

Indoor versus outdoor allergens in allergic respiratory disease

Thomas AE Platts-Mills*, Lisa M Wheatley* and Rob C Aalberse†

Immediate hypersensitivity to indoor or outdoor allergens is strongly associated with asthma or hay fever, respectively. Recent progress has defined the sequences, tertiary structures and enzymatic functions of many of the proteins involved; furthermore, the immune responses to these proteins have been examined; however, the mechanisms responsible for the dichotomous response remain elusive. The resolution of such mechanisms may explain the large increase in the prevalence of eosinophil-rich inflammation of the lower respiratory tract.

Addresses

*Asthma and Allergic Disease Center, Department of Medicine, University of Virginia, Charlottesville, Virginia, VA 22908, USA

†Central Laboratory of the Netherlands Red Cross Transfusion Society, Department of Immunochemistry, P.O. Box 9190, 1006 AD Amsterdam, The Netherlands

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Abbreviations

IFN- γ interferon γ

NMR nuclear magnetic resonance

Introduction

There are two allergic diseases of the respiratory tract that are common enough and sufficiently well defined to allow epidemiological studies on their prevalence and causes — hay fever and asthma. Seasonal allergic rhinitis, or hay fever, was first described in the nineteenth century and became well recognized by 1920. Hay fever is by definition associated with sensitization to proteins derived from plant pollens or mold spores that are airborne during a specific season of the year. Asthma has been known for thousands of years but its incidence has increased dramatically over the last 40 years of the twentieth century. Asthma in children and young adults is also an allergic disease but the allergens associated with asthma are predominantly found indoors. Obviously, these two syndromes overlap and some individuals are sensitive to multiple allergens; nonetheless, understanding the reasons why indoor and outdoor exposure can give rise to different diseases could help to explain the role of allergens in asthma and their relevance to the increase in asthma prevalence.

The primary event in all allergic diseases is an immune response to a normally innocuous foreign protein. In the past ten years, many of the proteins involved have been defined both in terms of amino-acid sequence and by using monoclonal antibodies specific for these allergens. In the past year the tertiary structures of allergens from birch pollen and from dust mites have been reported. These structures allow analysis of B-cell epitopes and comparison of 'indoor' and 'outdoor' proteins. The next issue is

whether exposure to these allergens is sufficiently different to explain the disease associations. Immune responses to dust mite and pollen proteins include the production of IgE antibodies, IgG4 antibodies and T cells with a Th2 phenotype. Recent developments in delineating these responses include better understanding of the homing characteristics of T cells and insight into the reasons why IgG4 is monovalent. Thus issues relating to indoor and outdoor allergens include the physical and biological properties of the proteins, the quantitative aspects of exposure, the nature of the immune response and the epidemiology of asthma versus hay fever.

Physical and biological properties of allergens

The primary properties defined thus far for an allergen are the physical properties that facilitate delivery to the lymphoid tissue of the respiratory tract. These proteins are soluble in aqueous solutions, abundant in the source material and predominantly between 10,000 and 50,000 daltons in size [1•]. The major allergens of the dust mite, cat and cockroach have been defined (Table 1) and the sequence data have provided extensive information about homology with other proteins. In the past year, two further groups of cockroach allergens have been cloned: first the group I proteins Bla g 1 and Per a 1, that have a novel repeated structure; and second a glutathione-S-transferase, Bla g 5 [2,3•,4].

Proteins derived from remarkably diverse species can be allergens [1•,5]. Not surprisingly, most of these proteins have defined biological functions and many of them have sequence homology with known enzymes [1•,2,5,6]. This has led to speculation about the relevance of enzymatic activity to immunogenicity, although in most cases enzymatic activity has not been established. The mite allergen Der p 1 is a potent thiol protease and three different mechanisms have been proposed by which the activity of Der p 1 could enhance allergic responses: first, the enzyme can disrupt epithelial cell layers and enhance the passage of macromolecules; second, the enzyme can cleave CD23 at two sites — leading to release of soluble CD23 and also blocking the inhibitory effect of IgE on B cells [7]; finally, Der p 1 can act on CD25 thereby inactivating the IL-2-receptor on T cells [8••]. Comoy *et al.* [9•] have recently demonstrated an inhibitory effect of Der p 1 on IFN- γ production and Th1 responses in mice. Most of the experimental studies have used a dose of 10 or 20 μ g of Der p 1, which contrasts with the quantity found in a mite fecal particle, that is 0.1 ng [7,8••,9•,10]. Demonstrating that the enzymatic activity of allergens is relevant to immunogenicity in man will be very difficult: it is not easy, first, to estimate the concentrations that occur locally in the respiratory tract; second, to define conditions for activity; or third, to evaluate the effects of other proteases in the

Table 1

Structural and functional properties of indoor allergens.

Source	Allergen*	Molecular weight	Function, or amino acid homology†	Sequence data
House dust mite				
<i>Dermatophagoides spp.</i>	Der p 1, Der f 1‡	25 kDa	Cysteine protease	cDNA
	Der p 2, Der f 2‡	14 kDa	(Epididymal protein)	cDNA
	Der p 3, Der f 3‡	~30 kDa	Serine protease	cDNA
	Der p 4	~60 kDa	Amylase	Protein
	Der p 8	26 kDa	Glutathione-S-transferase	cDNA
<i>Euroglyphus maynei</i>	Eur m 1	25 kDa	Cysteine protease	PCR
<i>Lepidoglyphus destructor</i>	Lep d 2‡	14 kDa	(Epididymal protein)	Protein, DNA
Mammals				
<i>Felis domesticus</i>	Fel d 1‡	36 kDa	(Uteroglobulin)	PCR
<i>Canis familiaris</i>	Can f 1‡	25 kDa	(Taste perception)	cDNA
	Can f 2‡	27 kDa	Calycin	cDNA
<i>Mus musculus</i>	Mus m 1	19 kDa	Pheromone-binding protein	cDNA
<i>Rattus norvegicus</i>	Rat n 1‡	19 kDa	Pheromone-binding protein	cDNA
Cockroach				
<i>Blattella germanica</i>	Bla g 1‡	20–25 kDa	Unknown	cDNA
	Bla g 2‡	36 kDa	Aspartic protease	cDNA
	Bla g 4	21 kDa	Calycin	cDNA
	Bla g 5	22 kDa	Glutathione-S-transferase	cDNA
<i>Periplaneta americana</i>	Per a 1	20–25 kDa	Unknown	cDNA

*Revised nomenclature proposed by the World Health Organisation/International Union of Immunological Societies subcommittee. †Based on sequence-similarity searches of protein and nucleic acid databases. In most cases the allergen function has been confirmed by testing for biological activity (e.g. enzyme activity). Parentheses indicate structural homology, where functional activity has not been confirmed or established.

‡Allergens for which monoclonal antibodies are available and are suitable for immunoassay purposes. PCR, polymerase chain reaction.

lung [11]. It is also important to recognize that several major allergens (e.g. the lipocalins derived from rodents, horse and roach; or Der p 2 and Fel d 1) do not show any evidence that they are enzymes [12].

Models of the tertiary structures of allergens made by homology with proteins of known structure [5,6,12] do not define epitopes; however, using site-directed mutagenesis it is possible to reduce IgE binding without reducing T cell responses *in vitro* [13]. More exact tertiary structure can be obtained from X-ray crystallography or nuclear magnetic resonance (NMR). The tertiary structures of Bet v 1, Der p 2 and Der f 2 have been determined by NMR and used to define B-cell epitopes [14,15*,16*]; however, the results reinforce how far we are from predicting the physical dimensions of an antibody-binding site from the primary structure. The published structures of Der p 2 and Der f 2 have different β sheet patterns; however, the native structures of these molecules are so similar that IgE antibodies and monoclonal antibodies have difficulty distinguishing them [1*,13,14,15*].

Our conclusion is that diverse foreign proteins can be allergens and that, not surprisingly, many of these proteins show homology with enzymes. Despite this clear evidence that some of these proteins are enzymes, there is no evidence that the enzymatic activity is relevant in man. Currently it seems unlikely that the tertiary structure or biochemical properties of these proteins are critical for

their antigenicity or play a role in the difference between responses to indoor and outdoor allergens.

Exposure to an airborne allergen

For proteins to reach the lymph nodes draining the respiratory tract, they have to be inhaled in the form of particles. The number of particles, their size and the quantity of allergen they contain are all relevant. Like dust-mite allergens, the cockroach allergens Bla g 1 and Bla g 2 only become airborne during disturbance and behave as if they were carried on particles sized $>10 \mu\text{m}$ in diameter [17,18]. In most natural situations the quantity of allergen that is inhaled is estimated to be very low, that is 10–100 ng/day. Exposure to indoor allergens involves the following features that differ from outdoor ones: the air is generally still; the subjects are often sedentary or asleep; and exposure is year-round. These points are pertinent for the following reasons: rapid movement of the air that is encountered outdoors is thought to explain the fact that conjunctivitis is only common with seasonal allergens; prolonged exposure to indoor allergens may contribute to asthma because quiet breathing selectively exposes the lungs; and while exposure to pollen (or spores of the fungus *Alternaria*) outdoors can give rise to asthma, it is unusual for bronchial hyper-reactivity (BHR) to persist for more than a few weeks after the pollen season. Thus, an alternative explanation for the relevance of indoor allergens to asthma is that it takes many months of 'continuous' exposure to produce the chronic inflammation that presents as clinical asthma.

The immune response to inhaled allergens

In animal models and the few experiments that have been done in man, immunization with submicrogram quantities of soluble protein does not usually induce an immune response. Similarly, the majority of humans who are exposed to mite or pollen allergens do not make any detectable immune response [1*]. Exposure to cat and also rodent allergens is higher; IgG antibody responses to these proteins are also common among nonallergic individuals [19]. The primary issue is why allergic individuals make an immune response to very low quantities of protein. A weak antigenic stimulation is not very efficient but when the response is induced it defaults to the Th2 phenotype. Responses of this kind (i.e. to low quantities of antigen) are more easily evoked in atopic individuals. If these responses are becoming more common they must be occurring in some individuals who would have been considered genetically nonpredisposed [19,20]. Several other explanations have been proposed: that the local concentration at the site of contact of particles is much higher than would be expected from the quantity that is airborne; that enzymatic activity alters the local response; or that immunization occurs early in life at a time when the immune system is both responsive and prejudiced towards the Th2-type [21*].

The 'early-life' proposal has two forms. The first proposal, made some years ago, is that there is a window of opportunity in early childhood when exposure to either pollen or mite allergens has an increased chance of inducing a response. Perhaps the strongest evidence for this hypothesis comes from studies showing that children born at certain times of the year are more likely to become allergic. In relation to this view it has also been suggested that part of the immunological protection of the fetus is a bias towards Th2 responses. If so, the maturity of the immune system at birth may be critically important. The alternative proposal is that sensitization or some form of immune response to inhalants can occur *in utero*. The evidence for this proposal comes primarily from studies on cord-blood lymphocytes. Several groups have reported that these cells will proliferate *in vitro* when exposed to nonspecific mitogens or specific allergens [22]. The bulk of the studies has used dust-mite extract or dust-mite allergens. In addition, some of the studies have used serum-free media [23]. There are many problems with theories about *in utero* sensitization. The simplest is that only proliferative responses have been shown and it is not clear that they are related to subsequent IgE antibody responses or disease. The second problem is that the enhancing effect of serum-free media may be mite-specific [24]. The next problem is that proliferative responses to mite allergen have been reported with cord-blood cells from babies in geographical areas where exposure of the mothers to mite allergen was very low (i.e. <1 µg/gram of dust; [25]). This raises the prickly issue of how a baby can make an immune response *in utero* to a protein of which the quantity inhaled by the mother is only ~50 ng/day. The only likely scenario is that complexes

of allergen and maternal IgG antibody are transferred across the placenta. Although both IgG1 and IgG4 antibodies cross the placenta, this mechanism of transferring allergens to the fetus has not been demonstrated. In addition, the best established effect of maternal antibody on the newborn is to suppress immune responses to early vaccination. If maternal antibody was involved, one would expect a strong correlation between maternal sensitization and specific sensitization of the infant. Although there is a stronger inheritance of allergy from the mother than from the child's father, this effect is modest and is not antigen-specific [26]. Overall, the hypothesis that sensitization to inhalant allergens occurs *in utero* has to be considered speculative.

It has been known for some years both that the immune response to allergens includes IgG4 antibodies and that this isotype does not precipitate in gels or fix complement. Recent experiments *in vitro* have shown that IgG4 has these properties because the two halves of the molecule separate and exchange *in vivo* giving rise to molecules which are bispecific and therefore under most conditions functionally univalent [27]. Increasing the proportion of the antibody response that is IgG4 (e.g. by immunotherapy) could have a protective effect but this proportion has not been shown to differ between seasonal and perennial allergens. By contrast, it is very likely that the homing properties of primed T cells can influence the patterns of allergic disease, that is skin versus lungs or nose. The skin-specific markers (e.g. cutaneous T lymphocyte antigen [CTLA]) are well established but the lung-specific markers are less convincing (see Rolland and O'Hehir, this issue pp 640–645).

Animal models of asthma

Animal models of asthma have focused on the conceptual model that proteins inhaled by an allergic individual induce an inflammatory response that leads to bronchial hyper-reactivity. This has been a fruitful model in terms of understanding the immune mechanisms that control inflammation of the lungs in mice and, in particular, the mechanisms of eosinophil recruitment [28,29]; however, the calculated doses of protein used in mice are higher than natural exposure and are given as droplets rather than in the form of particles [28,29]. No model has reproduced the contrast in exposure that gives rise to seasonal hay fever or to perennial asthma. Equally, the bronchial hyper-reactivity induced in mice does not compare to the human disease either in chronicity or the magnitude of the differences.

The epidemiology of the relationship between IgE antibodies and allergic disease of the respiratory tract

The driving force for research on asthma is the astonishing increase in this disease that has been reported from so many parts of the Western world [30,31]. Although there are many questions about the size or nature of the increase, it is not in doubt that asthma has become a common problem and that vast numbers of patients receive treatment in the form of inhalers; furthermore in the USA, England and

Australia increasing numbers of patients have required hospitalization for asthma [30]. There are several possible descriptions of what has actually changed and many ideas about the cause or causes of the increase [30]. The bulk of the patients with asthma are allergic to one or more indoor allergens. Some studies have suggested that the increase in numbers of wheezing patients is simply a reflection of a progressive increase in the number of individuals who are allergic [32,33]. Thus, in some studies the prevalence of allergic rhinitis appears to have increased in parallel with asthma [34]. On the other hand, there is a series of studies that suggest an entirely different explanation. First there are studies, on rural communities, that have found an average, or even high, prevalence of positive skin tests to indoor allergens despite a very low prevalence of asthma [35,36]. Second, the prevalence of hay fever in students at American universities was ~10% as early as 1965, that is before the increase in asthma [37,38]. Indeed, most American authorities do not believe that there has been more than a marginal increase in nasal allergy over the past 40 years [39]. In keeping with the view that the real increase is in asthma among individuals who are allergic to indoor allergens, pollen sensitization plays little role in chronic bronchial hyper-reactivity [40,41,42*].

The epidemic of asthma that is occurring among African-Americans represents a real hurdle for many of the hypotheses. Why would asthma show a strong social-class bias towards low-income patients in the USA but not elsewhere in the world? Asthma in American inner cities is strongly associated with sensitization to dust-mites in Atlanta and to cockroaches in the northern cities [42*,43,44**]. Theories about the effects of air pollution, improving general health and family size fail to explain the evidence from North American cities. Similarly, theories involving the decrease in early childhood viral infections do not appear to apply to these cities. Changes in diet in North America could be relevant to asthma; however, studies on diet report changes in bronchial reactivity that are inadequate to explain the current increases in asthma [45,46]. There have been dramatic changes in lifestyle, including an increasing time spent indoors, decreasing physical exercise and major increases in obesity [47–49]. The recent report that asthmatic children in New York are more obese than average children in that city is striking because children with asthma were traditionally found to be thin [50**].

Conclusions

Over the past 40 years asthma has become a major public health problem and understanding the reasons for the increase presents a major scientific challenge. Studies from all over the world confirm that asthma is an inflammatory disease and that it is associated with immediate hypersensitivity to the perennial indoor allergens rather than to those allergens where exposure is seasonal and predominantly outdoors. Full definition of the indoor allergens has allowed study of the structure of the proteins, quantifica-

tion of exposure indoors and evaluation of the immune response; however, the results do not explain the changing epidemiology of allergic disease. There are now three major hypotheses to explain the reasons for the increase in asthma; these are detailed below. As is clear from the preceding discussion, these depend on strikingly different interpretations of the data.

The first hypothesis is that progressive changes in housing and lifestyle (including increased time indoors) have increased exposure to those perennial allergens that are most likely to produce chronic inflammation and increased reactivity of the lung. The second hypothesis is that changes in Western society—including antibiotic use, diet, immunization and public health—have changed immune responsiveness such that a larger number of individuals develop immediate hypersensitivity. These two hypotheses are based on the view that there has been a general increase in allergic disease in addition to a specific increase in asthma. The third hypothesis is that changes in lifestyle over the past 40 years have not only influenced immune responsiveness and exposure to indoor allergens but also influenced the physiology of the lungs. This hypothesis implies that there has been a progressive loss of a lung-specific protective effect and that this has contributed to an increased prevalence of asthma among allergic individuals [51*,52].

Understanding the reasons why there should be an epidemic increase of a disease that is so strongly associated with immediate hypersensitivity will involve sorting out interactions between the environment, the physiology of the lungs and the immune mechanisms that can give rise to inflammation in the lungs; however, all of these changes appear to be related to modern lifestyle. Thus, asthma should be included with hypertension, diabetes and arteriosclerosis as a disease of modern society. Which of the lifestyle changes are most important to the progressive rise in asthma remains unclear; however, it seems unlikely that the results of asthma management will improve further unless the lifestyle issues are addressed.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

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were analyzed both by protection assays and by using multiple substitutions of predicted surface amino acids with alanine. NMR analyses of proteins are limited both by the size of the molecule (generally it must be less than 18,000 daltons) and by the cost of labelling proteins with the heavy isotopes necessary for specific forms of resonance.

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