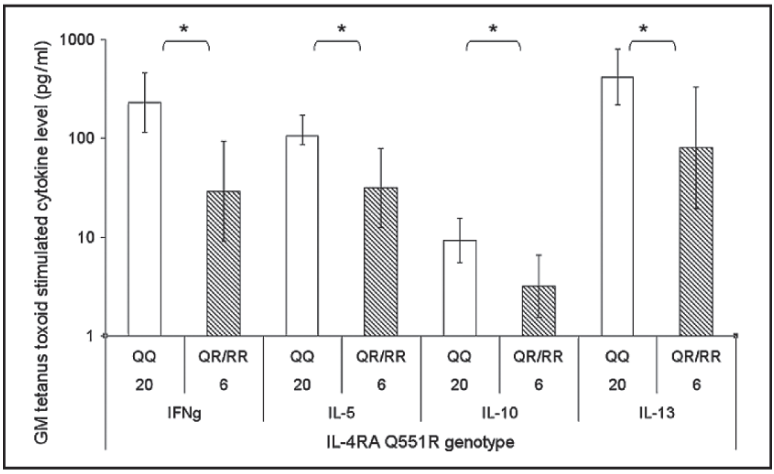

Fig. 1 Relationship between IL-4Ra Q551R and vaccine responses. Log geometric mean (GM) and 95% CI of levels are shown. All values corrected for time between last dose of vaccine and specimen collection. From [45] with permission. **a** Vaccine antigen-specific igg responses to diphtheria and tetanus toxoids for IL-4Ra Q551R in subjects exposed to parental smoking (PS) and unexposed (no PS) ($^{*}P < 0.05$). Genotypes for PS exposed but not for non-PS exposed subjects with lower levels of igg being observed for the "pro-atopy" genotypes (grey bars). Numbers on the *x* axes = number of subjects per genotype group. **b** Tetanus toxoid–stimulated cytokine responses versus genotype in subjects exposed to PS ($^{*}P < 0.05$). Cytokine levels lower for "pro-atopy" genotypes (gray bars)

to ETS in fetal or early life impairs T cell immune responses and that these could contribute to increased susceptibility to infections that produce wheezing at this time. Intriguingly, the genotypes in this study associated with the greatest reductions in Th1-related antibody and T cell responses are the "pro-Th2" alleles that have been associated with the supposedly Th2-related diseases atopy and asthma [46]. These data, therefore, demonstrate that genes with an apparent Th2 focus have covert affects on Th1 immunity and major implications to disease etiology in early life.

Th2 Genotype-Specific Associations with Responses to Antigens

The problem with pro-Th2 genotypes being associated with impaired specific IgG responses to antigens is also present in older children. In a Dutch study of children with recurrent otitis media, those with the "pro-Th2" allelic variants of CD14, IL-4, IL-4Rα, and IL-13 demonstrated reduced vaccine-specific specific IgG levels for each of the seven antigens of the pneumococcal vaccine employed in this study [47, 48]. These findings therefore support the findings noted above in younger children and also have broad implications to susceptibility to infection. Again, the mechanisms responsible for these findings are unclear. Linkage disequilibrium is not a likely explanation, as the relationships have been noted in several genes. Given that several genes are involved, a common and as yet unknown link between the mechanisms that control immunoglobulin production would seem probable.

Allergic Phenotypes and Wheezing in Children

T cell responses are strongly associated with wheezing phenotypes in children who wheeze. In general, children who have developed strong Th2 responses to allergens are more likely to wheeze than children without such responses. The relationships between Th2 responses and wheeze are particularly strong in children beyond the preschool age. These relationships are so strong that for many years the prevailing paradigm has been that asthma has been considered as an allergic disease and wheeze has been considered to be primarily an allergic phenomenon. Indeed, in many countries, allergists treat wheezy children especially in North and South America.

In general, the association between allergy and wheeze has been taken as evidence of causality although direct evidence for this has never been strong. In recent years, the assumption that allergy causes wheeze in the majority of children who wheeze has looked increasingly less convincing and as new evidence comes to light, the possibility that allergy has been overemphasized has been raised. Nonetheless, children who wheeze have a wide range of allergic phenotypes that vary with age, indoor and local environment, external environment, and ethnic group.

T Cell Phenotypes and Wheezing Phenotypes in Infants and Preschool Children

In infants, wheeze is much less likely to be associated with the presence of allergy [49]. There are several reasons why this might be so. Firstly and perhaps most importantly, in those destined to develop the enhanced Th2 responses that are common in older children with asthma, there is, as noted, an abnormality of the rate of development of the immune system. The lack of evidence that skewing toward Th2 responses is associated with wheeze in infants and preschool children

maybe one of the reasons that has led epidemiologists and others to determine that there is a different disease entity at this age and the term "transient viral-induced wheeze of preschool children" was introduced some years ago to cover this condition [49, 50]. There are several reasons why this categorization is misleading. First, as will be discussed later in this chapter, viruses are the main inducers of wheeze at all ages, even in adults [51] and older people with chronic obstructive pulmonary disease [52], so a diagnosis of viral-induced wheeze as a separate entity for infants and preschool children makes no sense when this is true at all ages. Secondly, at the time of presentation with wheeze in infants, there is no accurate way to determine which children will continue to wheeze later in life [53]. Thirdly, in those destined to develop enhanced Th2 responses by the age of 5 or 6 years, the abnormal ontogeny of the immune system in the preschool years means that there may be no sign of this happening in the early years of life and the known biomarkers of future allergic disease or atopy are not accurate in their predictions [3]. Finally, the transient nature of wheezing in response to viruses in many young children does not necessarily mean that the mechanisms producing wheeze are different from those in older children or that they have a different disease. Indeed, there is no evidence that the basic mechanisms by which viruses produce wheeze are age-related.

T Cell Phenotypes and Wheezing in Older Children

In older children, wheezing and T cell responses are more strongly related, and the majority of children demonstrate evidence of enhanced Th2 responses [54]. Why this is so in this age group has never been established, but the strong evidence of association between Th2 responses and allergy does not mean that either the measured Th2 responses or the allergic manifestations are the direct cause of wheeze. Furthermore, as mentioned above, the majority of wheezing exacerbations are associated with the presence of evidence of an acute respiratory viral infection.

A comprehensive study of T cell function was carried out in an unselected group of 147 11-year-old children who have been followed up from birth [55]. PBMC challenged with allergen showed responses dominated by IL-4, IL-5, IL-9, and IL-10 in those with atopy, whereas IL-10, TNF, and IFN γ responses were common in both atopics and nonatopics. Such specificity of response was not detected when T cells were subjected to nonspecific stimulation. These distinct T cell response patterns underline the differences between atopics and nonatopics. The T cell responses in atopics are also likely to be related to wheeze, as they were also associated with increases in AR.

Associations between T cell phenotypes and wheezing with respect to parental smoking or subjects' smoking is less clear in older children, although the relationship between recent or current smoke exposure and asthma remains [56]. Part of the problem in demonstrating these relationships is that the association between atopy

and asthma is so strong at this stage in life that determining whether the effect of smoke exposure works through affecting atopy is difficult to ascertain.

T Cell Phenotypes and Wheezing in Older Children: Genetic Factors

Age-specific relationships have been demonstrated between atopy and specific genotypes. Although only a few such relationships have been reported, this is more a reflection of the paucity of good long-term epidemiological longitudinal cohort studies than any lack of evidence or interest in this field [57]. Also, numbers in such studies are usually too small to allow the interrelationships to be worked out between T cell function, atopy, wheeze, and genotype. The CD14 variant that has been associated with increased specific IgE in children [58] is also associated with an increased prevalence of atopy (as determined by SPT) in midchildhood and adolescence, but not in young adults [59]. Other studies that have shown age-specific effects are for a CCR5 polymorphism and asthma [60], an IL12 promoter polymorphism and reduced lung function in females aged 10 and 14 years [61] and variations in the β-2 adrenoceptor gene and asthma [62] and asthma symptoms [49].

Many cross-sectional studies have shown relationships between genotypes in genes associated with T cell function and wheeze and the diagnosis of asthma. These studies are too numerous and there are too many genes to mention in detail here, but they have been summarized recently [63, 64]. As can be seen from Table 1, in which the top ten genes are listed according to the number of reported studies with positive associations between a polymorphism or haplotype in that gene and an asthma-related phenotype, there is a strong presence of genes with Th2 activity among these genes, although this would have been determined to a degree by researchers choosing to investigate these genes due to their known relationship with Th2 responses. Of particular note is the presence of several genes with a central role in the generation of Th2 responses: IL4, IL4Rα, IL13, and CD14. Also of interest is the chromosomal location of four of these top ten genes in the region of 5q23–32. This region is known to be chromosomal area in the human genome that is richest in cytokines. This is highly relevant to the relationships between T cell function and wheezing for several reasons. First, many of the cytokines that control the production of IgE appear to be located in this area. Second, many genome-wide screening studies have found linkage to wheeze-associated phenotypes in this region [63, 64]. Finally, as has been discussed previously in this chapter, the region has also been associated with parental smoking in a genome-wide screening study and linkage was only significant for asthma when the population was segregated according to the presence or absence of parental smoking [40]. These data fit well with epidemiological findings that have found highly consistent associations between parental smoking, especially maternal smoking, and wheezing in childhood [28, 65]. The cytokine region linkage data also emphasizes

Table 1 Top ten genes with variations with associations with asthma-related phenotypes in terms of number of positive reported studies.

Gene	c'some	+ve	*n*
CD14	5q31	16	24
IL4	5q23	19	34
IL13	5q23	18	21
ADRβ2	5q32-24	33	44
HLA-DQB1	6p21	12	18
HLA-DRB1	6q21	34	40
TNFα	6q21	17	30
FCER1β	11q12	18	30
IL4Rα	16q12	24	38
ADAM33	20p13	11	13

Genes are listed according to chromosomal position. Note the strong presence of genes with Th2 activity

c'some chromosomal position, + *ve* positive reported studies, *n* total number of reported studies, *CD14* monocyte differentiation antigen 14, *adrβ2* beta 2 adrenergic receptor, *HLA* human leukocyte antigen, *TNFα* tumour necrosis factor alpha, *FCER β1* high-affinity IgE receptor beta chain; *ADAM33* a disintegrin and metalloproteinase domain 33

Source: From Ref. [64] with permission

that genetic predisposition is only apparent if the environment reveals it. The lack of linkage found in many populations between this region and asthma phenotypes is almost certainly due to this.

T Cell Responses and Defense from Viral Respiratory Infections

The evidence that wheezing in humans is strongly related to acute viral respiratory infections has been increasing over recent years and includes epidemiological, immunological, and genetic data. In brief, the evidence suggests that in atopic individuals, along with their skewed Th2 responses to allergens, there are accompanying and significant decreases in Th1 immune responses that could account for the increased susceptibility of asthmatics to develop wheeze in response to acute viral respiratory infections. The data of the association between Th2 genotypes and reduced adaptive specific IgG and T cell immune responses was presented previously in this chapter [45, 47, 48]. Over the last five years or so, many other apparent defects in Th1 immune responses have been described. Relative impairment in responses have been shown to be present at birth, as CBMCs from infants with a family history of atopy produce less IFNγ in response to phytohaemaglutinen stimulation than those without such a history [3]. A reduced level of IFNγ has also been shown to be present at 3 months of age in those destined to have recurrent

wheezing by 1 year of age [66]. A reduced ability of PBMC to produce IFNγ in response to an induced infection with rhinovirus 16 in adult asthmatics compared with nonasthmatics has also been reported, along with accompanying reductions in IL12 [67]. In another study of induced rhinovirus infection in adult volunteers, reduced production or IFN-lambdas by rhinovirus was demonstrated in asthmatic primary bronchial epithelial cells and alveolar macrophages, and these findings correlated with severity of the induced asthma exacerbation and virus load in experimentally infected human volunteers [68]. More direct evidence that asthmatics have problems in handling viruses was inferred by a study of rhinovirus 16 infection of adult airway epithelial cells in which the virus was shown to have a substantially higher replication rate in cells from asthmatics than from nonasthmatics [69]. In a study of 76 cohabitating couples, one of whom had asthma, natural rhinovirus infection was studied and asthmatics were shown to have a delayed clearance of the virus from their airway [70]. In summary of this evidence, therefore, asthmatics (or future asthmatics) show relative defects in Th1 responses from birth, and evidence for these continues into childhood and adulthood.

Role of Acute Respiratory Viral Infection in Acute Wheezing in Children

Given the strong evidence of potential problems in Th2-related but Th1-mediated antiviral defenses that are present from birth, the finding over the last few years that there is evidence of an acute viral respiratory infection in the great majority of those presenting with a wheezing illness is not unexpected. Indeed, this appears to be true for the whole of life in humans. In the first year of life, a community study has shown that viruses were detected in 69% of acute respiratory infections and the most common infective agents were rhinoviruses (48.5%) and respiratory syncytial virus (RSV) (10.9%) [71]. In infants admitted to hospital with bronchiolitis in the first year of life, the most common virus detected is RSV [72], suggesting that while rhinovirus is likely to be the most common virus causing wheezing in the community in the first year of life, the most severe cases of bronchiolitis are those caused by RSV. In children presenting to an emergency department with wheezing, rhinovirus is by far the most common virus detected, being found in 60% of children in an Australian study [73]. Similar findings have been made in other studies [74–78]. The same is true in adult asthmatics, rhinovirus again being the most common virus isolated during acute episodes of wheezing being present in 60% of adults with acute asthma in a study from the UK [51]. In adults with chronic obstructive pulmonary disease, rhinovirus was again the most common infective agent detected, being present in 58.2% of those in whom an infective agent was found [51].

The above results pertaining to rhinovirus need to be interpreted in light of some new evidence related to rhinovirus detection [23, 24]. The first is that rhinovirus does not remain in the airway after more than 3 or 4 weeks even in asthmatic children who

have delayed clearance of this virus compared with nonasthmatics. This observation is important for two major reasons. First, it means that detection of rhinovirus in the airway in asymptomatic individuals is not evidence of a commensal infection, but rather evidence of an acute current asymptomatic infection [23]. Typically, in studies comparing patients with acute asthma versus controls, the control population has a relatively high frequency of rhinovirus detection. For example, in a study of asthmatic children presenting with acute asthma, rhinovirus was found in over 60% of the acute asthma group compared with 18.2% in asthmatic control subjects who were well at the time of testing [79]. Second, it suggests a mechanism for chronic asthma, since an infection that does not produce symptoms of a "cold" may still have the potential to produce an airway response that could contribute to chronic airway inflammation [80]. Rhinovirus has been shown to have a prolonged effect on increasing AR [81]. However, the most important observation of the new, more refined polymerase chain reaction (PCR)-based techniques used for detection of rhinovirus is that they were able to identify many new strains of rhinovirus in the known groups of A and B, but also a new group C, which was more likely to cause infection in younger children [23]. The strong implication of this is that all previous studies reporting rates of rhinovirus infection would have significantly underestimated the true number of infections with this virus and need to be repeated using this newer approach.

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Indoor Air Pollution and Airway Disease

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A good quality of indoor environment (dwellings, workplaces, schools, day care centers, bars, and discotheques) is a very important environment and health target, in so far as subjects in industrialized countries spend over 90% of their time indoors [1].

The quality of indoor environments depends on the quality of the atmospheric air that penetrates from outdoors and on the presence of indoor air pollution sources. Modern dwellings are often thermally insulated and have a low ventilation rate, to improve energy efficiency [1], but these aspects can deteriorate the indoor air quality. Indeed, pollutants are less diluted indoors than outdoors, possibly reaching higher concentrations. Moreover, the indoor environment is a result of the interaction between building system, construction techniques and materials, contaminant sources, and building occupants [2].

As regarding pollutants produced indoors, the most important are nitrogen dioxide (NO_2), carbon monoxide (CO), environmental tobacco smoke (ETS), particulate matter (PM), volatile organic compounds (VOCs), and biological allergens. In developing countries, relevant sources of indoor pollution include biomass and coal burning for cooking and heating. Almost 3 billion people worldwide (around 50% of the world's population) use biomass fuels as primary source for cooking, home heating, and light [3, 4]. A study on the global burden of disease attributable to major risk factors showed that, in 2000, over 1.6 million premature deaths, and about 3% of the global burden of disease, were attributable to indoor air pollution from solid fuels [5]. Moreover, in Africa, without systematic changes in the household biomass use,

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Supported in part by the Italian Agency for Medicines (AIFA) (contract no. FARM5JYS5A) and by the EU DG-SANCO projects EnVIE (contract no. SSPE-CT-2004-502671) and IMCA2 (contract no. 2005121).

R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_22, © Springer 2009

8.1 million lower respiratory infections deaths among young children and 1.7 million chronic obstructive pulmonary diseases (COPD) deaths of adult women were estimated by the year 2030 [6].

Recent studies showed clearly that the exposure to indoor air pollution is associated with increased respiratory symptoms/diseases, in particular with asthma and asthma-like symptoms [7–10].

The review in this chapter will cover the exposure to specific pollutants in indoor environments and their potential health effects.

Sources of Exposure to Indoor Air Pollution

To assess quantitatively the exposure to indoor air pollutants, several factors such as time spent at home, different sources of pollutants, household activities, presence of pets, indoor conditions suitable for mites, fungal and insect growth, infiltrations of outdoor pollutants, and presence of ventilation systems [2] have to be considered.

Indirect and direct methods for assessing indoor exposure have been used in epidemiological studies. Indirect methods, more widely used in population studies, include indoor source inventories, questionnaires, or time activity logs [11–13]. Direct methods include stationary passive or active sampling [7, 9, 14], biological monitoring (in blood, urine, or saliva) [15], or exhaled air [16].

Combustion Processes

The primary sources of indoor pollutants are combustion processes, such as cooking, heating with unvented gas or kerosene heaters, wood burning, and ETS. In developing countries, other principal sources of indoor pollutants are biomass-wood, crop residuals, animal dung and coal, used for heating and cooking [17].

The combustion process produces a mixture of pollutants, such as CO, NO_2, sulfur dioxide, aldehydes, polycyclic aromatic hydrocarbons, and inhalable PM, which have been associated with respiratory troubles [17]. In particular, PM includes inorganic acids (e.g., sulfates or nitrates), smoke (containing polycyclic aromatic hydrocarbons), fine dust, and residues of lead and asbestos. PM with an aerodynamic diameter $<10\,\mu m$ (PM_{10}) (i.e., inhalable particles) can be inhaled and accumulated in the respiratory system (trachea and large bronchi), and particles with aerodynamic diameter $<2.5\,\mu m$ ($PM_{2.5}$) (i.e., fine respirable particles) may be deposited in the smaller airways and alveoli [18].

Cleaning and Washing Products

Cleaning constitutes a large field of activities involving the general population and a large fraction of the workforce worldwide. Recently, it has caused increasing

concern. The exposure can be quite substantial [19] and knowledge of the potential toxicity of consumer products is limited.

During the process of cleaning, which can be considered equivalent to a chemical reaction, individuals are exposed to gases (e.g., formaldehyde and VOCs) and dust. VOCs include aromatic hydrocarbons, aldehydes, aliphatic halogenated hydrocarbons, and terpenes. During a cleaning activity, the overall VOCs level increases, affecting the indoor air quality; only ventilation (opening a window, turning on a fan or air conditioner) can reduce the exposure levels.

Little is known about long-term exposure to VOCs at levels generally detected inside dwellings [14]. In Europe, levels of VOCs, in public buildings, range from 21.7 $\mu g/m^3$ in Arnhem (Holland), 63.8 $\mu g/m^3$ in Catania (Italy), to 143.7 $\mu g/m^3$ in Salonicco (Greece) [20].

Other sources of VOCs can be paints, building materials, floor/wall coverings, cosmetics, adhesives, pesticides, tobacco smoke, mobile homes, and office equipment [14, 21, 22].

Indoor Allergens

Indoor allergens include domestic house dust mites, animal allergens, cockroach allergen, and molds. Indoor allergens today have increased in developed countries where homes have been insulated for energy efficiency, carpeted, heated, cooled, and humidified; these changes have made homes the ideal habitats for indoor allergens [23].

House Dust Mites

House dust is composed of several organic and inorganic compounds, including fibers, mold spores, pollen grains, insects and insect feces, and mites and mite feces [23].

The principal domestic mite species, *Dermatophagoides* and *Euroglyphus,* are particularly abundant in mattresses, box springs, pillows, carpets, or fluffy toys [24]. Mites proliferate in warm (above 20°C) and humid conditions (80% or higherrelative humidity) [24].

Pets

Cats and dogs are another important source of indoor allergens, released through secretions (saliva) and excretions (e.g., urine) [23]. The most studied cat allergen, *Fel d1*, is found in cat pelt (especially in the facial area), sebaceous secretions, and urine [25] and it may be airborne for many hours after emission. Dogs produce two

important allergenic proteins (C*an f1* and *Can f2*): their characteristics (allergen-carrying particles, ubiquity, etc.) are similar to those of cat allergens [23].

Cockroaches

In urban areas characterized by low-income status, cockroaches are a major indoor source of allergens. Most species of cockroaches live in tropical climates. The main allergens (*Per a1*, *Bla g1*, and *Bla g2)*, produced by dead bodies and fecal matter, have been frequently found in floor dust, kitchen cabinets, bathrooms, and basements [21].

Mold/Damp

Domestic molds are very important allergens and are most common in humid areas. Microscopic fungi present in homes are capable of producing spores all year round and are responsible for persistent symptoms, especially in hot and humid dwellings. They can also grow in aeration and climatization ducts (central heating and air conditioning) and water pipes. They are particularly abundant in bathrooms and kitchens. Molds also grow on plants, which are watered frequently, or on animal or vegetable waste, furnishings, wallpaper, mattress dust, and fluffy toys [24].

Respiratory Health Effects of Indoor Air Pollution

There is epidemiological evidence on the relationship (cross-sectional studies) and the causality (longitudinal studies) between indoor air pollution and respiratory health. Women and children seem to be more susceptible to indoor air pollution than their male counterparts. It could result from the fact that they generally spend more time indoors, but the differences in susceptibility between females and males should not be neglected [26].

Nitrogen Dioxide and Carbon Monoxide

The presence of commonly known NO_2 sources (i.e., gas appliances) is a risk factor for respiratory symptoms and asthma in children and in adults. Risks of having asthma, wheezing and bronchitis have been associated with an increase of 10 ppb (24 h average) of NO_2 concentration, measured in a living room, in females [27] (Table 1). An association of NO_2 exposure with acute respiratory illnesses (ARI) has also been found in adults living in a rural area of northern Italy and in an urban

area of central Italy [28] (Table 1). Taking into account only the sample of subjects living in the rural area, the authors showed a higher risk of having ARI for non-smokers and an association of NO_2 exposure with chronic bronchitis and/or asthmatic symptoms without fever and without ARI [29] (Table 1).

The association between an increase of 10 μg/m^3 of indoor NO_2 and current asthma, asthma attacks, and asthma medication was observed in 10 naturally ventilated schools in Shanghai [7] (Table 1).

In addition, the exposure of asthmatic children to CO_2 indoors was associated with an increased risk of wheezing attacks [30] (Table 1). Other symptoms linked to CO exposure were fatigue, headache, nausea, and vomiting. Moreover, people suffered from heart and pulmonary diseases; anemic subjects and pregnant women were the most susceptible subjects.

Table 1 Respiratory disorders caused by NO_2, CO_2, and particulate matter (OR, 95% CI).

Study	Country (*n*, sample)	Exposure	Health outcome	Measures (OR (95% CI)
Shima and Adachi [27]	Japan (842, females)	NO_2 (10 ppb = 18.8 μg/m^3 increasing)	Bronchitis	1.42 (1.06–1.90)
			Wheeze	1.90 (1.30–2.83)
			Asthma	1.63 (1.06–2.54)
Simoni et al. [29]	Italy (383, general population)	NO_2	ARI	2.18 (1.07–4.46)
			WFRI	1.77 (1.24–2.42)
	(291, never smoker)	NO_2	ARI	2.47 (1.14–5.34)
			WFRI	1.51 (1.04–2.18)
		$PM_{2.5}$	WFRI	1.83 (1.26–2.65)
Simoni et al. [28]	Italy (421, general population)	NO_2	ARI	1.66 (1.08–2.57)
		$PM_{2.5}$	ARI	1.62 (1.04–2.51)
			WFRI	1.39 (1.17–1.66)
Mi et al. [7]	China (1,414, children)	NO_2 (10 μg/m^3 increasing)	Asthma attacks	1.50 (1.11–2.02)
			Asthma medication	1.45 (1.08–1.94)
			Current asthma	1.51 (1.17–1.96)
Rabinovitch et al. [31]	Colorado (73, asthmatic children)			Percentage increment (95% CI)
		$PM_{2.5}$[a]	Bronchodilator usage at school	3.8% (0.2–7.4)
		$PM_{2.5}$[b]		2.7% (0.1–5.4)
Kim et al. [30]	Korea (26, asthmatic children)	CO_2 (10 ppb increasing)	Wheezing attacks	1.12 (1.02–1.28)

OR, odds ratio; 95% CI, 95% confidence interval; NO_2, nitrogen dioxide; $PM_{2.5}$, particulate matter with aerodynamic diameter <2.5 μm; CO_2, carbon dioxide; ARI, acute respiratory illnesses; FWRI, chronic bronchitis and/or asthmatic symptoms without fever and ARI.

[a] 12 μg/m^3 increasing of morning maximum.

[b] 6 μg/m^3 increasing of morning mean.

Suspended Particulate Matter

Exposure to suspended PM has a negative effect on respiratory health in both children and adults (Table 1). A relationship between $PM_{2.5}$ exposure and ARI and bronchitic/asthmatic symptoms was reported from a general population study, in adults [28, 29].

In asthmatic children, an increase of one interquartile range in morning maximum (12 μg/m^3) and in morning mean (6 μg/m^3) outdoor fine particulate levels was associated with an increase in bronchodilator usage at school; the stronger association was found for children with severe asthma with respect to those with moderate/mild disease [31].

However, few studies assessed the effects of ultrafine particles (UFPs) indoors, despite the fact that many indoor sources had been identified. The potential respiratory effects of such exposure could be really important because these particles cause oxidative stress and inflammation in the lungs. Thus, indoor UFP exposures may contribute to the exacerbation of asthma symptoms in susceptible individuals [32].

Wood/Coal Smoke and ETS

Women and young children are exposed to high levels of indoor air pollution every day, likely resulting in a high prevalence of chronic airway diseases [3, 33, 34] (Table 2). Also the respiratory risks of ETS are well documented (Table 2).

A recent study has investigated the effects of indoor pollution exposure to tobacco and home-heating in children. The results showed that maternal smoking and home-heating coal increased the risk of lower respiratory illness in the first 3 years of life, particularly in those non-breast-fed [12] (Table 2). A Chinese study showed that in children, the coal smoke was significantly associated with lower values of forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1) [35]. The same study showed that adults exposed to the coal smoke had increased risks of persistent cough, persistent phlegm, cough with phlegm, and wheeze, when compared to those not exposed [35] (Table 2).

Wood/Coal Smoke and Environmental Tobacco Smoke (ETS) is linked to several acute and long-term adverse respiratory effects, such as airway irritation, upper and lower respiratory tract infections, and respiratory symptoms or obstructive diseases in both children/adolescents and adults [36].

Women represent an important target of ETS exposure: living with smokers has been related to many respiratory symptoms/diseases, such as dyspnea, asthma, wheeze, asthma-like symptoms, obstructive lung diseases, current phlegm/cough, and rhinoconjunctivitis [8] (Table 2). Moreover, epidemiological data seem sufficiently consistent to suggest that exposure to ETS is an important risk factor for childhood asthma [37, 38] (Table 2).

Recently, the association between ETS and disease-specific mortality was examined in two New Zealand non-smoking adult cohorts. Significantly higher mortality

Table 2 Respiratory disorders caused by wood/coal smoke and environmental tobacco smoke (ETS).

Study	Country (*n*, sample)	Exposure	Health outcome	Measures (OR (95% CI)
Ekici et al. [34]	Turkey (596 woman)	Biomass	$FEV_1/FVC < 0.70$ or chronic bronchitis	2.5 (1.5–4.0)
Qian et al. [35]	China (2,360 adults)	Heating coal smoke	Persistent cough	1.10 (1.00–1.21)
			Persistent phlegm	1.12 (1.01–1.24)
			Cough with phlegm	1.10 (0.99–1.22)
			Wheeze	1.17 (1.00–1.37)
Baker et al. [12]	Czech Republic (452, 0–3 years)	Coal home heat	Lower respiratory illness	2.77 (1.45–5.27)
		ETS		2.52 (1.31–4.85)
Agabiti et al. [38]	Italy (18,737, children) (21,068, adolescents)	ETS	Asthma	1.34 (1.11–1.62)
			Wheeze	1.24 (1.07–1.44)
David et al. [36]	China (35,000 adults)	ETS	Asthma diagnosis	1.32 (1.13–1.53)
			Chronic bronchitis	2.87 (1.58–5.22)
			Chronic phlegm	2.38 (1.82–3.12)
			Chronic cough	2.80 (1.61–4.87)
Simoni et al. [8]	Italy (2,195, women, never smoker)	ETS	Dyspnea	1.61 (1.20–2.16)
			Shortness of breath	2.81 (1.83–4.30)
			Wheeze	1.71 (1.04–2.82)
			Attacks of shortness of breath with wheeze	1.85 (1.05–3.26)
			Asthma	1.50 (1.09–2.08)
			Any OLD	2.24 (1.40–3.58)
			Cough/phlegm	1.52 (1.07–2.15)
			Rhinoconjunctivitis	1.48 (1.13–1.94)

OLD, obstructive lung diseases; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; RR, relative risk.

risks for never smokers living in households with smokers were found for: cardiovascular disease, ischemic heart disease, in males and females (odds ratio, OR 1.25 and 1.18 in males; OR 1.35 and 1.27 in females, respectively) and cerebrovascular disease, only in males (OR 1.82) [39].

Volatile Organic Compounds

Potential health effects of VOCs include respiratory tract irritation and infections, irritation to eyes, allergic skin reaction, bronchitis, dyspnea [22], asthma, and asthma symptoms in children and adults [9, 13, 40]. Continuous low exposure to VOCs (mainly aromatic compounds) has been related to diagnosed asthma and attacks of wheezing (OR 1.63, 95% CI 1.17–2.27; OR 1.68, 95% CI 1.08–2.61, respectively), in American adults [9].

A recent Swedish study conducted in eight primary schools confirmed a significantly elevated risk of having diagnosis of asthma and asthma-like symptoms for a change of 1 μg/m^3 of VOCs concentration [13]. In particular, the study reported the following OR: 1.97 (95% CI 1.14–3.42) for asthma, 1.85 (95% CI 1.08–3.17) for wheeze, 2.39 (95% CI 1.15–4.96) for daytime breathlessness, and 5.71 (95% CI 2.04–16.02) for nocturnal breathlessness [13].

Cleaning and Washing Products

There are numerous indications that the products of indoor chemistry can impact both comfort and health, but the magnitude of these effects and the frequency with which they occur remain unclear [41]. Cleaning products constitute a common cause of both specific and non-specific symptoms in different sites and organs (eye, skin, nose, low respiratory tract, deep lung, and alveoli) [41]. Moreover, there is evidence that inhaled oxidant pollutants produce oxidative stress and can damage lipids, protein and DNA [41]. These chemical agents can constitute a respiratory hazard, particularly when used in poorly ventilated areas [42].

A recent study suggested that cleaning and washing products could be sources of UFP in an indoor environment. UFP, as described earlier, might play an important role in the exacerbation of asthma [32].

Domestic female cleaners with a recent history of asthma and/or chronic bronchitis, were assessed for short-term effects on respiratory symptoms. Elevated risks for lower respiratory tract symptoms were associated with exposure to diluted bleach (OR 4.4, 95% CI 1.8–11), diluted ammonia (OR 3.0, 95% CI 1.0–9.1), degreasing sprays/atomizers (OR 6.9, 95% CI 2.9–1.6), gas cleaning sprays/atomizers (OR 2.9, 95% CI 1.3–6.4), air fresheners sprays/atomizers (OR 7.8, 95% CI 2.6–6.4) and decalcifiers (OR 3.6, 95% CI 1.6–8.4). Moreover, these symptoms were more common among those doing daily cleaning >8 h [43].

Domestic cleaning work has an important health impact, not only on professional cleaners but also on those undertaking cleaning tasks in the home [42].

The maternal use of chemical-based products in the prenatal period has been associated with airway problems among pre-school children, in particular with the presence of persistent wheezing (OR 1.06, 95% CI 1.03–1.09) [44]. Moreover, children whose mothers had high usage of chemical products were more than twice as likely to have persistent wheeze, in early childhood, than children whose mothers had low usage of chemical products (OR 2.30, 95% CI 1.20–4.39) [44].

Indoor Allergens

At home, in public buildings and at school the most common allergens include mites, pets, cockroaches, and molds.

In a study of subjects suffering from chronic rhinitis, the proportion of etiological allergens identifiable by the skin prick test was evaluated; 63% of the patients had positive reaction to dust mites, 23% to cockroaches, 14% to cats, 5% to dogs, and 3% to molds. Comparing the medical history of patients who were positive and negative to any skin prick test, the former had the onset of symptoms at an earlier age with a likelihood of a history of eczema and asthma [45].

House Dust Mites

Sensitization to house dust mites has been epidemiologically associated with development of asthma (in susceptible children), and with exacerbation of asthma [46]. Continuous exposure to house dust mites can contribute to chronic bronchial hyper-reactivity [1, 47] (Table 3).

Table 3 Respiratory disorders caused by house dust mites, mold, and cockroaches exposure.

				Measures
Study	Country (*n*, sample)	Exposure	Health outcome	(OR (95% CI)
Miraglia et al. [46]	Italy (1,426, children)	House dust mites	Asthma	4.84 (2.42–9.60)
Wong et al. [47]	China (608, children)	House dust mites	Bronchial hyper-responsiveness	3.67 (1.93–6.97)
Davey et al. [10]	Ethiopia (7,649, general population)	House dust mites	Wheeze	1.21 (1.00–1.51)
			Asthma	4.09 (2.86–5.84)
		Cockroach	Wheeze	1.27 (1.00–1.62)
Salam et al. [57]	California (691 children)	Cockroach	Asthma	2.03 (1.03–4.02)
Silva et al. [56]	Brazil (73 young children)	Cockroach	Wheeze	7.6 (1.4–41)
Simoni et al. [59]	Italy (2,016 children 13,266 adolescents)	Mold	Wheeze	1.98 (1.47–2.66)
			Current asthma	1.39 (1.00–1.93)
	Children		Rhinoconjunctivitis	1.46 (1.01–2.09)
	Adolescents		Eczema	1.44 (1.09–1.91)
			Current cough/ phlegm	1.86 (1.19–2.91)
			Early wheeze	1.56 (1.15–2.11)
			Asthma	1.62 (1.00–2.62)
			Rhinoconjunctivitis	1.78 (1.30–2.45)
Skorge et al. [49]	Norway (2,401, adults)	Mold	Wheezing	2.3 (1.46–3.47)
			Cough/phlegm	1.7 (1.08–2.64)
			Chronic cough	2.0 (1.17–2.48)
			Dyspnea (2° grade)	2.3 (1.35–3.85)

A study of a general population sample of Ethiopian children and adults showed an elevated association between dust mites exposure and asthma and wheeze in the past year [10] (Table 3).

Data from the European Respiratory Community Health Survey (ECRHS) have indicated an association between pulmonary function and house dust mites: asthmatic subjects, sensitized to mites, had a lower FEV_1 and FEV_1/FVC ratio than non-sensitized asthmatics [48].

Pets

There is sufficient evidence of a causal relationship between pet allergen exposure and asthma exacerbation, but not with asthma development [1]. The recent Allergic Rhinitis and Its Impact on Asthma (ARIA); guideline on allergic rhinitis reports that cats and dogs are major allergens triggers in asthma, rhinitis or rhinoconjunctivitis, and cough [24].

A Swedish study on an adult population sample showed an association between keeping a cat or dog, in childhood, and grade 2 dyspnea, in adulthood, as well as between keeping a cat, in childhood, and attacks of dyspnea, in adulthood [49] (Fig. 1).

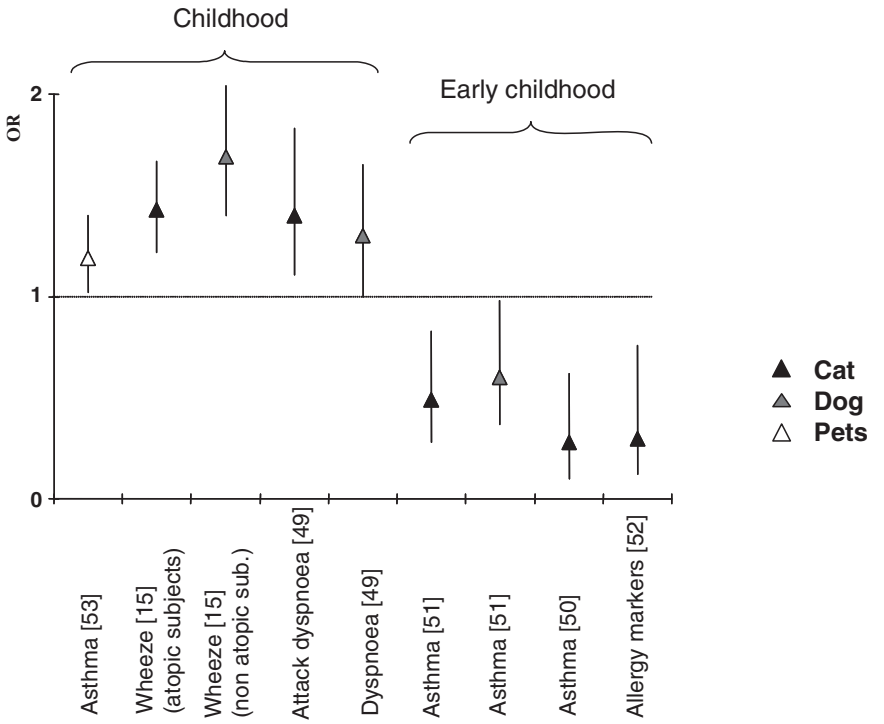

Fig. 1 Respiratory disorders caused by exposure to pets.

In contrast, other scientific evidence seems to suggest that intensive exposure to cats in early childhood may have a protective effect for developing asthma [50, 51] and prevent allergen sensitization [52], while later exposure in childhood may have an opposite effect [53] (Fig. 1).

This health effect can vary according to the type of pet and to the individual's allergic sensitization [15]. The ECRHS study showed that atopic subjects, exposed to cats in childhood, have a high risk of developing wheeze and other asthma-like symptoms. The same effects in non-atopic subjects were due to dog exposure [15] (Fig. 1).

The inconsistency of findings from several studies may be partly due to different study design (cohort, case–control, and cross-sectional studies), type of exposure (early or current pet ownership and allergen concentrations), health outcome (sensitization, presence of wheeze, or asthma) [54], recall, or selection bias.

Cockroaches

Exposure and sensitization to cockroach allergens have been repeatedly associated with the onset of asthma or exacerbation in many countries [10, 55–57] (Table 3).

A study on a sample of 2- to 4-year-old children showed that the exposure to cockroach allergen in the kitchen was associated with three or more wheezing episodes in the past 12 months [56]. In the USA, children aged 2 months to 10 years, not previously identified as atopic, were evaluated to assess the association between the prevalence of positive skin test to common allergens and the presence of wheezing. Although dust mite was the most common allergen to which the children were sensitized, only cockroach sensitivity showed a significant correlation to wheezing [58].

Molds/Dampness

Epidemiological data seem to indicate that molds play an important role as a risk factor for respiratory symptoms/diseases, mainly asthma exacerbation [1].

Self-reported mold exposure was associated with an increased risk of cough with phlegm, chronic cough, dyspnea and wheezing, in adults [49] (Table 3).

Most studies have been performed among children and adolescents. The exposure to molds was associated with wheezing, asthma, rhinoconjunctivitis, eczema, cough, and phlegm [59] (Table 3). This association seems more evident in children than in adolescents, and when the exposure occurs early in life. Moreover, the population attributable risk % (PAR) for mold exposure was computed. Avoiding early mold/dampness exposure would abate wheeze by 6%, asthma or cough/phlegm by 7% and rhinoconjunctivitis by 4%, in children; in adolescents, asthma would be abated by 6% and wheeze by 4% [59].

Conclusion

The American Thoracic Society (ATS) [60] and World Health Organization (WHO) [61] have suggested several options for achieving acceptable indoor air quality. Guidelines and recommendations on indoor air quality in dwellings are also reported in the EFA (European Federation of Allergy and Airways Diseases Patients Associations) final document of the THADE (Towards Healthy Air in Dwellings in Europe) project [2, 62].

Despite the presence of guidelines on indoor air quality, people cannot be obliged to respect them, as they have the right to live in their own homes as they wish. But it is important that people be aware of the health risk due to indoor pollution, so that they can try to reduce it.

Recommended actions toward healthy air in dwellings are: improve ventilation, improve cleaning methods and housing hygiene, avoid wall-to-wall carpeting, control moisture to prevent accumulation of mold, control the sources of pollution (e.g., tobacco smoke and emissions from building and consumer products), carry out education and information campaigns [2, 63–65]. Most of these prevention strategies are valid regardless of cultural and climatic differences.

In conclusion, recent epidemiological studies have clearly shown that indoor pollution largely affects respiratory health worldwide and that preventative public health programs should be implemented. More research is needed about the long-term effects of indoor environments, to elucidate the mechanisms by which pollutants induce damage in exposed subjects, and on the cost-effectiveness of preventative and remedial measures related to indoor air quality.

Acknowledgement Support in part by the Italian Agency for Medicines (AIFA) (contract no. FARM5JYS5A) and by the EU DG-Sanco projects ENVIE (contract no. SSPE-CT-2004-502671) and IMCA2 (contract no. 2005121). In order to have some space to introduce acknowledgement try to put name of all Authors in the same line continuing with name of Institutions.

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Impact of Tobacco Smoke on Asthma and Allergic Disease

Eric Livingston and Neil C. Thomson

The prevalence of asthma and other atopic diseases are rising. Genetic factors are unable to explain the increase in prevalence over the last few decades. It seems very likely that there is a strong environmental influence on the expression of allergic diseases [1]. These exposures may occur in childhood or adult life but environmental exposure during pregnancy may also dictate patterns of disease in later life. The risk factors that might have contributed to this increase include active and passive tobacco smoking, which are on the rise in certain age groups and in females. Allergic conditions share numerous characteristics including the production of IgE antibodies in response to allergens, an impaired balance between cytokines of the Th1 and Th2 lymphocytes, a positive family history of atopic diseases and sometimes the expression of several atopic conditions [1]. In this chapter, the effect of exposure to environmental tobacco smoke (ETS) and active smoking on the development and clinical manifestations of asthma and other allergic conditions will be reviewed.

Cigarette Smoking

Cigarette smoking is common in the general population, but the prevalence varies between countries. The World Health Organisation has estimated that there are 1.25 billion smokers worldwide, with approximately two-thirds living in developing countries. Prevalence figures for the USA in 2005 show rates of 23.9% in men and 18.1% in women [2]. In the UK, the prevalence rates in 2001 were reported as 28% in men and

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_23, © Springer 2009

26% in women [3]. In both the USA and UK, the prevalence in smoking has reduced since the 1970s [3, 4]. In the UK, however, the rate of decline slowed until it levelled out between 27% and 28% in the 1990s [3]. Prevalence rates of smoking are higher in countries with lower incomes, and among young adults, especially females [5].

When a cigarette is smoked, two types of cigarette smoke are produced. Smokers inhale mainstream cigarette smoke. When they are not inhaling, the smouldering end of the cigarette produces sidestream cigarette smoke [6]. ETS consists mainly of sidestream smoke with the addition of a smaller amount of mainstream exhaled smoke. Over 4,000 individual chemicals are found in tobacco smoke [7] although the biological activities of only a few hundred are known. Many of the constituents are carcinogens, of which some affect development of reproductive organs and others are neurotoxic [8]. Mainstream cigarette smoke differs both quantitatively and qualitatively from sidestream smoke. Some combustion products are enriched in sidestream smoke, however dilution by room air reduces markedly the concentrations that are subsequently inhaled [8].

Smoking and Airway Inflammation

A fundamental component of the pathophysiology of asthma is airway inflammation [9–11]. Increased inflammatory cells are seen within the central and peripheral airways [12, 13] as well as within the lung parenchyma [14]. Inflammation is present even in those with mild disease [15, 16], those with relatively few symptoms [17] and in newly diagnosed asthma [15]. Many cell types have been implicated in asthmatic inflammation, particularly eosinophils [18, 19], T lymphocytes [20, 21] and mast cells [22], but neutrophils and macrophages may also play a role [23].

Inflammation associated with asthma may be modified by cigarette smoking. However, there is limited data on the influence of active smoking on airway pathology. In non-asthmatic smokers without airflow obstruction, cigarette smoking induces airway inflammation [24–27], with increases in T-lymphocytes and macrophages within the airway wall and higher neutrophil numbers within the bronchial secretions. Eosinophil counts in induced sputum from heavy smokers with mild asthma are lower compared with non-smokers with mild asthma [28], although some studies have reported similar counts to those found in non-smokers with asthma [29–31]. The reason for the reduced counts is unknown, but could be explained by the exogenous nitric oxide in cigarette smoke increasing the apoptosis of activated eosinophils. Nicotine within tobacco smoke may have immunomodulatory effects on eosinophil function by inhibiting the release of proinflammatory cytokines from macrophages [32].

Reduced numbers of CD38 + ve mature dendritic cells and B lymphocytes have been found in bronchial biopsies from smokers with asthma compared to never smokers with asthma, although similar numbers of Langerhan's cells have been found [33]. It has been speculated that an increase in the frequency of lower respiratory tract infections in asthmatic smokers may be a result of fewer dendritic cells and B lymphocytes in the bronchial mucosa with a resultant reduced Th-1 immunity [33].

It has been suggested that smoking induces bronchial immune modulation at least in occupational asthma as smokers with occupational non-atopic asthma having reduced bronchial CD4 + T cell density and eosinophil numbers compared to non-smokers with occupational asthma [34]. The airway longitudinal elastic fibre network is increased in specimens obtained from smokers compared to non-smokers dying from causes other than asthma [35], suggesting that airway remodelling may be more severe in smokers with asthma. Smokers with asthma have an increase in the expression of arginase I in the airway epithelium and smooth muscle, which may explain the low levels of F_ENO [36].

The effect of smoking cessation on airway inflammation in healthy smokers shows a dose-dependent relationship between smoking and airway inflammation [37]. In contrast to this, there was minimal change in airway inflammation in patients with chronic obstructive pulmonary disease after smoking cessation [38–40]. Recently, a study in asthmatic smokers have shown that smoking cessation was associated with a fall in neutrophil count in induced sputum at 6 weeks [41].

Active cigarette smoking also alters the cytokines and mediators in asthmatic subjects. Sputum interleukin (IL)-8 levels are increased in asthmatic smokers and the concentrations are positively correlated with smoking history in pack years and negatively correlated with forced expiratory volume in 1 second (FEV_1) % predicted [28]. This would suggest indirect evidence for an association between smoking, airway inflammation and reduced lung function in asthmatic smokers. IL-18 is a cytokine that is involved in the development of Th-1 lymphocyte responses and is thought to have a regulatory role in asthma by inhibiting Th-2 lymphocyte responses. Smoking is associated with a significant reduction in sputum IL-18 levels in both normal and asthmatic subjects compared with non-smokers, with the effect being more pronounced in asthmatics than in normal subjects [42]. IL-18 mRNA expression was reduced in asthmatic smokers compared with non-smokers. These results suggest that cigarette smoking may in part, by altering the balance of Th-1/Th-2 cytokine secretion, modify airway inflammation.

The combined inflammatory effects of asthma and cigarette smoking are likely to contribute to the airway pathology of smokers with asthma.

Active Smoking and Asthma

Causal Effect of Active Smoking on Development of Asthma

Active cigarette smoking has been associated with the development of asthma in some but not all studies. Little information is available on the effect of smoking in childhood. In asymptomatic teenagers, the development of asthma-like symptoms over a 6-year period was independently associated with active tobacco smoking with an odds ratio (OR) (95% CI) of 2.1 (1.2 to 3.8) as well as atopy and bronchial hyperresponsiveness to methacholine [43]. However, a cross-sectional study carried out in Hong Kong schoolchildren aged 12–15 years found no association between active smoking and

physician-diagnosed asthma. The prevalence of asthma was 8.6% among children who reported smoking six or more cigarettes per week compared with 8.1% among children who had never smoked giving an OR (95% CI) of 1.18 (0.76–1.83) [44]. In adults, studies have shown that active smoking is associated with an increased incidence of adult-onset asthma in females but not in males [45]. Smoking has also been shown to be strongly associated with the development of asthma in non-atopic individuals [46] and a risk factor for the development of asthma in older adults [47].

Effect of Active Smoking on Established Asthma

Active smoking in asthma is common with the prevalence of smoking in the asthma population being between 17% and 35% [47–54]. Adults presenting to the hospital emergency departments with acute asthma had the highest rates [50]. A further 22% to 43% of asthmatics are former smokers [48, 49].

Smokers with asthma tend to have more severe respiratory symptoms such as cough, wheeze and dyspnoea compared to non-smoking asthmatics [29, 31, 51]. Global asthma-specific quality of life scores are similar in smokers and non-smokers, although specific domains for breathlessness and mood are worse in smokers [51]. Indices of asthma severity are higher in smokers with asthma [49]. Admission rates to hospital for asthma and hospital-based care are increased in smokers [51, 55], although this may not be the case in younger adults [43]. There is some conflicting evidence as to whether current smoking is a risk factor for fatal or near-fatal asthma [43, 56–58]. However, the 6-year mortality rate is higher for smokers than non-smokers following a near fatal asthma attack, with an age-adjusted OR (95% CI) of 3.6 (2–6.2) [59].

Active smoking causes an accelerated decline in lung function in asthmatic smokers [60–62]. This effect of smoking is additive to the decline in lung function already seen in asthmatic subjects. The Copenhagen City Heart Study included longitudinal measurement of FEV_1 over a 15-year period, and found that the average decline in FEV_1 was greater in asthmatic smokers than non-smokers [60]. A further study of 4,000 adults initially aged between 18 and 30 years, which was followed-up over 10 years, showed that the combination of asthma and smoking 15 cigarettes per day had a synergistic effect on the decline in lung function [54].

Smoking and Asthma Therapy

It has been known for many years that smoking was a major cause of altered drug interactions [63]. Smoking has been shown to alter the pharmacokinetic and pharmacodynamic properties of many drugs although the clinical significance of most of these interactions is not clear. The mechanism involved in most interactions between cigarette smoking and drugs is through the induction of several drug-metabolising enzymes [4]. Smoking alters the effects of drugs used in a variety of conditions, including asthma [64].

Corticosteroids are the most effective medication available for the treatment of asthma and international guidelines emphasise the importance of inhaled corticosteroids in the management of the disease [65]. Until recently, there has been little information on the effect of cigarette smoking on the response to asthma therapy as most studies have excluded asthmatics who smoke. Several studies now have suggested that the efficacy of both inhaled and oral corticosteroids is reduced in asthmatics who are active cigarette smokers [30, 66–69].

The first observation that smoking had an effect on the response to corticosteroids was made during a study investigating the long-term effects of inhaled corticosteroid therapy for asthma on selected blood markers of asthmatic inflammation [68]. Subsequent analysis of the results suggested an adverse effect of smoking on the response to inhaled corticosteroids, with the smoking group having no effect of low- or higher-dose budesonide on FEV_1, histamine provocative concentration causing a 20% drop in FEV_1 (PC20) or rescue medication use. However, this study was not placebo-controlled and the effect of smoking on the response to inhaled corticosteroid was an incidental finding and not the main aim of the study. Despite the study being long (9 months duration), there was no data available on asthma exacerbation rates; however, it did raise an important question regarding the effect of smoking on corticosteroid responsiveness.

The first randomised, controlled trial looking at this effect of smoking on the response to inhaled corticosteroids showed that asthmatic smokers had a reduced response compared with non-smoking asthmatics [67]. In this study using fluticasone propionate 1000 µg daily or placebo for 3 weeks, the asthmatic non-smokers showed a significant improvement in morning peak expiratory flow (PEF) (Fig. 1),

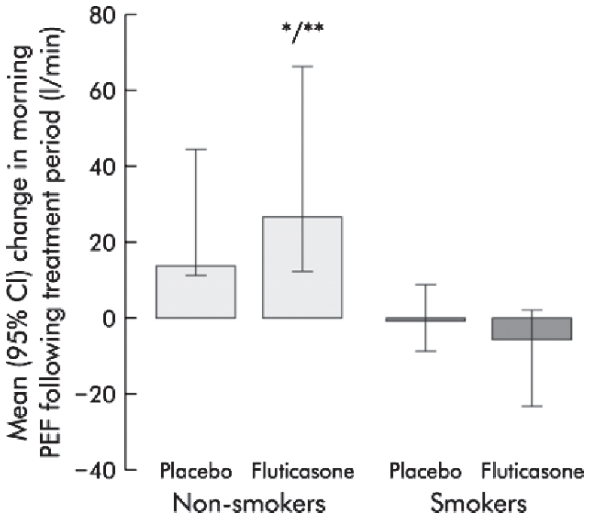

Fig. 1 Mean (95% CI) peak expiratory flow (l/min) in non-smoking and smoking asthmatic patients following treatment with inhaled placebo or fluticasone propionate 1000 µg per day. $^{*}p = 0.016$, greater than non-smokers after placebo; $^{**}p = 0.001$, greater than smokers after fluticasone. Reproduced with permission from Ref. [67]

FEV_1, bronchial hyperreactivity and sputum eosinophil count, while no significant changes in these measurements were found in the smoking asthmatic group.

A further randomised double-blind, parallel study assessed the effect of inhaled corticosteroids in smoking and never-smoking asthmatics over a 12-week period [69]. They assessed the effect of low- and high-dose beclometasone on 95 asthmatics. After 12 weeks of inhaled therapy, there was a considerable difference between the mean morning PEF measurements and numbers of exacerbations of smokers and never-smokers with asthma. The differences however were less marked in those receiving high-dose beclometasone. A large study of 83 asthmatics carried out in the USA using inhaled beclometasone for 8 weeks has confirmed a lack of responsiveness in a number of parameters in asthmatic smokers [30] (Fig. 2).

Even the efficacy of short-term oral corticosteroids is reduced in cigarette smokers with chronic stable asthma [66]. In this randomised, placebo-controlled cross-over study, the effect of oral prednisolone 40 mg daily or placebo for 2 weeks was studied in 50 asthmatic patients (smokers, never-smokers and former smokers). There were significant improvements in FEV_1, morning PEF and asthma control score after oral

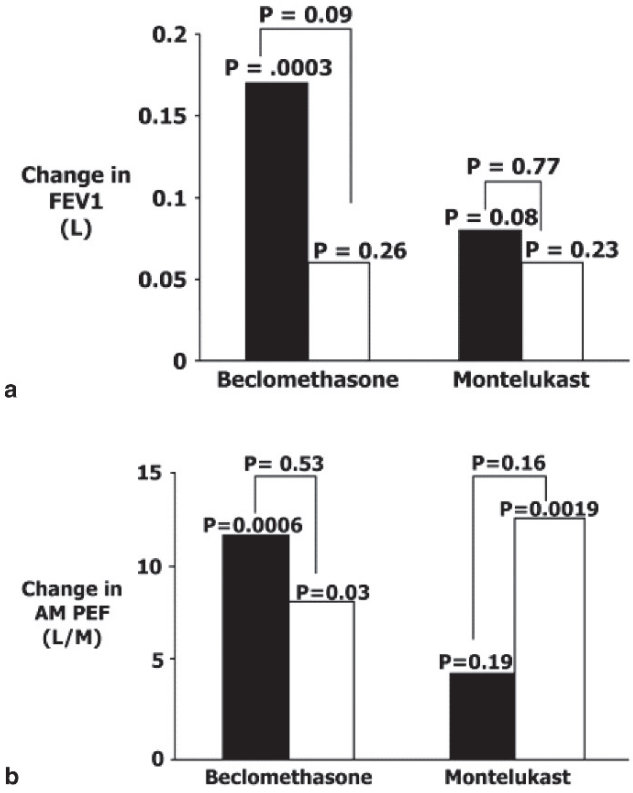

Fig. 2 Effect of 8 weeks of double-blind treatment on **a** FEV_1 and **b** PEF. Solid bars, nonsmokers; open bar, smokers. Reproduced with permission from Ref. [30]

prednisolone compared with placebo in the non-smokers but no change in the smokers (Fig. 3). Of interest, the former smokers had significant improvements in morning and night-time PEF, suggesting that the effect of smoking in asthma may be partially reversible.

The mechanisms behind the reduced responsiveness to corticosteroids in asthma is not fully known [70], but may include one or more of the pathways implicated in asthmatic non-smokers and other inflammatory conditions including inflammatory bowel disease and rheumatoid arthritis [71–75] (Fig. 4). Despite cigarette smoke-inducing enzymes involved in the metabolism various drugs, the metabolism of prednisolone, prednisone or dexamethasone is not affected by smoking [76].

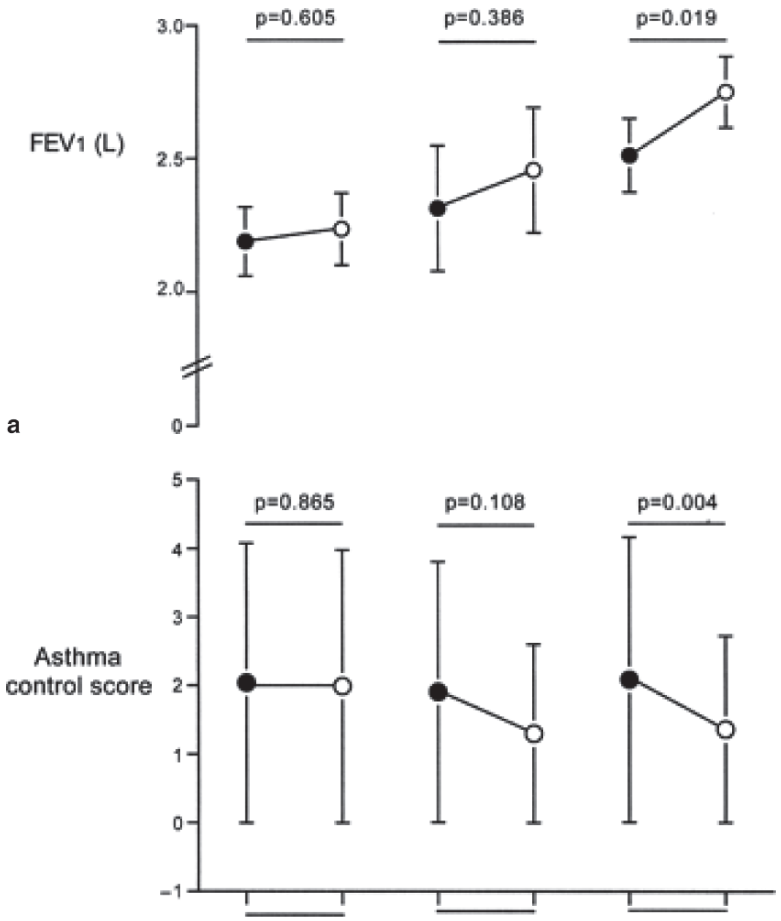

Fig. 3 Mean difference (95% confidence interval) after placebo (*closed circles*) and after prednisolone (*open circles*) in smokers with asthma, ex-smokers with asthma, and never-smokers with asthma for **a** change in FEV_1, L and **b** asthma control score. A reduction in the score implies an improvement in asthma control. Reproduced with permission from Ref. [66]

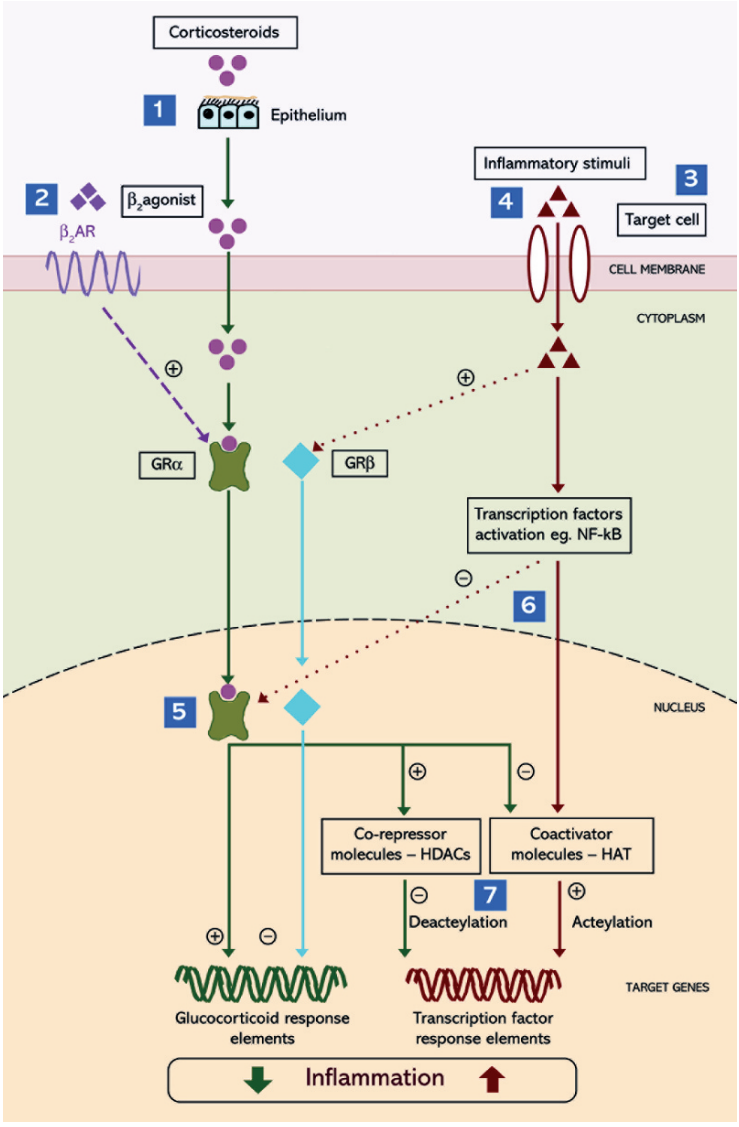

Fig. 4 The anti-inflammatory effects of corticosteroids are mediated by activation of cytoplasmic glucocorticoid receptors (grs) that act as ligand-activated transcription factors, which translocate into the nucleus to suppress or induce glucocorticoid target genes. GR-α acts by directly binding to DNA sequences (transactivation) or by interacting with pro-inflammatory transcription factors (transrepression). GR-β, which does not bind ligand, is predominately located in the nucleus and cannot transactivate glucocorticoid-sensitive genes. Potential pathways and mechanisms of corticosteroid resistance in asthmatic smokers include the following: (1) corticosteroid pharmacokinetics, e.g. Increased airway mucosal permeability, increased bronchial secretions; (2) corticosteroid and β2-adrenergic receptor (β2AR) interactions, e.g. Down-regulation of b2ar function;

As mentioned above, cigarette smoke alters the number and function of airway inflammatory cells [77, 78], with some smokers with asthma having a reduced sputum eosinophil count and/or an elevated sputum neutrophil count. A reduction in sputum eosinophils or an elevation in airway neutrophils may be associated with a poor (but not absent) response to corticosteroids [79]. Corticosteroids require histone deacetylase (HDAC) activity for maximal suppression of cytokine induction [80]. It has been shown that oxidative stress in smokers results in reduced HDAC2 activity, resulting in the potential for increased inflammatory gene expression [81] and reduced sensitivity to corticosteroids. There are two naturally occurring glucocorticoid receptor (GCR) isoforms: GCR-α (the functional GCR) and GCR-β (not ligand binding), which result from alternative splicing of exon 9 of the GCR gene [82]. It has been suggested that corticosteroid resistance is associated with an overexpression of GCRβ [83–85], a reduction in GCR-α numbers [86] or a reduction in the GCRα:GCRβ ratio [85, 87]. The expression of GCR-β is increased following exposure to pro-inflammatory cytokines and mediators such as IL-2 [84], IL-4 [84], IL-8 [88] and TNF-α [89], which are increased on exposure to cigarette smoke. Smoking-induced alterations in cytokine expression may alter the GCR binding affinity, thus altering response to corticosteroids. Corticosteroid-resistant asthmatic patients have a reversible defect in peripheral blood mononuclear cell GCR binding affinity, which may be sustained in vitro by the combination of IL-2 and IL-4 [90].

A recent study has examined the effect of the oral leukotriene receptor antagonist montelukast in asthmatic smokers [30]. Montelukast produced a statistically significant increase in morning PEF and a decrease in PEF variability in smokers with asthma. These changes were significantly greater than its effects seen in non-smokers (Fig. 2). The authors explained this finding by enhanced leukotriene synthesis or sensitivity in smokers [30]. Smoking has been found to increase urinary leukotriene E_4 (LTE_4) in patients with asthma, but not in normal subjects or patients with chronic obstructive pulmonary disorder (COPD) [91]. Urinary LTE_4 excretion is closely correlated with the number of cigarettes smoked per day and urinary LTE_4 levels increase significantly in non-smokers who smoke 6 cigarettes in 12 hours [92]. It has been postulated that asthmatic smokers may have chronically elevated leukotriene levels that may render them responsive to treatment with leukotriene receptor antagonists such as montelukast [30].

The effect of smoking on theophyllines is well known. Smoking has been shown to cause a 58–100% increase in theophylline clearance, resulting in an almost two-fold

Fig. 4 (continued) (3) inflammatory cell phenotypes, e.g., increased airway neutrophil or CD8z lymphocyte numbers, reduced airway eosinophil numbers; (4) cytokine and mediators levels, e.g., increased production of interleukin (IL)-4, IL-8, tumour necrosis factor-α, decreased production of IL-10, increased nitrosative stress; (5) grs, e.g., overexpression of GR-β, reduced expression of GR-α; (6) pro-inflammatory transcription factor activation, e.g. Nuclear factor-kb (NF-kb), activator protein-1, signal transduction-activated factor; and (7) corticosteroid cell-signalling systems, e.g. Reduced histone deacetylase activity (HDAC), increased p38 mitogen-activated protein kinase activity. HAT: histone acetyltransferase. Reproduced with permission from Ref. [5]

decrease in half-life compared with non-smokers [93, 94]. Problems can be encountered upon cessation of smoking, as within 7 days of stopping smoking, the clearance of theophylline falls by 35% [95].

With both national and international guidelines advocating the use of inhaled corticosteroids as the mainstay of asthma management, in view of the studies mentioned above, should corticosteroids still be used in asthmatic patients? The studies have assessed the effect of corticosteroids on lung function and symptoms, but corticosteroids may influence other important outcomes such as exacerbation rates or rate in decline of lung function [96]. Results from observational studies suggest that a decrease in lung function induced by smoking in asthma may be reduced by inhaled corticosteroids [97, 98], although this effect appears to be lost in heavy smokers [98]. The recent data on the benefits of montelukast in smokers with mild asthma is interesting [30], although the beneficial effects on morning PEF was not large. It is not known whether a similar effect would be seen in asthmatics with more severe disease. More research into drug treatment of smokers with asthma is undoubtedly warranted.

One important factor in the management of smokers with asthma is smoking cessation advice. In a recent study, former smokers gained considerable short-term improvement in lung function and a decline in sputum neutrophil count compared with those who continued to smoke [41] (Fig. 5). Smoking cessation is also associated with improved asthma control [41, 99].

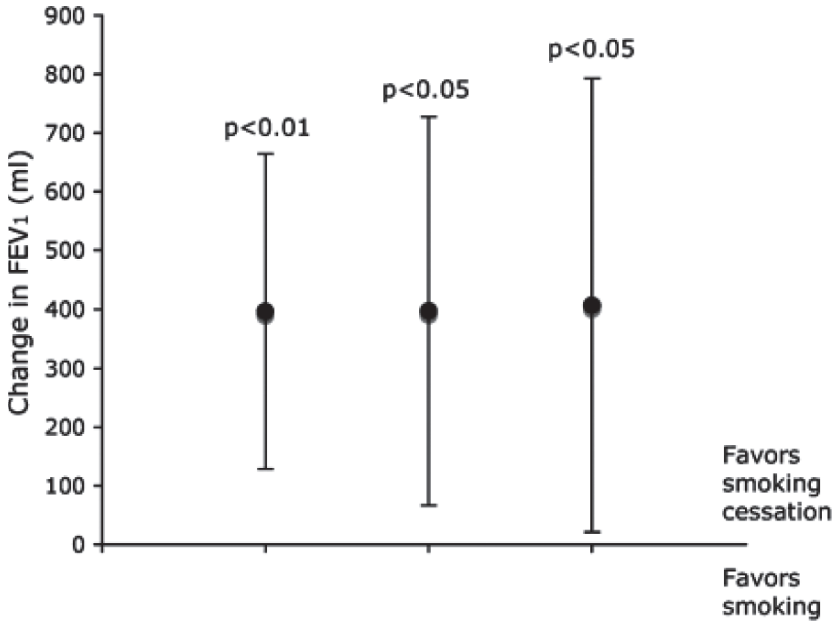

Fig. 5 Mean (95% confidence interval) difference between quitters and control smokers in change in FEV_1 (ml) compared with baseline. Reproduced with permission from Ref. [41]

Environmental Tobacco Smoke and Asthma

ETS exposure can be assessed by a variety of methods. Questionnaires are fairly reliable but can be influenced recall bias. More objective means of assessing ETS exposure include cotinine measurements in the urine, serum and saliva or nicotine in hair or nicotine badges [100–102]. Exposure to ETS in the general population is high with the majority of exposure in homes and workplaces with a lesser extent in public places such as bars and restaurants [103], although this will reduce as bans on smoking in public places come into effect in increasing numbers of different countries. In the USA, the National Health and Nutrition Examination Survey in 2001–2002 reported that 43% of the non-smoking general public was exposed to ETS on a regular basis [104]. Parents have been responsible for 90% of a child's exposure to ETS and exposure is higher in lower income households [103, 105] and those with low education levels [103, 105, 106].

In asthmatics, there is less information on exposure to ETS. Data does however suggest significant exposure levels. Studies have indicated that between 17% and 80% of non-smoking asthmatics are exposed to ETS [100, 105, 107–109], with those being admitted to hospital with acute asthma having higher rates of exposure.

Causal Effect of ETS on Development of Asthma

Children

In the 2006 Surgeon General's Report on the Health Consequences of Involuntary Exposure to Tobacco Smoke, a systematic review of 41 relevant studies concluded that there was sufficient evidence to infer a causal relationship between parental smoking and ever having asthma among children of school age with an OR (95% CI) of 1.23 (1.14–1.33) [103]. Other meta-analyses have shown similar ORs [110–113]. Results from several studies not included in the Surgeon General's report indicate that the strongest effect of ETS on the induction of asthma occurs *in utero* and during early life [114, 115]. A large UK study of 11,562 children between the ages of 4 and 6 years found that a larger number of active smokers in the home increased the likelihood of a child having wheezed in the last year with an OR (95% CI) of 1.2 (1.0–1.4) for one smoker and an OR (95% CI) of 1.4 (1.2–1.4) for two smokers [116]. Based on these findings, the authors suggested that assuming a causal relationship, 8% of asthma in children aged 4–6 years could be attributable to ETS at home [116]. Smoking during pregnancy has been shown in a case–control study to increase the risk of asthma in the first 5 years of life with an OR (95% CI) of 1.6 (1.0–2.6) [117]. In the same study, mothers who stopped smoking before becoming pregnant were no longer at risk of the child developing asthma with an OR (95% CI) of 0.9 (0.5–1.5) [117].

Adults

There appears to be conflicting evidence as to whether exposure to ETS causes the development of asthma. The 2006 Surgeon General's Report concluded from 14 studies between 1988 and 2001 that evidence is suggestive but not sufficient to infer a causal relationship between exposure to ETS an development of asthma in adult life [103]. A further systematic review including studies between 1993 and 2004 concluded that there was sufficient evidence to support a causal relationship between ETS exposure and development of asthma in adolescents and adults [113]. Childhood exposure to ETS has interestingly been shown to be associated with an increased risk of developing asthma as an adult [118]. A Norwegian study of 15- to 70-year-old individuals over an 11-year period reported an increased risk of developing asthma if the mother smoked during childhood with an OR (95% CI) of 1.9 (1.6–3.2) [118].

Effect of ETS on Established Asthma

As with active cigarette smoking, exposure to ETS has been shown to have a number of adverse effects on asthma control and severity in children. In children with asthma, ETS exposure results in increased asthma symptom scores, exacerbation frequency, use of reliever medication, hospitalisation rates and number of life-threatening attacks [112]. In adults, the effect of ETS is still controversial. The Surgeon General's Report in 2006 was suggestive but not sufficient to infer a causal effect between exposure to ETS and worsening asthma control [103]. However, another review of seven studies published between 1998 and 2002 concluded there was evidence to consider that ETS exposure is causally associated with exacerbations of asthma [113].

A recent study from Tayside, Scotland, looked at the effect of the ban of smoking cessation in public places on airway inflammation and quality of life in bar workers. Asthmatic bar workers had less airway inflammation with a reduction in exhaled nitric oxide from 34.3 ppb to 27.4 ppb 1 month after the ban (0.8-fold change; 95% CI, 0.67–0.96 ppb; P =.04), and Juniper quality-of-life scores increased from 80.2 to 87.5 points (7.3 points; 95% CI, 0.1 to 14.6 points; P =.049) [119].

A clinical trial in children with mild persistent to severe asthma that compared the administration of drug treatment either at school or away from school concluded that ETS is associated with reduced therapeutic response to inhaled corticosteroids. Improvements in the number of symptom-free days, need for rescue medication and quality of life score were evident among children who were not exposed to ETS, but not amongst the children exposed to ETS at home, although this was identified in a post hoc analysis [120].

ETS and Asthma Therapy

Very little information is available on the effect of ETS on asthma therapy. Some evidence is available on the effect of ETS on theophylline metabolism. Increased clearance of theophylline has been reported in patients chronically exposed to ETS

[121], although acute exposure to ETS has been shown to have no effect on theophylline clearance [122].

Smoking and Other Allergic Diseases

While total IgE is known to have a strong genetic component, it has been suggested that total IgE might also be influenced by the environment. A number of epidemiological studies have shown that cigarette smoking is associated with elevated concentrations of total serum IgE [123–126]. Studies looking at the effect of ETS on IgE levels are conflicting. Some studies report significant relationship between ETS exposure and increased total IgE levels [127, 128] but other studies have been unable to show this relationship [129] or only been able to show the effect in a small subgroup [124].

Different hypotheses have been proposed to explain the relationship of smoking to IgE [124]. These include a direct effect on IgE regulation at the cellular level and an indirect action, which may lead to an increased airway permeability of airways to allergens [130]. IgE is controlled by the Th-2 interleukin IL-4, which has been shown to be elevated by cigarette smoking [131]. The permeability of bronchial epithelium is increased in smokers [132], thus facilitating penetration by small aeroallergens that may in turn sensitise the subject and stimulate IgE production.

Studies on the effects of smoking (both active smoking and exposure to ETS) in allergic disease have mainly focussed on the effects on asthma. There is however some, but limited, data on the effect of smoking on some of the other allergic diseases including atopic eczema, allergic rhinitis and food allergy. The studies mainly look at the effect of maternal smoking in the development of the disease; however, the effect of active smoking on the development of allergic rhinitis has also been studied.

Active Smoking and Non-Asthmatic Allergic Disease

Other than the effect of active smoking on asthma, the data is limited. A large French study of over 15,000 adolescents found that active smoking was associated with an increase risk of having current rhinoconjunctivitis OR (95% CI) 2.1 (1.5–2.0), lifetime hay fever OR (95% CI) 1.5 (1.1–2.2) and current eczema OR (95% CI) 1.8 (1.2–2.7) even after controlling for passive smoking [133] (Fig. 6). Previous observational studies have indicated that both allergic rhinitis and eczema are more commonly reported by current smokers among adolescents [134] and by former smokers among adults [135, 136]. In a large Swedish postal questionnaire survey, smoking was an independent risk factor for the development of hand eczema with an OR (95% CI) of 1.35 (1.04–1.75) [137]. However, a large Swiss study of over 8,000 subjects showed significantly higher prevalences of atopy and hay fever in non-smokers compared to smokers [138].

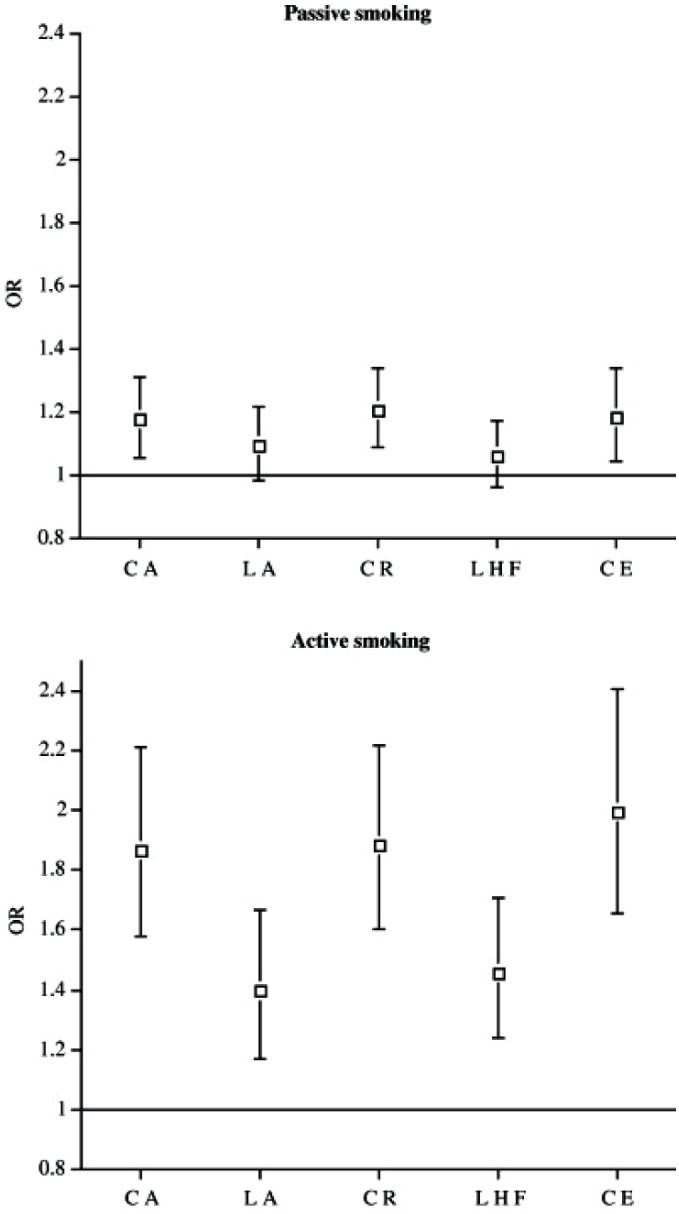

Fig. 6 Relationships of active and passive smoking to asthma and allied diseases in adolescents after adjustment for potential confounding factors in France. Figures are odds ratio (OR) obtained with logistic models. The model predicting factors associated with passive smoking was adjusted for age, sex, region, active smoking (and familial history of allergy when appropriate) whereas the model predicting factors associated with active smoking was adjusted for age, sex, region, passive smoking (and familial history of allergy when appropriate), respectively. CA, current asthma; LA, lifetime asthma; CR, current rhinoconjonctivitis; LHF, lifetime hay fever; CE, current eczema. Reproduced with permission from Ref. [133]

There is limited data on the effect of active smoking on the symptoms and severity of the other non-asthmatic allergic diseases mentioned above. One study did find that active smoking was highly associated not only with severe asthma but also with severe rhinoconjunctivitis [133]. Another study has concluded that active smoking can lead to exacerbations of eczema [139].

Environmental Tobacco Smoke and Non-Asthmatic Allergic Disease

Data are available on the effect of ETS on the development of allergic diseases other than asthma. This includes the effect of *in utero* exposure to cigarette smoke. Exposure to ETS has been associated with the development of atopic eczema [140, 141], allergic rhinitis [133] and food allergy [141–143].

A prospective observational study of 342 children in Germany showed that at 3 years, children who were exposed to ETS *in utero* and postnatally had a significantly higher risk of sensitisation to food allergens with an OR (95% CI) of 2.3 (1.1–4.6) [142]. Interestingly, children who were only postnatally exposed by a smoking mother also had an increased risk with an OR (95% CI) of 2.2 (0.9–5.9), although this was not statistically significant. A further study of 678 German preschool children found that maternal smoking during pregnancy or lactation was associated with an increased risk of atopic eczema, with an OR (95% CI) of 2.3 (1.3–3.1) [140]. Earlier studies have associated smoking during pregnancy to

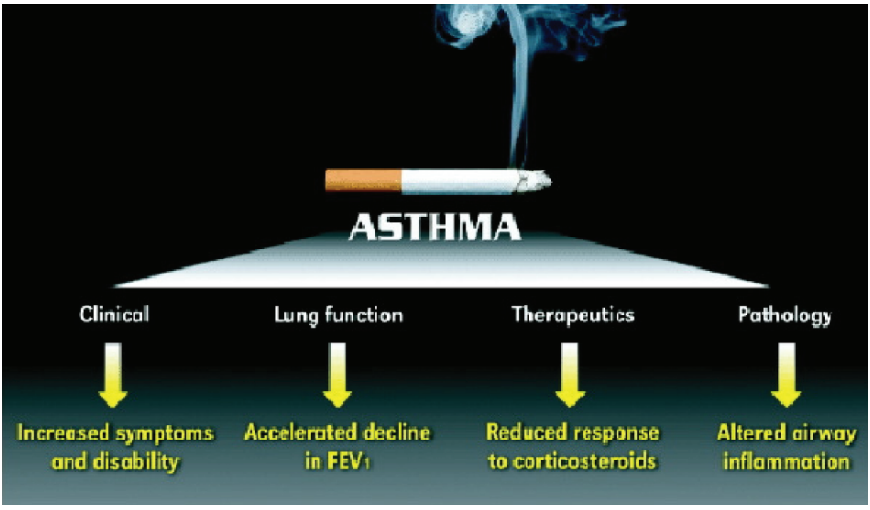

Fig. 7 Interaction of active cigarette smoking and asthma. Reproduced with permission from Ref. [70]

alterations in the child's skin [144]. The cumulative incidence during the first 5 years of life for hospitalisation for diseases of the skin was 22.5/1000 live births in the group of mothers who smoked during pregnancy (n = 1,821) and 8.2/1000 in the non-smoker group (n = 1,823; $p < 0.0001$). Eczema and urticaria were observed 4.7 times more often in the smoker group [145].

Little is known about the effect of smoking on the therapeutics of these other allergic diseases. Corticosteroids remain the mainstay of treatment. It is likely that the altered response to corticosteroids in cigarette smokers is a systemic effect [31] and so it is possible that the systemic effects of treatment may also be impaired in smoking subjects. Further work in this area is required.

In conclusion, allergic disease is increasing and although there is a strong genetic predisposition, environmental factors, including smoking, may be responsible for this increase. Good evidence is available for the detrimental effects of smoking on asthma morbidity and mortality [70] (Fig. 7). Reduced response to oral and inhaled corticosteroids is now well demonstrated, however, recent evidence suggests that this reduced responsiveness may be reversible on cessation of smoking [41]. Smoking cessation must therefore be an integral part of our management of these difficult patients.

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Socioeconomic Status and Asthma in Children

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Socioeconomic Status and Asthma in Children

Social factors have long been suggested to contribute to childhood asthma, and recently research has begun to provide some intriguing empirical evidence to support this hypothesis. Characteristics of a child's larger social environment, including neighborhoods with high levels of violence, occurrences of acute and chronic stress, and negative family environments have all been linked to asthma onset or morbidity in children [1–3]. These findings suggest that in addition to traditional risk factors such as genetics and environmental exposures, the social environment maybe an important component to a fuller understanding of asthma pathogenesis. For a graphical representation of how social contributors interact to shape asthma, see Fig. 1.

One broad social environment factor that maybe important to childhood asthma is socioeconomic status (SES). SES refers to a family's position within a larger social hierarchy, and can be defined in terms of prestige (e.g., parent's education or occupation) as well as resources (e.g., family income or assets) [4]. Across all social factors, SES exhibits one of the most robust and consistent associations with physical health outcomes. Individuals from lower SES families have poorer health than individuals from higher SES families. This relationship holds true across a variety of diseases, across many different countries, and throughout the life span [5, 6]. Despite this striking pattern, the relationship of SES with childhood asthma remains unclear. Asthma is one of the few diseases for which evidence of a reverse gradient (higher SES being associated with greater disease prevalence) has been presented. Thus the aims of the present chapter are to review evidence regarding the direction of association of SES with childhood asthma and to discuss plausible pathways between SES and asthma.

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R. Pawankar et al. (eds.) *Allergy Frontiers: Epigenetics, Allergens and Risk Factors,*
DOI: 10.1007/978-4-431-72802-3_24, © Springer 2009

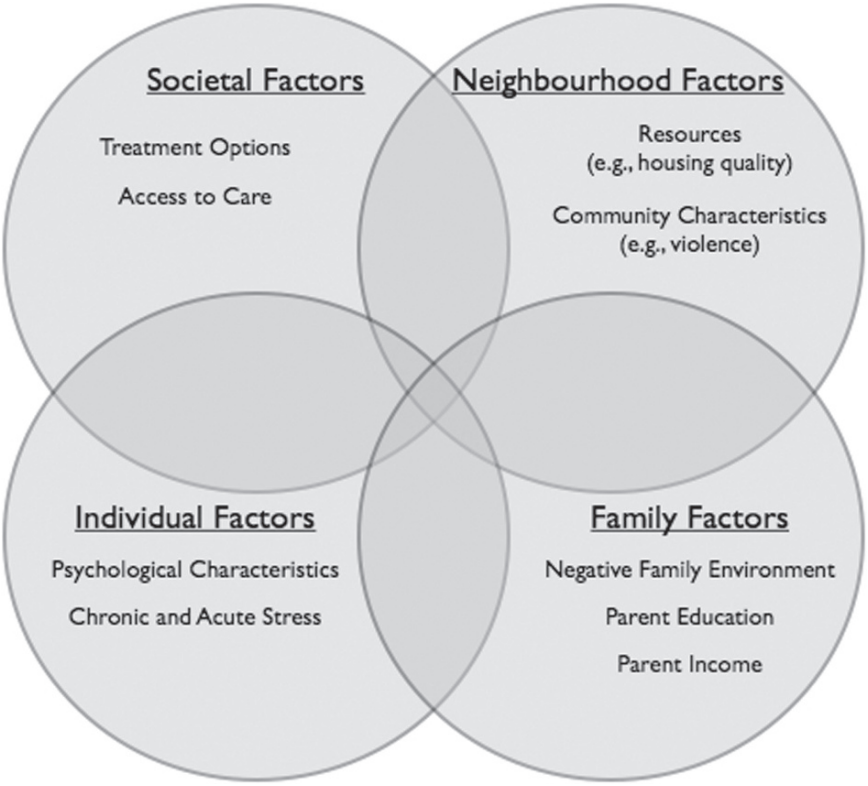

Fig. 1 Numerous social contributors interact to shape asthma

Epidemiological Evidence for SES and Asthma Associations

In this chapter, we focus on childhood asthma and elaborate primarily on more recent articles discussing SES and asthma. We refer the reader to a previous review that discusses articles prior to 2000 in greater depth [6]. With respect to types of asthma outcomes, one important distinction is between morbidity outcomes in children with existing asthma versus the diagnosis of asthma found in community samples. Because the relationship of each with SES could differ, we present findings separately for these outcomes.

Asthma Morbidity

Across numerous studies, there is quite consistent evidence that lower SES increases risk for poor asthma outcomes among those who are already diagnosed with asthma. For example, poor children with asthma and children from lower

income families are significantly more likely to be hospitalized for asthma, have greater asthma symptoms, and have more severe asthma episodes compared to nonpoor/higher income children with asthma [7–9]. Fewer years of parent education also have been associated with greater risk of asthma hospitalizations and emergency department visits in children with asthma [10,11]. Longitudinal studies show similar patterns. For example over a 6-year follow-up period, children whose fathers had less prestigious occupations were more likely to be hospitalized due to asthma compared to those whose fathers had more prestigious occupations [12]. At the neighborhood level, neighborhoods with lower income levels and higher unemployment rates have been found to have higher rates of pediatric asthma hospitalizations [13–15]. Finally, some studies utilize population-based survey designs but include questions related to asthma morbidity (even though only a small proportion of children in the survey have asthma). These studies show that lower SES (as indicated by either parental education or neighborhood characteristics) is associated with greater risk of asthma emergency department visits, asthma hospitalizations, and more severe asthma in children [16,17]. Finally, the above studies are consistent with earlier studies that documented associations between low SES and greater morbidity and severity of asthma [6].

Asthma Prevalence

Although the patterns are not as consistent, there are a number of studies that document that low SES is associated with an increased prevalence of asthma. For example, children whose parents have fewer years of education are more likely to have a physician diagnosis of asthma [17]. Children from poorer families are also more likely to have a diagnosis of asthma [7,9]. At the neighborhood level, children who live in inner city or low-income neighborhoods are more likely to have current asthma [14, 16, 18, 19]. Because many of these studies rely on parents to report diagnoses or symptoms, some studies have taken the approach of utilizing more objective measures of lung function. One such study found that lower parental occupation was associated with a greater likelihood of exercise induced bronchospasm in school children [20]. Finally, our previous review documented that among earlier studies on SES and asthma prevalence, associations of low SES with higher prevalence rates of asthma were more likely in cohorts of children aged 9 years and younger [6].

In contrast, several studies have found no evidence for an association between SES and asthma prevalence. For example, neighborhood income showed null or weak associations with childhood asthma prevalence rates in two studies [21, 22]. Occasionally, studies have found higher SES groups to be more likely to have asthma, for example finding that school children who were not eligible for a federal free lunch program had higher rates of asthma than those who were eligible [23]. Earlier studies suggesting a reverse gradient typically focused on occupational status, finding that parents in nonmanual occupations were more likely to have a child with asthma than those in manual occupations [24].

Overall, these studies suggest that the relationship between SES and asthma prevalence is much more mixed than relationships with asthma morbidity. One reason why this maybe is that there may be two distinct groups who are more likely to have a diagnosis of asthma. One would be the same group that is more likely to have adverse asthma outcomes; that is, low SES children, by virtue of being more likely to suffer from severe asthma, are also more likely to have a diagnosis of asthma. A second group maybe more likely to detect symptoms and seek treatment for their children, resulting in physician diagnoses of asthma. In this case, higher SES parents maybe more vigilant about monitoring symptoms, which may result in their children being more likely to be diagnosed with asthma, although in many cases, the asthma may be quite mild. Thus when prevalence (presence or absence of asthma) is the focus of study, both low and high SES groups may appear at risk; however, when considering severity, only low SES children are at risk. If true, the mixed patterns with respect to prevalence could be due to differences in the range of SES sampled in a particular study, leading to negative relationships in some studies, positive relationships in other studies, and perhaps no linear relationships in studies that span the entire SES spectrum.

Given these patterns, we focus the remainder of the chapter on explaining how SES might affect outcomes among those who have already been diagnosed with asthma. We first discuss possible biological pathways by which SES could get "under the skin" to affect clinical outcomes in a child with asthma, and then we address environmental and behavioral pathways that could affect these biological processes.

Effects of SES on Biological Processes

Asthma is an inflammatory disease of the airways, and certain cytokines have been hypothesized to be important for the orchestration of cellular events related to airway inflammation and hyperresponsiveness [25, 26]. These cytokines are produced by T helper (Th) cells, often in response to an external stimulus such as allergen exposure. Th cells have two phenotypes known as Th-1 and Th-2. Th-1 cells generally initiate and coordinate cellular immune responses by deploying cytokines such as IL-2 and IFN-γ. By contrast, Th-2 cells promote B cell proliferation and differentiation, which leads to a humoral response involving antibody synthesis. Th-2 cells release cytokines such as IL-4, IL-5, and IL-13. Th-2 cells have been implicated in asthma. For example, secretion of IL-4 and IL-13 induces B cells to produce IgE antibodies, which initiates an inflammatory cascade, leading to airway constriction and mucus production [27]. In addition, secretion of IL-5 has been found to increase eosinophil production, which also promotes airway inflammation and obstruction [28, 29].

Our research group has investigated whether SES is associated with cytokine production in two studies of children and adolescents diagnosed with asthma. In the first study, adolescents diagnosed with persistent asthma were recruited from either low-SES or high-SES neighborhoods (based on the percentage of people living below poverty in each neighborhood). Peripheral blood was drawn, and cells were stimulated with a combination of phorbol myristate acetate and ionomycin to

induce the production of cytokines. Adolescents with asthma from low-SES neighborhoods displayed significantly greater production of IL-5, and marginally greater production of IL-4 compared to adolescents with asthma from high-SES neighborhoods [30]. In a second study, children and adolescents diagnosed with asthma were recruited from a broad spectrum of SES backgrounds in order to test whether linear relationships between family SES and biological markers existed. This study found that SES was inversely and linearly associated with the production of IL-5 and IL-13, as well as with eosinophil counts [31]. Thus as family savings decreased, stimulated production of IL-5 and IL-13 increased in a linear fashion. In addition, as family savings decreased, children's eosinophil counts increased in a linear fashion. These findings suggest that low-SES adolescents with asthma exhibit heightened inflammatory profiles, and that the specific nature of these immunologic responses is consistent with pathways to more severe exacerbations of asthma.

Given the few studies of immune variables in children with asthma, we also mention several relevant adult studies on this topic. In a cohort of women who either had asthma or allergic disease or whose spouse had asthma/allergies, one group of researchers investigated whether SES was related to IgE profiles. SES was categorized at the neighborhood level using census data based on the zip code in which each participant lived. Women who lived in neighborhoods with higher rates of poverty, lower household incomes, and less educated individuals had higher levels of total serum IgE [32]. Women who lived in higher poverty neighborhoods also had greater immunologic sensitivity to cockroach, cat, dog, and ragweed allergens [33]. Other studies of healthy adults have demonstrated that low SES is associated with greater inflammatory profiles, as indicated by elevations in markers such as C-reactive protein, and cytokines such as IL-6 and TNF-α. These are reviewed in greater detail in Ref. [34].

Taken together, these patterns indicate that there is a socioeconomic patterning to immune profiles in children with asthma as well as in adult populations. The larger social environment appears to be able to get "under the skin" to alter immune function in a manner that has implications for asthma. In particular, low SES has been associated with greater stimulated production of Th-2 cytokines, higher eosinophil counts, and higher IgE levels. Among children already diagnosed with asthma, this type of profile would also be consistent with greater morbidity due to asthma, and hence could provide a biological explanation for how SES comes to affect clinical asthma outcomes in childhood.

The next question that arises is how SES comes to be associated with biological changes such as immune dysregulation. In the next section, we review more proximal causes of immune system alterations that could also be affected by SES.

Possible Pathways between SES and Immune Function

There are a number of pathways by which SES could alter biological profiles in children with asthma. In this chapter, we discuss three broad categories, including environmental exposures, psychological factors, and medical care characteristics.

Environmental Exposures

One commonly proposed explanation for associations between SES and asthma is that living in a low SES neighborhood may mean greater exposure to both outdoor and indoor environmental triggers of asthma. These include smoking, allergen exposure, and air pollution, to name a few.

For example, lower family income, parent education, and parent occupational status are all associated with greater smoking in the homes of children with asthma [10, 35]. In turn, exposure to tobacco smoke can lead to wheezing and asthmatic symptoms in vulnerable individuals [36]. Furthermore, some researchers have argued that parental smoking largely accounts for SES differences in asthma morbidity [10].

With respect to allergen exposure, cockroach allergen represents one type of exposure that has been linked to both asthma morbidity and low SES. Low SES is associated with both greater sensitivity and exposure to cockroach allergens [37, 38]. In particular, inner-city homes, where rates of asthma are high, also frequently have high levels of cockroach exposure. For example, among inner-city children with asthma, 50% of homes had high levels of cockroach allergen in the bedroom. Furthermore, children who had high exposure and were allergic to cockroach allergen were significantly more likely to be hospitalized, have unplanned medical visits for asthma, and have more asthma symptoms during a 1-year follow-up [39].

Finally, exposure to outdoor air pollutants such as nitrogen dioxide (NO_2) and sulfur dioxide (SO_2) can cause significant respiratory morbidity [40–43]. Previous research has found that children with asthma who live in multifamily houses (likely lower in SES) were exposed to greater amounts of NO_2 compared to those who lived in single-family housing [44]. Moreover, the effects of exposure to NO_2 (in males) and SO_2 (in females) on asthma hospitalization frequency were significant only in a low SES group [45].

In sum, a number of indoor and outdoor asthma triggers have been linked to low SES, including greater exposure to smoke, cockroach allergen, and air pollution. In turn, these triggers are recognized by immune cells, which initiate a cascade of events leading to the aggravation of airway inflammation and asthma symptoms. Hence one pathway by which SES may come to affect immunologic profiles lies in the environmental exposures to which low SES children may be vulnerable.

Psychological Stress

A handful of research studies have evaluated whether stress amplifies the immune responses implicated in asthma. In a study of college students with asthma during periods of high stress (final examination period) and low stress (no major examination) [46], patients inhaled increasing dosages of allergens to which they were sensitized (ragweed, cat, or dust mite) until their pulmonary functioning declined by ≥20%. There was evidence of a greater immune response to challenge under

stressful conditions. During the session that occurred around final examinations, the allergen challenge elicited greater numbers of eosinophils in both sputum and blood. A parallel finding emerged for in vitro production of IL-5 in sputum treated with phytohemagglutinin. These findings provide support for the notion that stress changes the way in which the immune system responds to challenge, making the individual potentially more vulnerable to asthma exacerbations.

In another set of studies, high school students were tested before an examinations (baseline period of low stress) and after examinations (high-stress period), and their peripheral blood cells were stimulated in vitro with various mitogen cocktails. In one study, students with asthma had greater production of IL-5 postexamination compared to students who were healthy. In contrast, there were no group differences in IL-5 production at baseline. This suggests that under conditions of low stress, individuals with asthma do not differ from healthy individuals in their responsiveness to mitogen, but that periods of stress heighten the responsiveness of Th-2 immune cells to mitogens specifically in individuals with asthma [47]. A second study from this group documented that examination stress was associated with reduced production of Th-1 cytokines (IFN-γ and IL-2) but increased production of the proinflammatory cytokine IL-6 (argued to represent Th-2) across both a sample of students with asthma and healthy students [48].

In one of the few studies of young children, one group investigated 2-year-old children with a family history of asthma or allergy. Immune cells were stimulated in vitro with allergens (dust mite, cockroach) as well as phytohemagglutinin (PHA). Because of the age of the children, stress was assessed in their caregivers. Children whose caregivers appraised their lives as high in stress had greater stimulated production of the proinflammatory cytokine TNF-α, and reduced production of the Th-1 cytokine IFN-γ [49]. Importantly, these effects were prospective, with stress temporally preceding immune response.

Finally, in our previous work in which we demonstrated that low SES was associated with increased production of Th-2 cytokines and higher eosinophil counts in children with asthma, we also measured children's chronic stress levels as well as their tendency to perceive ambiguous situations as stressful. When statistical analyses were conducted to evaluate relationships among these processes, they showed that chronic stress and stress perception formed an indirect pathway between low SES and inflammatory responses [30, 31]. In other words, data were consistent with the notion that that low SES children experience more chronic stress and perceive events in a more stressful manner, and that in turn these stress experiences amplify asthma inflammatory responses.

Collectively, these studies suggest that among patients suffering from asthma, stress can heighten the Th-2 cytokine response to asthma triggers and mitogen cocktails, and in some cases also blunt the release of Th-1 cytokines. In addition, psychological stress appears to form a significant pathway linking SES to asthma-specific inflammatory responses. In turn, these stress-induced inflammatory patterns may make children vulnerable to more frequent or severe asthma symptoms.

Interestingly, one additional study suggests that stress may interact with the physical environment to affect asthma. In this study, children with high levels of stress,

as defined by greater experiences with neighborhood violence, who were also exposed to high levels of NO_2, were at elevated risk for a diagnosis of asthma [50], suggesting that it maybe important to consider environmental exposures in combination with psychological stress in understanding contributors to asthma.

Medical Characteristics

Another pathway by which low SES may affect asthma inflammatory responses relates to differences in medical care and in the management of asthma in low versus high SES families.

First, low SES children maybe less likely to receive a diagnosis of asthma. For example, enrollment in a federal free school lunch program was associated with a higher rate of undiagnosed frequent wheezing in adolescent schoolchildren [51]. Second, low SES children who have asthma are less likely to have a regular source of medical care [52]. Third, low SES children with asthma are prescribed medications differently from high SES children. Children who have been diagnosed with asthma but who come from lower income families are less likely to receive inhaled corticosteroid prescriptions, independent of asthma severity. Moreover, this was a sample of Canadian schoolchildren, where all children were insured through the same provincial drug plan [53]. Furthermore, a substantial proportion of inner-city children with asthma are undermedicated, as evidenced by the fact that over 50% of children who had been to the emergency room in the past 6 months were untreated or only on beta agonists for their asthma [54]. Finally, low SES children often receive different asthma treatment regimens from high SES children. Children who had been hospitalized for asthma and who came from lower income families or had less educated parents were less likely to have seen an asthma specialist, have had pulmonary function measured, or have been prescribed an anti-inflammatory agent posthospitalization compared to higher SES children who had also been hospitalized for asthma [52].

In addition, low SES families show differences in how they manage asthma compared to high SES families. First, low SES families utilize asthma medications differently from high SES families. Children with asthma from low SES families on Medicaid were less likely to have filled prescriptions for inhaled corticosteroids or mast cell stabilizers [55]. Children with asthma from lower income families also were less likely to be using an inhaled corticosteroid [38]. Second, low SES families adhere to asthma medication regimens differently from high SES families. Among children aged 5–12 years, families that were lower in SES (receiving social assistance) were more likely to have a child who was nonadherent to national asthma guidelines for medications [56]. Similarly, among adults with asthma, lower levels of education were associated with a greater likelihood of nonadherence to national asthma guidelines [57], and lower levels of education and income were associated with poor adherence to daily inhaled corticosteroid medications, even when all patients were given prescriptions and medications without cost [58].

Adults with lower income or less education also more frequently use short-acting beta agonists, independent of the severity of their asthma [59]. Finally, low SES families access medical care in different ways from high SES families. Children from lower SES families, as determined by Medicaid status, were more likely to receive care in the emergency department rather than through outpatient, nonurgent visits [55, 60].

In sum, differences are apparent in how low versus high SES families interact with and receive care from the medical system as well as how they manage asthma at home. These differences likely impact the ability of families to contain asthma-related inflammation in their children, which in turn will have implications for clinical asthma outcomes. See Fig. 2 for the possible pathways that may lead from SES to asthma exacerbations.

Taken together, these findings suggest that SES may operate through a variety of pathways to affect immune function, and in turn, asthma morbidity. At the community or societal level, SES is associated with the health care that a patient has access to and receives. At the neighborhood level, SES is associated with the amount of exposure to various environmental pollutants. Finally, at the individual level, SES is associated with the psychological experiences of chronic stress and stress perception that a child has. Though each of these pathways is quite distinct, they all have implications for asthma inflammation, and provide plausible models for how SES gets "under the skin" of a child with asthma.

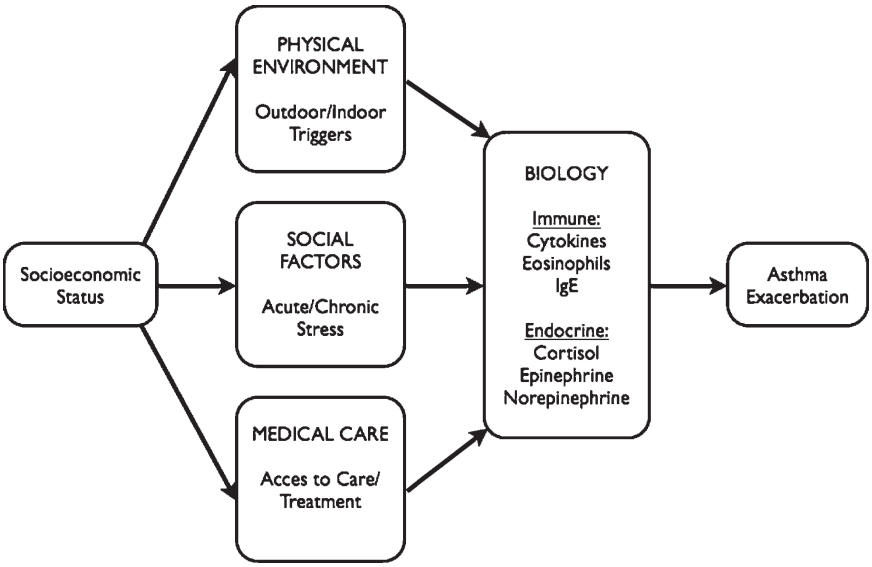

Fig. 2 Pathways linking socioeconomic status (SES) and asthma outcomes

Conclusions and Future Directions

In this chapter, we reviewed evidence documenting that low SES is associated with poorer asthma outcomes, such as increased likelihood of hospitalization, among children already diagnosed with asthma. In contrast, the association between SES and incidence or prevalence of asthma is more mixed. It appears that both low SES and high SES children maybe at risk for a diagnosis of asthma. Low SES children maybe at greater risk for the same reasons that they are at greater risk for experiencing greater asthma morbidity. High SES children maybe at greater risk by virtue of having parents who are more vigilant for symptoms and more proactive about seeking medical attention for their child.

With respect to pathways, a number of studies have shown that low SES is associated with inflammatory profiles that are detrimental for asthma. Specifically, low SES is linked to increased production of Th-2 cytokines, as well as to higher levels of IgE and eosinophil counts. These findings indicate that the larger social environment is able to affect biological systems in an individual child, thus providing a plausible explanation for how SES can shape physical health outcomes, and in particular, asthma pathogenesis. In addition, studies have shown that SES may exert its effects at a variety of levels. At the societal level, SES may affect the type and quality of health care a family has access to. At the neighborhood level, the type of house and neighborhood families can afford to live in will affect their degree of exposure to environmental pollutants and allergens. At the individual level, SES affects both psychosocial characteristics such as stress, as well as behaviors such as a child's adherence to asthma medications. All of these factors in turn contribute to asthma inflammation and morbidity.

Now that research has begun to establish some of the factors through which the social environment comes to influence asthma, it would be important for future research to begin testing more comprehensive models. Future research that is able to simultaneously assess multiple levels of factors (health care characteristics, environmental factors, psychosocial factors, biological markers) will be critical for developing a better understanding of the relative importance of each of these factors to childhood asthma. In addition, research that simultaneously assesses multiple factors will also allow researchers to determine whether there are interactive effects such that certain combinations of factors are most detrimental to asthma.

In addition, many of the studies described above were cross-sectional. It is important for future researchers to conduct longitudinal studies that are able to draw firmer conclusions about directionality. For example, what happens when families improve in SES? Which other factors change subsequent to movements in SES, and do these factors precede changes in clinical symptoms or functional outcomes related to asthma? Finally, when possible, it would be important to conduct experimental investigations of SES and asthma. While it is not ethically possible to assign study participants to SES, it could be possible to take advantage of naturally occurring social experiments (e.g., welfare-to-work programs) and to assess the effects of SES manipulations on childhood asthma outcomes. These types of studies would permit firmer conclusions about causality.

In sum, evidence points to the importance of the larger social environment, such as low SES, for childhood asthma. In addition to risk factors traditionally recognized in asthma, such as the role of allergens and genetics, there is a need for incorporating social factors such as low SES and stress [61, 62] in order to develop more comprehensive models of the trajectory of asthma in childhood.

Acknowledgment This study was supported by funding from the Michael Smith Foundation for Health Research and the Human Early Learning Partnership.

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