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Allergens and their role in the allergic immune response

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Summary: Allergens are recognized as the proteins that induce immunoglobulin E (IgE) responses in humans. The proteins come from a range of sources and, not surprisingly, have many different biological functions. However, the delivery of allergens to the nose is exclusively on particles, which carry a range of molecules in addition to the protein allergens. These molecules include pathogen-associated molecular patterns (PAMPs) that can alter the response. Although the response to allergens is characterized by IgE antibodies, it also includes other isotypes (IgG, IgA, and IgG4), as well as T cells. The challenge is to identify the characteristics of these exposures that favor the production of this form of response. The primary features of the exposure appear to be the delivery in particles, such as pollen grains or mite feces, containing both proteins and PAMPs, but with overall low dose. Within this model, there is a simple direct relationship between the dose of exposure to mite or grass pollen and the prevalence of IgE responses. By contrast, the highest levels of exposure to cat allergen are associated with a lower prevalence of IgE responses. Although the detailed mechanisms for this phenomenon are not clear, it appears that enhanced production of interleukin-10 in response to specific Fel d 1 peptides could influence the response. However, it is striking that the animal sources that are most clearly associated with decreased responses at high allergen dose are derived from animals from which humans evolved more recently (~65 million years ago). Although the nose is still recognized as the primary route for sensitization to inhalant allergens, there is increasing evidence that the skin is also an important site for the generation of IgE antibody responses. By contrast, it is now evident that delivery of foreign proteins by the oral route or sublingually will favor the generation of tolerance.

Keywords: IgE, allergy, inhaled particles, peptide-specific T cells, glycosylation, protein structure

Introduction

When Charles Blackley published his 'Researches on the causes and nature of *catarrhus aestivus*' in 1873 (1), he provided the first systematic investigation of an allergen source. Those studies not only identified grass pollen grains as the cause of hay fever, but they also provided estimates of the number and weight of pollen grains inhaled. Furthermore, Blackley pioneered the pollen count and proved that pollen grains could float up to 2000 feet. In the first half of the 20th century, many different allergen sources were identified

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including pollen, fungi, insects, and domestic animals (2, 3). In addition, there was full awareness that food could become an important source of allergens. It was clear at that time that most of the soluble allergens were proteins; however, some pollen allergens were so resistant to heat that it was suggested that they could not be proteins. Further understanding of the chemical properties of allergens had to wait until the 1960s when full purification became possible (4, 5). Those studies provided new insight into the aerodynamic properties of allergens and formed the foundation for our rapidly evolving knowledge of the diversity and complexity of allergens.

In this review, we evaluate the relevance of allergen exposure to the immune response and the subsequent symptoms in allergic individuals. It is now evident that exposure includes not only the quantities and properties of the individual proteins, but also the particles on which they are inhaled, and the biological/immunological activity of the other constituents of the particles. We also briefly consider the relevance of other routes of exposure including the skin and gastrointestinal tract. A primary focus is how immunoglobulin E (IgE) antibody responses are generated and influenced by different allergens, since most of the evidence links IgE antibodies to the manifestations of allergic disease; however, the antibody response to allergens also includes other isotypes (IgG1, IgA, and IgG4). We also explain how allergen properties may favor the induction and amplification of T-helper 2 (Th2) responses that promote allergic disease. Our aim is to highlight elements of the immune system (B cells and T cells) that are pivotal to understanding the nature of the immune response to allergens.

The aerodynamic properties of allergens

Purification of the first grass pollen allergen in 1965 by David Marsh (4) allowed the first detailed investigation of the properties of pollen grains relevant to exposure. Among other things, Marsh provided accurate estimates of the quantities of allergen inhaled (approximately 10 ng/day or approximately 1 µg/year) and also demonstrated that pollen allergens elute rapidly from grains in aqueous solution (6). Similarly, purification of cat allergen by Ohman *et al.* (7) led to the first studies on airborne exposure. When the first dust mite allergen (Der p 1) was purified in 1980, it was used as the basis for an inhibition radioimmunoassay to study both the biology of the allergen and to measure exposure in homes (8, 9). Those studies established that: (i) a large proportion of the Der p 1 accumulating in cultures was in the form of fecal particles (10), (ii) Der p 1 elutes rapidly, i.e. 90% in 2 min, from the

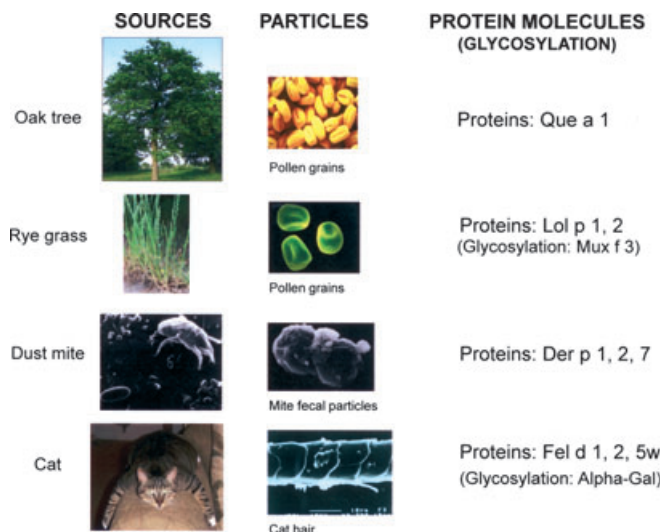


Fig. 1. Overview of allergens, sources, particles, and proteins. For all the major inhaled allergens, there is a source, the particle that becomes airborne, and a variety of molecules that are present in the particle. Although protein epitopes are the main target for IgE antibodies, there are also significant responses against glycosylated moieties on both plant-derived proteins (e.g. MUXF3) and animal-derived proteins (e.g. galactose- α -1,3-galactose).

particles in aqueous solution (10), and (iii) these particles only become airborne during domestic disturbance, and that in keeping with their size, they fall rapidly (within 10 min) (9). Thus, it became clear that mite fecal particles and pollen grains were similar in size and in the quantities of allergen 'delivered' to the nose. Each type of particle is typically 20–40 µm in diameter and contains approximately 0.2 ng of the major allergen(s) (Fig. 1).

The major allergens purified since 1960 have all turned out to be proteins or glyco-proteins (Table 1). Over the past 20 years, many allergens have been purified, defined and cloned, revealing a great diversity of proteins, as judged by sequence homology, crystal structure, or biochemical analysis of their properties (<http://www.allergen.org>) (11–13). The question is whether these molecules possess properties that are relevant to their allergenicity. First and foremost, we should consider their physical properties including molecular weight and solubility in aqueous solution. Almost all the defined inhalant allergens are freely soluble and between 10 000 and 50 000 Daltons in size (Table 1). This fits the known permeability properties of the basement membrane in the nose and suggests that delivery to dendritic cells (DCs) in the nasal epithelium may be the primary requirement (14). Moreover, it is clearly essential that the protein is 'foreign' and that it may also need to be accompanied by Toll-like receptor (TLR) ligands to activate the response (15).

Table 1. Representative allergens from grasses, weeds, domestic animals, mites, cockroaches, and fungi

Source	Name	Airborne particles	Size in microns	Allergen(s)	Molecular weight (kilodaltons)	Protein function or homology
Rye grass	<i>Lolium perenne</i>	Pollen	30–40	Lo l p 1	27	Beta expansin
Ragweed	<i>Ambrosia artemisiifolia</i>	Pollen	20–30	Amb a 1	38	Pectate lyase
Oak tree	<i>Quercus alba</i>	Pollen	20–36	Que a 1	17	–
Birch tree	<i>Betula verrucosa</i>	Pollen	20–27	Bet v 1	17	PR-10
				Bet v 2	15	Profilin
Dust mite	<i>Dermatophagoides pteronyssinus</i>	Feces	15–30	Der p 1	24	Cysteine protease
				Der p 2	15	MD-2 (secreted TLR4 helper protein)
Cockroach	<i>Blattella germanica</i>	Debris	15–40	Bla g 1	46	–
				Bla g 5	23	Glutathione-S transferase
Cat	<i>Felis domesticus</i>	Dander	2–20	Fel d 1	18	Uteroglobulin
				Fel d 2	69	Albumin
				Fel d 5w	400	Cat IgA
Dog	<i>Canis familiaris</i>	Dander	2–20	Can f 1	24	Lipocalin
				Can f 3	69	Serum albumin
Alternaria	<i>Alternaria alternata</i>	Spores	20 × 14	Alt a 1	16	–
Aspergillus	<i>Aspergillus fumigatus</i>	Spores	2 × 2	Asp f 1	18	Mitogillin family
				Asp f 5	40	Metalloprotease

We first consider the properties of the mite allergen, Der p 1. The role of Der p 1 in the digestive tract of mites was strongly suggested by identification of this protein in fecal pellets and by fluorescence microscopy showing this protein in glands around the gut (16). When Der p 1 was cloned, it became clear that this protein had extensive homology with cysteine proteases (17). Subsequently, it became clear that Der p 1 is an active protease and that this could influence the immunogenicity of the molecule in a variety of ways (18–20) (Table 2). As already mentioned, any analysis of responses to inhaled allergens has to start with dosage issues. Although the quantity inhaled is generally very low (i.e. ≤ 10 ng/day) the concentration in the particles may be very high, approximating 0.2 ng in a 20-micron sphere, which is roughly equal to 2 mg/ml. Thus, the effects of the enzyme may be highly relevant at the site of impact of the particle. By contrast, biopsy of a random area of the lung would be very unlikely to identify any changes attributable to the effects of mite exposure. In addition, the fact that

mite fecal particles contain multiple proteins (at least 14 fully defined allergens) raises the question of whether the effects of an enzyme locally would relate to that protein alone or could influence the response to other mite allergens (21).

If we next consider allergens from domestic animals, the best defined mammalian allergen source is the cat *Felis domesticus*. These enigmatic animals are present in millions of homes, and indeed in some communities, they may be present in as many as 50% of the homes. Exposure to cat allergens is dramatically different from mite or pollen allergens. Once assays for the major cat allergen Fel d 1 were established, it became clear that this protein is airborne most of the time in homes with a cat (22). In these homes, we have estimated that children can inhale as much as 1 μ g Fel d 1/day, i.e. as much as 100 times the quantity of mite or pollen allergens (23). The skin protein Fel d 1 is somewhat mysterious; although it is described as a uteroglobulin on the basis of amino-acid sequence homology, the biological role of this protein in the skin is not known. Up to 90% of individuals who are allergic to cats have made an IgE antibody response to Fel d 1.

Patients who are allergic to cats, as judged by skin tests or serum IgE antibodies, report developing nasal, eye, and lung symptoms within half an hour or less of entering a house with a cat. The assays for airborne Fel d 1 provided an explanation for this reaction (22, 23). Fel d 1 is airborne on particles that are aerodynamically 'smaller' than mite particles or pollen grains. In keeping with that, they remain airborne or float for long periods of time with little or no disturbance of the air in a room (24). This of course is a large part of the explanation for the high levels of overall exposure but also explains why symptoms often develop rapidly. The other important feature of cat allergens is that the dander particles appear to be 'sticky'

Table 2. Biological and adjuvant activity present in fecal particles: allergens and PAMPs

- I. Der p 1: is an active cysteine protease, which can cause
 - (i) Increased permeability of the epithelial barrier secondary to enzymic digestion of tight junctions.
 - (ii) Cleavage of lymphocyte surface receptors, including the IL-2 receptor (CD25) and the low affinity receptor for IgE (CD23)
 - (iii) Digestion of other proteins as well as Der p 1 itself producing fragments with altered allergenicity.
2. Der p 2: is a homolog of the adapter protein MD-2 and can facilitate LPS-mediated signaling through TLR4.
3. Pathogen-associated molecular patterns (PAMPs) and their targets
 - (i) Mite DNA – unmethylated; can act on TLR9
 - (ii) Bacterial DNA – unmethylated; can act on TLR9
 - (iii) Endotoxin – ligand for TLR4
 - (iv) Chitin – C-type lectins (and/or FIBCD1)

and are easily transferred on human clothing to homes, schools, or other public buildings where there are no cats. Indeed, it is common to measure cat allergen in the floor dust, as well as airborne, in houses without a cat (23, 25, 26). Some of these estimates suggest that there is as much airborne Fel d 1 in homes without a cat as there is airborne mite allergen in homes that are infested with mites (23). As we discuss later, the evidence about airborne cat exposure is highly relevant to understanding immune responses to cat allergens.

Factors influencing prevalence of sensitization and IgE antibody levels: 'what makes the best allergen?'

Many or most forms of immunization do not induce persistent or high titer IgE antibody responses. Thus, most viral or bacterial infections, and all successful vaccines, do not induce IgE antibodies. As an example, it is safe to re-immunize a large population of children with diphtheria and/or tetanus toxoid 1, 3, or 10 years later, without significant risk of inducing anaphylaxis. Equally, attempts to demonstrate IgE responses to influenza or rhinoviruses have generally not resulted in any positives. For respiratory syncytial virus (RSV), although early studies reported some positive IgE responses, subsequent studies failed to confirm those findings (27, 28). Similarly, if we consider food antigens, the normal reaction to food proteins such as milk, egg, and wheat often includes IgG antibodies, but only in a small minority of individuals (approximately 2%) is there also an IgE antibody response.

In early animal experiments, it was clear that the optimal immunization strategy to induce IgE antibody responses was low dose (i.e. submicrogram) antigen in alum (29, 30). By contrast, using antigen with complete Freund's adjuvant (CFA) produces high titer precipitating IgG antibody responses without detectable IgE antibodies (31, 32). This appears to be true for all animals tested, i.e. rabbits, rats, goats, and mice. The question then is what feature of the immune response to protein in CFA is so effective at preventing an IgE antibody response? High dose immunization in CFA or an equivalent adjuvant is considered to be the optimal technique for inducing germinal center (GC) production in lymph nodes draining the site of injection. The question then is whether there is a specific reason why GCs should not be good for IgE production. In 2005, Aalberse and Platts-Mills published an hypothesis that the primary route for long term IgE production (most commonly to inhalants) was switch from B cells bearing IgM (B μ) to B ϵ occurring outside GCs, giving rise to plasma cell precursors that home to protected sites in the bone marrow (33, 34). It was argued that the

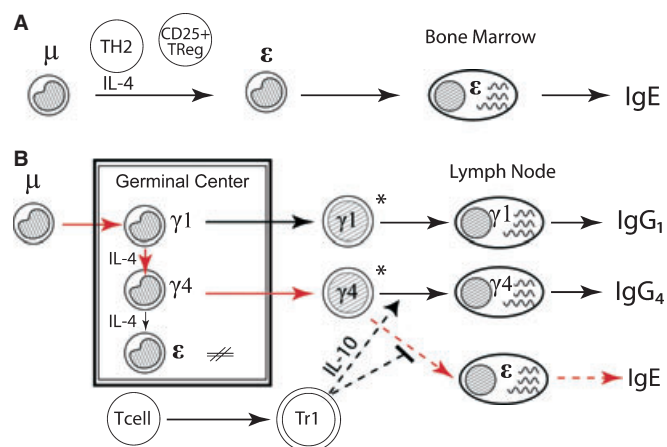


Fig. 2. There are at least two possible routes to IgE production. (A) Repeated low dose immunization can induce switching of B cells from μ or γ to ϵ with subsequent prolonged production of IgE by plasma cells localized to protected sites in the bone marrow. (B) With higher dose immunization, sequential switch from μ to $\gamma 1$, to $\gamma 4$, to ϵ can occur within a germinal center. However, IgE memory B cells are not protected from apoptosis and there are very few IgE B cells in the circulation. IgG4 memory B cells can transform to IgG4 plasma cells enhanced by IL-10 or transform to IgE B cells and plasma cells. The latter route to IgE production requires IL-4 and is inhibited by IL-10.

problem with GCs was that B cells that switch to ϵ were not protected from apoptosis. This appears to be because the membrane portion of IgE is defective and does not transmit the signals necessary to protect B cells from apoptosis (Fig. 2). This model suggests that there are two routes to IgE production: (i) low dose immunization inducing switch to IgE outside GCs, and (ii) switch from IgM $\rightarrow$ IgG $\rightarrow$ IgG4 inside GCs with subsequent switch from IgG4 to IgE production in the periphery. Saxon et al. (35), some years ago, provided evidence that the switch to IgE could occur sequentially through IgG4. The important feature of this hypothesis is that a 'good' allergen is one that can induce an IgE antibody response without generating GCs. However, it is obviously not simply the nature of the proteins that matters, but the size of the particles and the possible adjuvant activity of other substances in the particles are equally important.

It is important to recognize that the three dimensional structure of proteins within a family may remain very similar for hundreds of millions of years although the amino acid sequence and function has changed extensively (Fig. 3). We focus our discussion on three of our 'favorite' allergens: grass pollen, dust mite feces, and the airborne dander derived from cats.

Grass pollen

The properties of windborne pollen reflect their biological role. They are released when drying conditions favor their dispersal,

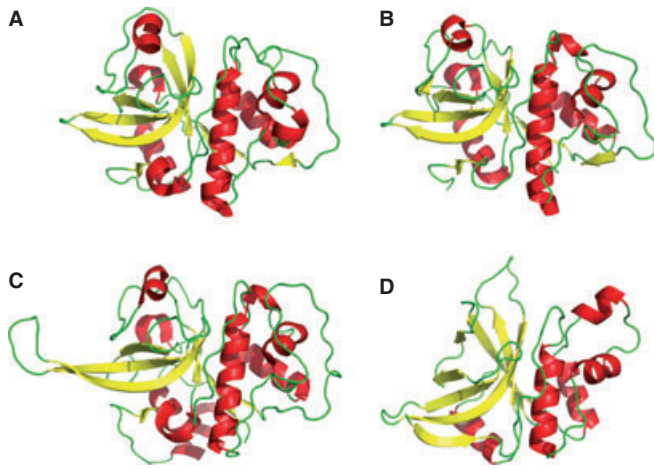


Fig. 3. Molecular structure of proteins in the same family as Der p 1. (A) Der p 1 (dust mite cysteine protease), (B) human cathepsin K, (C) falcipain-2 from *Plasmodium falciparum*, and (D) thiol proteinase from *Staphylococcus aureus* V-8. Images by and with permission of Maksymilian Chruszcz at the University of Virginia.

they are of a size that allows them to be carried by the wind, and on contact with a wet surface, e.g. a stamen, they rapidly release recognition proteins that can specifically trigger the production of a pollen tube. These same features are relevant to human IgE antibody responses and to the clinical features of allergic rhinitis (3). While some of the proteins released by pollens are species specific, there is also extensive cross reactivity within both grasses and weeds. Thus, the major allergen of rye grass, Lol p 1, cross reacts extensively with other grasses. The allergens are named according to an IUIS nomenclature (36). The first allergen described from *Lolium perenne* is Lol p 1 (see <http://www.allergen.org> for full lists of defined allergens and the correct nomenclature). It is important to note that structural homology between groups of allergens such as the Bet v 1 superfamily, or the family including Der p 1, can be present even when there are only low degrees of amino acid sequence homology ($\leq 20\%$) (Fig. 3). However, immunological cross reactivity, as judged by IgE antibody binding, is normally not present until amino acid homology approaches 65%.

For many of the grasses, there are now >10 allergens that have been defined and cloned. More recently, elegant tertiary structures have been defined for many different pollen allergens, from which it is may be easy to identify cross reactivity. In 1900, it was considered that pollen was toxic. Indeed, when Noon developed immunotherapy in 1911, he thought that he was creating immunity to pollen toxins (37). It is of course likely that pollen constituents have the ability to stimulate a range of pattern recognition receptors (PRRs). However, innate responses to pollen constituents have not been extensively studied. At the time of the original work on IgE,

Ishizaka and others recognized the presence of IgG and IgA antibodies to pollen allergens. That work was done primarily with antigen E from ragweed purified by King (5). Antigen E (now correctly called Amb a 1) and Lol p 1 were used extensively to measure serum and nasal antibodies in patients with hay fever (38). The results provided clear evidence that the patients mounted both local and systemic IgG and IgA antibody responses, as well as an IgE response (39).

In many countries where grass grows well, e.g. New Zealand, northern California in the US, and the UK, up to 30% of the population will have positive skin tests and/or serum IgE antibodies to pollen allergen. Furthermore, in areas with the highest pollen counts, the titers of IgE antibodies to grass pollen may be very high. Indeed, in northern California, we have shown a close correlation between the titer of IgE antibody to grass in IU/ml and the total IgE in IU/ml (40). This strongly suggests that the specific IgE response is a major determinant of total serum IgE in that area. By contrast, in areas where pollen growth is poor, the prevalence of sensitization and the titers of IgE to grass may be very low (<5%). The important conclusion here is that higher exposure leads to progressive increases in both prevalence and titer of IgE antibodies to pollen allergens.

All the data on the pollen-related diseases, hay fever and seasonal asthma, relates to IgE antibodies specific for protein antigens. However, it has been known for some time that there are also IgE antibodies directed against oligosaccharides on plant proteins (41, 42). These oligosaccharides can be responsible for cross reactivity, and they have been called cross reactive carbohydrate determinants (CCDs). However, for reasons that are not well defined, CCDs derived from plants do not appear to contribute to allergic symptoms.

Dust mites

In 1967, Spieksma and Voorhorst (43) demonstrated that the dust mite *Dermatophagoides pteronyssinus* (roughly translated as skin eating and feather loving) was a major source of allergens in house dust. That finding opened up a large area of research on the relationship between perennial indoor allergen exposure and asthma (44). Equally, the ability to accurately measure the major allergen Der p 1 made it possible to study the influence of exposure on the development of disease (44, 45). The fecal particles were known to contain high concentrations of several allergens including the cysteine protease Der p 1. However, not surprisingly, the fecal particles also contain mite DNA, bacterial DNA, endotoxin, and chitin (Table 2). As discussed previously, there are good reasons for considering

that the enzymatic activity of Der p 1 would influence the immunogenicity of these allergens. There have also been excellent studies carried out on the properties of chitin and Der p 2 (46, 47). It is important to note that both bacterial and mite DNA are unmethylated. However, the real conclusion is that mite fecal pellets contain a wide range of substances, including the allergens themselves, that could act as adjuvants by triggering innate signaling pathways including TLR4 and TLR9 (47, 48). In relation to this point, Der p 2 has recently been shown to bind to TLR4 and exert an 'autoadjuvant' effect, owing to its ability to mimic the lipid binding component of the receptor, MD-2 (47).

In many areas of the world, dust mites are the dominant allergen associated with asthma, as judged by the prevalence of positive skin tests (49–51). However, there is even stronger data based on high titer serum IgE antibodies. In New Zealand, the UK, south eastern USA, and also in Costa Rica, serum IgE antibodies greater than class 3 (or 3.5 IU/ml) have been strongly associated with asthma (52). There are other areas of the world and communities where mite sensitization is uncommon and not related to asthma. These include not only areas where mites do not survive because of low humidity (e.g. Northern Sweden, Los Alamos, New Mexico, and high rise apartments in Chicago) but also tropical countries where allergic asthma is absent apparently because of 'poor hygiene'. Thus in rural areas of Ethiopia, Kenya, or Ecuador, there is a low prevalence of asthma, no association with dust mite sensitization, but perhaps most strikingly almost no sera with high titer IgE antibodies to mite (53, 54). In these areas, it is not unusual to have high total serum IgE levels >1000 IU/ml. Thus, in most of these tropical situations, IgE antibodies to mite represent a very small percentage of the total (54–56). By contrast, in temperate countries or tropical countries where basic standards of hygiene have been established, total IgE levels are lower and IgE antibodies to mite in the sera of patients with asthma often represent $\geq 10\%$ of total IgE (55, 57).

Cat dander

The importance of cat allergens to rhinitis and asthma has been obvious for over 100 years. However, the data on cat allergens is not nearly as straightforward as that for pollen or dust mites. In houses with a cat, the quantity of cat allergen inhaled per day can approach estimates of pollen allergens inhaled per year. Despite this high exposure, in countries with 'high' exposure to pollen, mite, and cat allergens, the prevalence of sensitization to cats is lower than that for mite or grass pollen (49, 57). Thus, we could consider that cat

allergens are less successful or less immunogenic than other allergens. On the other hand, in most studies, the presence of IgE antibodies to cat or positive skin tests is strongly associated with asthma.

Homo sapiens has only been separate from the other mammals for 65 million years and, consequently, many proteins derived from cats and other mammals still have extensive homology with human proteins. Thus, Spitzauer (58), when discussing the allergenicity of foreign proteins, referred to mammalian proteins as 'at the borderline between foreign and self'. By contrast, humans have been separate from birds for 300 million years (Fig. 4). It is partly for this reason that chickens are used to make monoclonal antibodies to those human proteins that are only weakly immunogenic in mice (59). The proteins of mites and grasses, not surprisingly, are completely foreign to the human immune system, since human has been separate from those groups for 600 million and 1500 million years, respectively (Fig. 4).

The protein that is the major allergen of cats, Fel d 1, is highly conserved throughout the cat family but has little or no homology with other mammalian proteins. This of course may be the reason why it is such an important or dominant allergen. In many studies, the quantitative correlation between IgE antibodies to an extract made from cat dander and IgE

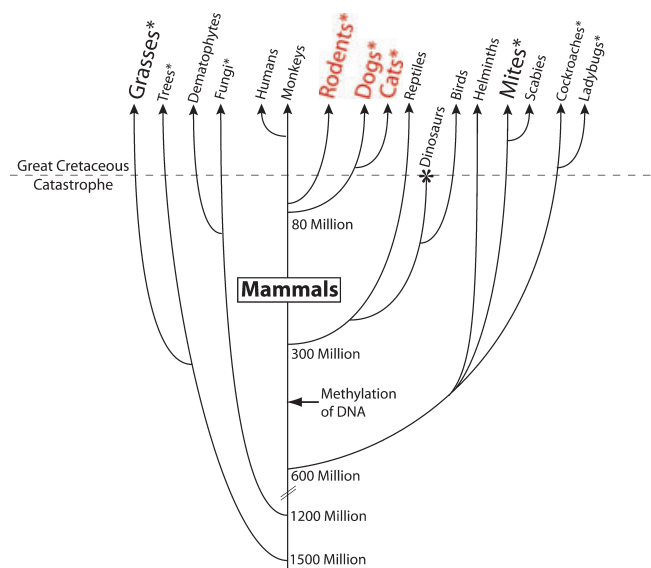


Fig. 4. Evolutionary distance and immune or allergic responses to foreign proteins. Most protein families were established before plants and animals diverged [i.e. about 1500 million years ago (MYA)]. With a fairly predictable 'speed,' these proteins change amino acid sequence, so that species separated by more than 100 MYA generally have very little (i.e. <20%) amino acid sequence homology. By contrast, many proteins of mammals show considerable homology with human proteins and thus, may be less immunogenic. The data and model were modified from figures in *The Ancestors Tale* (Au: Richard Dawkins).

Table 3. Three clinical syndromes in patients with positive serum IgE assays for 'cat': relevance of specific IgE to three different proteins

IgE antibody specificities	Cat allergic asthma		Pork/Cat Syndrome*	Delayed anaphylaxis to red meat†
	i	ii		
Cat extract	130	55	3.87	62
Fel d 1	110	23	<0.35	<0.35
Cat albumin	<0.35	12	4.18	<0.35
Cat IgA	<0.35	<0.35	<0.35	6.0
Alpha-gal	<0.35	<0.35	<0.35	52
Pork	<0.35	6	2.3	13
Asthma	Yes	Yes	No	No
Allergic reactions to pork or beef	No	No	Yes (in 30 min)	Yes (in >3 h)

Values that indicate the primary features of each response are shown in bold.

*Cross-reactivity of protein epitopes on different mammalian albumins.

†Cross-reactivity of IgE to the oligosaccharide galactose- α -1,3-galactose (α -gal). Data from S.P. Commins, L.A. Kelly, H.R. James, E. Ronmark, and T.A.E. Platts-Mills (in preparation).

antibodies to Fel d 1 is as high as 0.9 (60). However, there are other significant proteins in cat extract that can be the target of IgE antibodies. Two of these, cat albumin (Fel d 2) and cat IgA (Fel d 5w) are interesting because: (i) they illustrate different patterns of cross reactivity, and (ii) the route of primary exposure may be different. If a child lives in a community where cats are common and makes an IgE antibody response to Fel d 1, it is always assumed, almost certainly correctly, that this response was induced by inhaling particles of cat dander carrying Fel d 1. On the other hand, if a patient's serum has IgE antibodies to albumin that cross react with cat, dog, beef, and pork albumins, it may not be clear which exposure caused the primary sensitization (Table 3). The situation for cat IgA is more complex, because most of this IgE is directed against the oligosaccharide galactose α -1,3-galactose (α -gal) (61). Recently, we became aware of this response because of cases in the USA that presented with anaphylaxis (62, 63). In these cases, serum assays are generally positive for IgE antibodies to cat, dog, cow's milk, beef, and pork, i.e. mammalian proteins that carry α -gal (63, 64). However, it seems unlikely that any of these induced the development of IgE. In addition, these patients do not experience inhalant symptoms on exposure to cats or dogs (65). There is now extensive evidence that this IgE response to α -gal is induced by the bites of the tick *Amblyomma americanum* (66) (Table 3).

A house with a cat accumulates large quantities of cat allergen in carpets, furniture, etc., as well as on the cat itself. This allergen is easily measurable both in dust samples and airborne (22–24). However, it is also possible to detect cat allergen in homes without a cat, particularly if there is direct movement from houses with a cat to the house without a cat. In addition, cat allergen can be detected in schools and other public places. Thus, it is not surprising that children raised in houses without a cat may become allergic. In 1999, Hesselmar

and Bjorksten (67) reported data showing that Swedish children who lived in a house with a cat were less likely to be allergic to cats, as judged by skin tests. Initially, this report was received with astonishment; however, over the next few years the basic observation was confirmed by other groups and in several different countries (68–71). Two of these studies provided further insight into the phenomenon. In studies of Middle School children in the USA, it was clear that many of the children who had high exposure at home (i.e. cat in the house) had made IgG antibodies to Fel d 1, even if they were skin test negative and had no detectable IgE antibodies (68). In addition, we found that both the allergic children and the 'tolerant' children had made IgG4 antibodies to Fel d 1 (68). Analyzing the results for a birth cohort in Detroit, Ownby et al. (69) found that children who had been brought up in a house with more than one animal were not only less allergic but had better lung function than children who had lived in a house without animals. Recently, the same cohort has been analyzed at the age of 18 years. That analysis suggested strongly that the first year of life was critical for the 'tolerance' inducing effects of cat ownership (72). In that study, children who had a cat in the house for the first year of life were less than half as likely to be allergic to cat allergens at age 18 years.

Over the last 10 years, there have been multiple studies on the relevance of cat or dog ownership to specific sensitization. The results have not been consistent, in that many show decreased sensitization among children raised with an animal, while others do not (73, 74). However, careful analysis of the results on children over age 5 years show that very few of the studies have reported increased sensitization with cat ownership (67–74). Thus, there is not a simple relationship of increased exposure leading to increased sensitization. Furthermore, we have consistently confirmed the high prevalence of

IgG (and IgG4) antibodies to Fel d 1 among children raised in a house with a cat, at least half of whom do not have detectable IgE antibody production. In addition to the data on prevalence, we also reported that the titers of IgE antibodies to cat allergens are lower than those for mite (57). We consider that the analysis we published in our 2002 editorial is a correct representation of the data (75). Furthermore, since the IgG4 isotype is fully dependent on the Th2 cytokine IL-4, we believe that the tolerant state induced by high exposure to cat allergens is best described as a 'modified Th2 response' (68).

Other routes of allergen exposure

One of the traditional assumptions about allergic disease is that the route of exposure that causes symptoms is a good guide to the route that gave rise to sensitization. Thus, we tend to assume that a patient with seasonal rhino-conjunctivitis caused by exposure to airborne pollen grains was also sensitized by inhaling pollen. Equally, it is generally assumed that IgE responses to the major food allergens in childhood are induced by eating those foods. However, in most cases, the sensitization phase occurs over a period of months or years, and it is impossible to exclude a role for antigenic proteins entering the body by another route. In addition, there are well-documented cases of inhalant symptoms occurring secondary to food allergy, and cases of food allergy occurring secondary to an assumed inhaled sensitization. For example, there are patients who become sensitized to proteins in duck eggs and who subsequently experience inhalant symptoms on exposure to ducks (76). Alternatively, anaphylactic reactions have been reported after eating mite-infested flour in a patient with sensitization to inhaled mite allergens (77, 78). In general, inadequate attention has been paid to alternative routes of exposure. The two most obvious routes are transdermal and sublingual.

Intracutaneous or transdermal exposure

The fact that proteins applied to the skin can induce inflammation typical of eczema has been clear for many years. Exposure of the skin to allergen extracts or purified proteins can cause local infiltration of eosinophils and basophils as well as histological eczema (79, 80). It is also likely that permeability of the skin in eczema is responsible, at least in part, for the very high levels of IgE and IgE antibodies seen in patients with atopic dermatitis (AD) (81, 82). Equally, there are excellent models in mice of sensitization occurring through the skin. Spergel et al. (83) reported that transdermal immunization could sensitize the lungs. In 2003, Lack et al. (84) reported a

striking association between the use of ointment containing peanut antigen and the development of sensitization to peanut. It is obvious that stinging insects such as honey bees, yellow jackets, jumping ants, and fire ants can all induce IgE antibody responses following exposure through the skin. However, it is important to recognize the difference between 10 µg of phospholipase A₂ (PLA₂) injected in venom, which could induce a response systemically, and fire ant stings that produce an intense local response in the skin, which may be followed by sensitization and IgE antibody production. Two recent developments have strongly supported transdermal exposure as an important route for primary sensitization.

First, the investigations of food allergy in London found that the avoidance of oral peanut exposure was not an effective method of preventing sensitization to peanut, unless all peanuts and peanut products were excluded from the home. They proposed that early exposure through the skin could be an important route for sensitization, if it occurred before the onset of oral exposure (85). Second, as discussed earlier, we have recently reported that tick bites, particularly those of *Amblyomma americanum* (the lone star tick), can induce high titer IgE antibodies to galactose α -1,3-galactose (66). What is relevant here is that this response correlated best with pruritic responses at the site of the tick bites that lasted for weeks or months (66). The implication is that an inflammatory response in the skin may be highly relevant to the production of IgE antibodies.

Sublingual and oral: a major element in the 'balance' between sensitization and tolerance

That the oral route of exposure usually induces tolerance is obvious, because we all eat a wide range of foods regularly and 95% of the population does not experience allergic reactions. These foods include many different proteins, e.g. ovalbumin, which is perfectly capable of inducing sensitization and IgE antibody production if introduced to the body by a different route. In 1965, Geoffrey Asherson (86) coined the term 'immune deviation' to describe the effects of oral exposure on subsequent attempts to induce contact sensitivity to dinitrochlorobenzene. The remarkable feature of those experiments was that oral exposure of mice to the chemical would induce prolonged tolerance to exposure by the transdermal route. Evidence that similar mechanisms apply in man comes from a Danish report that girls who had braces on their teeth (oral nickel exposure) before their ears were pierced were less likely to develop contact sensitivity to nickel (87).

We have already mentioned the evidence that peanut exposure through the skin occurring prior to oral exposure could be an important cause of sensitization. However, similar differences in the sequence of exposure could be relevant to a wide range of foods. There are not many foods that become as widely distributed on surfaces in a house as peanut butter does. The question that follows from this is whether oral exposure to an inhalant allergen could induce tolerance to subsequent exposure through the respiratory tract. The obvious example is cat, where the allergen is present all over the house and is inevitably licked and ingested by an infant. Thus, it is perfectly possible that high oral exposure to cat allergens in the first year of life explains the effects of early life exposure to a cat in the house. Interestingly, this could provide a model for inducing tolerance prior to sensitization. Several studies are currently underway using oral or sublingual drops in early childhood to test whether this approach can reduce sensitization.

Thousands of patients, particularly in Europe, are now receiving allergen-specific immunotherapy in the form of sublingual drops (88, 89). There is extensive evidence that the sublingual route is more effective than allergen swallowed directly (90). In addition, recent studies suggest that there are important features of DCs in the oral/sublingual mucosa that may confer tolerance following allergen encounter (91). However, the mechanisms in already sensitized patients are unlikely to be the same as in a naive infant. Although sublingual immunotherapy can decrease allergic symptoms, this is not likely to be the same as inducing tolerance through natural exposure in early childhood (88, 89). Moreover, there is no evidence that the changes achieved by the sublingual route in a sensitized individual persist any longer than those achieved with traditional subcutaneous immunotherapy. It is clear that there is an urgent need to understand the mechanisms of oral/sublingual tolerance.

Environmental allergens as a trigger for Th2 responses

In the last two decades, much has been learned about the immune mechanisms that underlie allergic sensitization. Molecular cloning and sequencing of allergens provided a major impetus in this regard. In the following sections, we elaborate on what we have learned about how allergens impact the immune system at the cellular level. Allergic sensitization is a manifestation of immune processes mediated by pathogenic Th2 effector cells. These cells secrete a variety of cytokines, most notably IL-4, IL-5, and IL-13, which play a pivotal role in the recruitment of inflammatory cells to anatomic sites of allergen exposure, and contribute to the

development of bronchial hyperreactivity and mucus secretion. Importantly, IL-4 induces IgE class switching and thus is pivotal to IgE-mediated mast cell activation and the resulting 'pro-inflammatory' sequelae.

Despite what we have learned, it is still puzzling how an assortment of such diverse allergen molecules preferentially induces a Th2 response. The T-cell response is influenced by complex gene/environment interactions that are discussed in detail elsewhere (92, 93). This section will focus on T cells from the allergen perspective. As already discussed, there are a variety of allergen properties to consider including protein sequence, structure, and function, and secondary modifications such as glycosylation. Beyond these, are the numerous 'contaminants' that associate with these molecules. These factors combined endow each allergen with its own unique attributes.

Allergens as ligands for pattern recognition receptors

DCs play a pivotal role in the initiation of Th2 responses to allergen (94). These cells possess an array of surface receptors that influence DC function and thus T-cell outcomes (Fig. 5). Among these are TLRs that recognize molecular patterns derived from microorganisms. The hygiene hypothesis states that microbial exposure is protective for the development of atopy. From its inception, this theory implied that microbes could inhibit allergen-induced Th2 responses, possibly through a DC-mediated pathway. While this idea was substantiated in mice co-exposed to allergen with lipopolysaccharide (LPS), it was apparent that the dose of bacterial products was critical to the T-cell outcome. At high dose, LPS resulted in a Th1 response. By contrast, at low dose, LPS favored Th2 induction, and LPS was actually required for the development of Th2 responses to inhaled allergen through TLR4 signaling (15). The first direct evidence that an allergen itself could trigger TLR signaling came from the observation that the dust mite allergen Der p 2 acts as a functional mimic of MD-2, the LPS binding component of the TLR4 signaling complex (95). New ligand-binding sites continue to be identified within allergens that could also facilitate TLR signaling (96, 97). Thus, by virtue of their structural properties, some allergens are inherently 'dirty' and have the capacity to activate DCs through innate pathways.

Carbohydrate receptors also facilitate binding of diverse allergens to DCs. Among these, the C-type lectin receptors dectin-2, mannose receptor (MR) and DC-SIGN have been implicated in uptake of allergens from a variety of sources including house dust mite, pollen, dog, cockroach, and

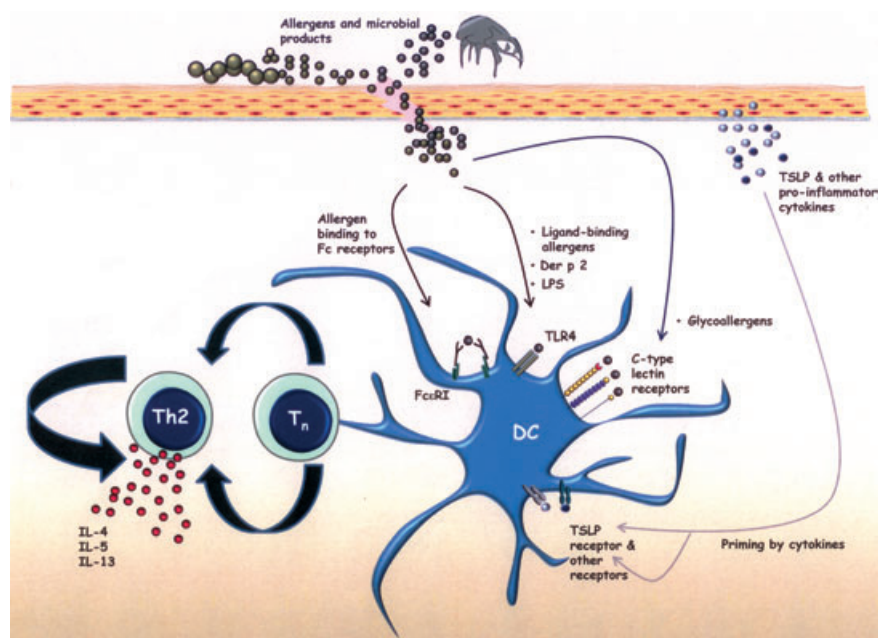


Fig. 5. Allergens license dendritic cells to induce Th2 responses. Allergens from diverse sources have the capacity to condition dendritic cells for Th2 induction by engaging an array of surface receptors. This may occur directly through Toll-like receptors and C-type lectin receptors, or else indirectly through cross-linking of Fc receptors by allergen/antibody complexes. FcεRI, high affinity IgE receptor.

peanut (98–101) (Fig. 5). Several lines of evidence point to a role for lectin receptors in the generation of Th2 responses to allergen. Studies which involved silencing of MR gene expression in human monocyte-derived DCs demonstrated the capacity to reverse Th2 cell polarization driven by Der p 1 (98). This appeared to involve upregulation of the activity of indoleamine 2,3-deoxygenase, an enzyme that has been implicated in Th skewing. Other work in human DCs reported activation of the DC-SIGN signaling components Raf-1 and ERK kinases triggered by Bermuda grass pollen allergen and concomitant production of TNF- α (99). Similar studies using soluble peanut antigen observed marked induction of DC maturation markers on monocyte-derived DCs by glycosylated but not deglycosylated preparations. Moreover, the purified peanut allergen Ara h 1 but not its deglycosylated counterpart induced Th2 skewing of naive T cells (100). In those studies, the capacity for Ara h 1 to bind DC-SIGN and for this receptor to mediate allergen uptake was confirmed. The findings implied that Th2 differentiation could be induced by a single purified allergen in a glycan-dependent manner.

Protein sequence and the T-cell response to allergens

In the last few years, there has been less emphasis on addressing if and how the amino acid sequence of allergens is relevant to Th2 induction. It is clear that allergens from diverse sources

contain immunodominant T-cell epitopes that are recognized by atopic and non-atopic subjects with HLA-diverse haplotypes (102). Early on, many studies failed to identify differences in patterns of T-cell epitope recognition or cytokine responses directed against these epitopes among allergic and non-allergic subjects. However, studies using rigorous experimental design and analyses performed in the context of specific HLA haplotypes indicated that differences do exist at the T-cell epitope level (103–105). Some major T-cell epitopes appear to be protective, as judged by T-cell stimulatory capacity or induction of the regulatory cytokine IL-10. For certain allergens, such as the cat allergen Fel d 1, cytokine responses to immunodominant epitopes were found to segregate within the molecule, regardless of atopic status. Notably, the amino-terminal region of polypeptide chain 2 preferentially induced the regulatory cytokine IL-10 in cultures from both allergic and non-allergic subjects with diverse HLA types. By contrast, IL-5-inducing epitopes localized to chain 1 (105). Moreover, cell cultures from cat-tolerant subjects who were DR7⁺ produced high levels of IL-10 in response to a chain 2 epitope that contained a predicted HLA-DR7 binding motif. Collectively, these findings supported an association between the intrinsic properties of Fel d 1 and the immune response to this allergen. The implication was that T cells directed against chain 2 were present at a higher frequency in individuals with tolerance to cats as compared with cat-allergic subjects. A key question is

whether such intrinsic properties are a feature of other allergens. If we consider the important mite allergens Der p 1 and Der p 2, only a few well-designed T-cell epitope mapping studies have been performed and information is limited. (106).

Until recently, it was not possible to fully assess the relevance of the protein sequence to the T-cell response. With the advent of peptide/MHC tetramer technology, it is now feasible to analyze allergen-specific T cells directed against specific epitopes in subjects with defined HLA types and known atopic status (107, 108). Approaching this from another angle, allergen sequence variants can now be engineered relatively easily for T-cell studies. Recent work suggests that single amino acid substitutions within the cockroach allergen Bla g 2 can drastically alter the T-cell response (A. Pomes & J.A. Woodfolk, personal observations).

Perhaps the strongest evidence of a role for protein sequence in the allergic response in humans comes from *in vivo* studies involving intradermal injection of allergen peptides in cat-allergic asthmatics. In those studies, peptides administered through the skin exacerbated asthma, pointing to a key role for Th2 cells with defined TCR specificities in disease pathogenesis (109). The implication is that injection of a single peptide can efficiently activate memory Th2 cells in the lung in subjects who are already cat-sensitized. However, these experiments tell us nothing about what role the injected peptide might play, if any, in Th2 induction. In relation to this point, there is convincing data in mice that a single epitope of Der p 1 is sufficient to induce all the cardinal signs of asthma following airway sensitization. Notably, this occurred in the absence of systemic priming or adjuvants (110). Thus, the intrinsic features of different allergens may be critical to Th2 initiation as well as driving Th2 responses in established disease.

A key question is how might a specific peptide preferentially induce a Th2 response? Factors to consider include: (i) the nature of the epitope generated during antigen processing, (ii) the resulting affinity of this peptide for a given MHC class II molecule, (iii) the density of the peptide/MHC complexes on the surface of the antigen presenting cell, and (iv) the avidity of the peptide/MHC/TCR interaction. These issues have been widely debated in the literature and remain somewhat controversial. Features of the host are also relevant including inherent differences in the T-cell repertoire resulting in the presence or absence of T cells with defined TCR specificities as well as genetic factors such as HLA type. Adding to the complexity, it is likely that the contribution of the protein sequence to the T-cell outcome is weighted differently according to the particular allergen. Thus, when considering cat and

dust mite, the propensity to induce different T-cell responses could be explained in part by involvement of carbohydrate receptors versus TLRs, respectively. For the glycoprotein Fel d 1, carbohydrate receptors may not only impact the DC through signaling pathways triggered by allergen binding but also could influence the epitopes generated, by enhancing allergen uptake and facilitating presentation of specific T-cell epitopes at high density at the APC surface. Such a scenario could favor tolerance induction. By contrast, the dust mite allergen Der p 2 may favor Th2 induction through triggering of the TLR4 pathway. A final consideration is that some allergens, such as Der p 2, could in theory co-ligate carbohydrate and TLR receptors. How convergence of these pathways would influence DC function and the resultant T-cell response to allergens is worthy of further investigation.

Allergens and thymic stromal lymphopoietin

Thinking beyond DCs, epithelial cells provide a critical barrier between the host and the environment. These cells are the first site of allergen encounter, and as such, are pivotal to immune outcome. Ligation of surface receptors including TLRs, as well as mechanical injury, results in the release of an array of pro-inflammatory cytokines from epithelial cells (111, 112). Among these, the IL-7-like cytokine thymic stromal lymphopoietin (TSLP) has garnered much interest. In humans, this cytokine induces differentiation of Th2 cells from naive T cells through a DC-mediated pathway reported to involve OX40/OX40 ligand (113). Animal models strongly support a key role for TSLP in the development of allergic disease. Mice engineered to express TSLP in the skin spontaneously develop eczematous lesions, while in an asthma model, animals lacking the TSLP gene or its receptor show attenuated disease (114–116). Further evidence of a role for TSLP in allergic disease comes from increased levels of TSLP expression in skin lesions in atopic dermatitis (AD), as well as in bronchial epithelial cells derived from the asthmatic lung (111, 117–119). Collectively, these findings raise the question of whether local antigen is actually necessary for initiating the Th2 response. Work in mice indicates that full development of airway inflammation requires both TSLP and antigen, pointing to an adjuvant effect of TSLP (120). Consistent with this notion, work in humans showed that Th2 responses were markedly amplified when DCs were co-primed with TSLP and allergen, as compared with either stimulus alone (121, 122). This process involved upregulation of TSLP receptor and enhancement of the TSLP pathway mediated by allergen binding to Fc γ RI on DCs. Such

observations could also be relevant to amplification of Th2 responses in established disease through the effects of allergen binding to the high affinity IgE receptor, FcεRI (Fig. 5). It is tempting to speculate that other surface receptors on DCs that are triggered by allergen can also act in concert with TSLP to induce Th2 responses to allergens.

Other influences on the quality of the T-cell response to allergens

When considering the complex makeup of allergens, it is evident that multiple factors contribute to Th2 responses and that these strike a delicate 'balance', depending on such subtleties as adjuvant type and dose as well as the mode and type of receptor signaling pathways triggered. It should be stressed that while Th2 responses play a key role in the development of allergic disease, pathogenic responses to allergens are more complex than a 'classical Th2' response and that the nature of the T-cell response to allergen differs depending on the type and stage of disease. A possible role for other effector T-cell subsets including Th9 and Th17 cells continues to emerge. Furthermore, Th2 cells themselves constitute a heterogeneous population. As an example, allergen-induced Th2 cells associated with anaphylactic food allergy to peanut and those with eosinophilic gastrointestinal disease are distinguishable based on whether or not they express IL-5 (123). Thus, the nature of the T-cell response to allergen is highly heterogeneous.

Site of exposure and allergen dose

Key elements in driving a T-cell-mediated inflammatory disorder are the site of allergen exposure and the dose. These factors are not mutually exclusive, and their influence on driving allergic sensitization versus tolerance has already been discussed. Here we focus on how these factors translate to T-cell/DC behavior. When considering natural exposure, the dose of allergen received will depend on the efficiency of allergen delivery to APCs, which is directly related to the route of exposure. Based on the central role for DCs, it follows that the milieu in which these cells encounter allergen will also be important for determining the quality of the T-cell response that is generated. Thus, the site of allergen encounter and the types of DCs that migrate to or reside at these sites are each important considerations.

Anatomic sites relevant to allergic disease include the skin, respiratory tract, mouth, and gut. Exactly how much contribution each site plays and how each are connected remains unclear in humans. As already suggested, exposure through

the skin is likely an important route of T-cell priming for 'obvious' allergens (e.g. the major bee venom allergen PLA₂), as well as for less obvious allergens such as mite and peanut. Recent work implicates a role for Th17 cells in driving lung disease following sensitization through the skin (83, 124). Such a phenomenon could explain the high prevalence of asthma among patients with eczema. In patients with this disease, the skin provides a microenvironment that is excellent for activating DCs and driving T-cell responses to allergen. Many AD patients suffer from bacterial superinfections that may arise from physical defects in the skin barrier. Loss-of-function mutations of a gene encoding the structural protein flaggrin play a pivotal role in compromising the skin's integrity in this disease (125). This factor, coupled with an acquired deficiency in anti-microbial peptides, sets the stage for DC activation through multiple surface receptors (125, 126). Thus, it is not surprising that different types of effector T cells have been identified in lesional skin, including Th1, Th2, and Th17 cells (127–129).

As already discussed, high level exposure to cat allergen in the environment is associated with development of a modified Th2 response to cat allergen. It is tempting to speculate that high dose exposure is sufficient to induce tolerance through ingestion or else absorption through the oral mucosa. Indeed, 'pro-tolerogenic' sites have been identified in the oral cavity that are populated by T cells that preferentially express TGF-β, IL-10, IFN-γ, and IL-17, as well as DCs expressing TLR2 and TLR4 (130). Studies in patients receiving sublingual immunotherapy (SLIT) also support the view that exposure to allergens in the oral cavity favors tolerance induction mediated by resident APCs. For example, uptake of the major grass pollen allergen Phl p 5 by oral Langerhans cells increases their production of TGF-β1 and IL-10 and decelerates their maturation, consistent with a tolerogenic phenotype (91). Extending this further, SLIT using house dust mite extract has been reported to increase the presence of circulating regulatory T cells with suppressor function (131). While TLR signaling mediated through DCs provides one mechanism for tolerance induction to allergen in the gut, there is also evidence that carbohydrate signaling pathways can fulfill this role. In an animal model of food-induced anaphylaxis, binding of carbohydrate moieties to the C-type lectin receptor SIGNR-1 expressed on DCs in the lamina propria, was shown to induce oral tolerance (132). Further work is warranted to define the functional properties of DCs that reside at different anatomic sites. Such cells will likely differ markedly from their counterparts in the blood.

Lessons from injected allergen

When considering factors influencing the quality of the T-cell response, we would be remiss in not mentioning allergen immunotherapy (IT). Conventional IT involves an up dosing regimen of subcutaneous injections of allergen extract over a prolonged period. This approach has proven clinically efficacious for a variety of allergens. As such, it exemplifies how the immune response can be skewed away from a Th2 response to a protective response by repeated injection of allergen through the skin. Regulatory T cells (Tregs) appear to hold the key to the development of tolerance during IT. These cells are reviewed elsewhere (133). However, it should be stressed that the nature and function of Tregs involved in allergen tolerance remain controversial. This is attributable to the blurred distinction among different Treg types and the fact that there is no reliable surface marker to distinguish these cells from activated effector cells. One thing that can be agreed upon is that levels of the regulatory cytokine IL-10 are increased following injection of allergen using both rush and conventional IT regimens. Other changes include diminished T-cell proliferation and production of Th2 cytokines to allergen in *in vitro* cultures, along with increased levels of IgG4 antibodies. IL-10 likely orchestrates many, if not all, of these changes. IL-10 induced during IT suppresses T-cell responses to allergens both *in vitro* and *in vivo* (134–136). Multiple pathways have been implicated in this process which could reflect a direct suppression of effector T cells via IL-10, or else indirect effects mediated by DCs, as well as involvement of different Treg types. In relation to the mode of induction of Tregs, recent work indicates a clonal ‘switch’ in allergen-specific Th2 cells to IL-10-secreting T cells (137). A key property of IL-10 relevant to allergen tolerance is its ability to selectively enhance IL-4-induced IgG4 while suppressing IgE (138). The generation of IgG4 antibodies is significant since this isotype can block allergen binding to B cells (139). Thus, these antibodies can directly modulate allergen delivery to APCs, which in turn may suppress or skew the T-cell response. Taken together, these findings point to orchestration of allergen tolerance by a complex immune network, in which IL-10 plays a key role.

Summary and conclusions

When Clemens Von Pirquet coined the word ‘allergen’ in 1906, he intended that the word allergen should cover all foreign antigens, but he stressed that this should include those substances that gave rise to ‘supersensitivity without immunity’ (140). Subsequently, the word allergen came to be

applied selectively to the substances that give rise to ‘supersensitivity’. The interesting question is what he meant by ‘without immunity’. It could be argued that an immune response to pollen does not provide immunity because the antigen is inherently benign. Von Pirquet was certainly aware that the sera of patients with wheal and flare skin tests did not have precipitins against pollen extract, and reagins (reactive substance later shown to be IgE) were not discovered until 1921 (141). We now know that the lack of precipitins reflects the fact that the serum IgG response includes IgG4 (142). Furthermore, at that time, it was well known that most common allergens did not give rise to delayed hypersensitivity skin tests. Thinking about why some antigens give rise to supersensitivity, i.e. IgE antibody responses, we have emphasized that the proteins that have been recognized as allergens are extremely diverse. Although these proteins have a wide range of biological properties, it is not clear that any specific properties of the proteins predict their allergenic capacity. In addition, it is meaningless to view the proteins in isolation, since other factors contribute to the immune response during natural exposure. The common features of the two most ‘effective’ allergen sources (pollen and dust mites) are the relatively small number of particles inhaled, the size of the particles, the fact that proteins elute rapidly, and the complex mixture of foreign proteins, glycoproteins, and lipids that they contain. Mite allergens are inhaled in the context of a range of substances that could act as adjuvants. While it is well established that the immune response to proteins can be altered by concomitant exposure to TLR ligands, it has recently been emphasized that these need to be in close physical proximity to the protein (47, 143). This appears to reflect the need for the antigen to be taken up in the same phagosome. However, we assume that as with endotoxin and ovalbumin, the presence of TLR ligands and proteins within the same particle represents sufficient physical proximity to activate a Th2 response (15). Thus, the major conclusion is that the proteins that induce IgE are delivered to the nose in small packets that contain high concentrations of ligands for pattern recognition receptors, along with the allergens which may also have relevant biological activity.

The fact that many allergic children raised in a house with a cat do not become allergic to cat allergens was originally compared to the effects of early exposure to farm animals (69). This implied that the effects of a cat could be related to high endotoxin exposure (144). However, the effect of a cat is primarily an effect on the IgE antibody response to cat allergens. Moreover, airborne endotoxin levels are not increased in a house with a cat (23, 145). Thus, it is more likely that the effects of

cat exposure relate to the higher inhaled dose of Fel d 1 and the modest evolutionary distance between cats and humans (68, 75, 146). Interestingly, there appear to be at least three separate forms of individuals who do not have symptoms on exposure to cats: non-responders who have no detectable antibodies to cat proteins; those with a 'modified Th2 response'; and finally some individuals with IgE antibodies who, despite exposure to cat allergens, do not experience symptoms. This last group may be comparable to successful desensitization.

A key question which remains is whether sensitization occurs during a critical developmental window early in life or else can occur at any time and is strictly related to the timing of allergen exposure. This is a difficult question to address, because most children are exposed within the first 2 years of life. With cat allergen, it appears that sensitization develops during this period (72). However, there is evidence that both sensitization and tolerance can develop within the first few months following exposure to a new allergen. In keeping with the latter explanation, there are good examples where exposure to a novel antigen in a new environment (e.g. experimental work with moths or grasshoppers or new exposure to Asian lady beetle allergens) can lead to sensitization in adult life (147). This evidence suggests that the immune system is capable of making IgE responses *de novo* during later life. If so, the persistent lack of response to a perennial allergen is more likely to represent a form of active suppression or tolerance than the inability of the adult immune system to respond. To further support this viewpoint, our recent data on the IgE antibody response induced by tick bites, confirms that IgE antibody responses can occur at any time, provided the new antigen is presented in the right way (66).

The main route for IgE production in modern Western society is inhalation through the nose, with IgE antibody production occurring in the local lymph nodes or from plasma cells in protected sites in the bone marrow. However, the results with tropical helminth parasites such as schistosomes and hook worm larvae, in patients with AD, in peanut allergy, and now with tick bites, suggest that antigen presentation through the skin is also a very effective route for inducing IgE production.

While our understanding of how allergens may trigger Th2 responses continues to evolve, our understanding of the mechanisms of tolerance induction and how this is maintained is inadequate. Obviously, we are hampered in studying B cells or plasma cells in the human, because it is 'difficult' to study either lymph nodes or bone marrow; however, the techniques for characterizing and monitoring peripheral blood T cells are steadily improving. Many of the markers of Th2 responses are well defined, and the relevance of IL-10 as a controller of IgE antibody responses and an enhancer of IgG4 is well established. Nonetheless, identification of markers unique to human regulatory T cells continues to pose a challenge.

The central question about allergens is why this group of foreign antigens selectively induces IgE antibody responses in such a large proportion of the population. The factors that are definitely relevant are as follows: (i) the number and size of particles inhaled, which reflects the prevalence of the animal or plant that releases the particles and the aerodynamic properties of the particles, (ii) the mixture of foreign substances in the particle, including allergens, adjuvants, and many molecules which can act as both, and (iii) the dose of allergen exposure. Importantly, people do not become allergic without exposure; however, the quantities inhaled are inherently small, ranging from 1 ng up to 1 µg/day.

In real life, no proteins are inhaled on their own, and the plant, fungal, and arthropod species that give rise to the 'best' allergens evolved separately from humans over 600 million years ago. The response to mammalian allergens, particularly cat and dog, is more complicated, because the species are much less foreign (only 65 million years) and the doses inhaled are much larger. While these allergens can induce high titer IgE antibodies, such responses are actually more common among children exposed to low level allergen by virtue of no cat in the home. Finally, while we believe that the presence of adjuvants in allergen particles such as endotoxin, chitin, DNA, proteases, and lipid-binding proteins are important determinants of allergenicity, we are not convinced that the allergen proteins themselves must be endowed with adjuvant properties. Ultimately, it is the delivery of relatively small quantities of a foreign protein in the context of other adjuvant molecules that induces the allergic response.

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