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## Allergens, sources, particles, and molecules: Why do we make IgE responses?



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## Abbreviations:

USA, United States of America; UK, United Kingdom; kDa, kilodalton; OLIN, Obstructive Lung Disease in Northern Sweden Studies; NC, North Carolina; TN, Tennessee; AK, Arkansas; MI, Missouri; VA, Virginia; GOS, galacto-oligosaccharide; IU/mL, international units per milliliter; APC, antigen presenting cell; DCs, dendritic cells; IL, interleukin; GC, germinal center; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3 grabbing non-integrin; MD2, lymphocyte antigen 96; Th2, Type 2 (helper T cells); TSLP, thymic stromal lymphopoietin; DNCB, dinitrochlorobenzene; TGF, transforming growth factor; TLR, Toll-like receptors; IFN, interferon; CCD, carbohydrate cross-reactive determinants; MMXF<sup>3</sup>, horseradish peroxidase; MUXF<sup>3</sup>, Bromelain; MMF<sup>3</sup>F<sup>6</sup>, Insect core 3-fucosylated N-glycan; GalNAc, N-Acetylgalactosamine; GlcNAc, N-Acetylglucosamine

## ABSTRACT

Allergens are foreign proteins or glycoproteins that are the target of IgE antibody responses in humans. The relationship between subsequent exposure and the allergic symptoms is often or usually obvious; however, there is increasing evidence that in asthma, atopic dermatitis and some forms of food allergy the induction of symptoms is delayed or chronic. The primary exposure to inhaled allergens is to the particles, which are capable of carrying allergens in the air. Thus, the response reflects not only the properties of the proteins, but also the biological properties of the other constituents of the particle. This is best understood in relation to the mite fecal particles in which the contents include many different immunologically active substances. Allergic disease first became a major problem over 100 years ago, and for many years sensitization to pollens was the dominant form of these diseases. The rise in pediatric asthma correlates best with the move of children indoors, which started in 1960 and was primarily driven by indoor entertainment for children. While the causes of the increase are not simple they include both a major increase in sensitization to indoor allergens and the complex consequences of inactivity. Most recently, there has also been an increase in food allergy. Understanding this has required a reappraisal of the importance of the skin as a route for sensitization. Overall, understanding allergic diseases requires knowing about the sources, the particles and the routes of exposure as well as the properties of the individual allergens.

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## Introduction

In public usage an allergy is a reaction that follows exposure to a foreign agent with a predictable time relationship. In many cases, this implies a rapid or immediate relationship but patients can be “allergic” to poison ivy, which takes 6–24 h, or to red meat in the alpha-gal syndrome which takes 3–6 h.<sup>1,2</sup>

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Allergic diseases can be characterized by the nature of the immune response that gives rise to the unwanted symptoms or by the form of the clinical presentation (Table 1). Many of these diseases have rapid onset after exposure, which greatly simplifies identifying the cause. Thus, exposure to a cat can cause sneezing, eye itching and wheezing within a few minutes; similarly the sting of a wasp or yellow jacket can cause generalized hives within 10–15 min, and eating peanuts can cause full-blown anaphylaxis within 20 min. On the other hand, there are many allergic diseases for which the symptoms take longer to develop, and the symptoms may be much less obviously related to the relevant exposure. In addition, there are now several situations in which the route of exposure that gives rise to sensitization is not the same as the route that precipitates symptoms (Table 2)

The three diseases included in Table 2 illustrate the fact that the skin is an excellent route for inducing IgE antibodies. The first example was actually schistosomiasis. In this case, Taliafero and Taliafero demonstrated in 1931 that the serum of patients with the disease could transfer skin sensitivity into the skin of non-infected individuals.<sup>3</sup> The importance of the skin as a route of sensitization has now been established for three diseases: i) peanut allergy<sup>4</sup>; ii) delayed anaphylaxis to red meat in patients with IgE to galactose alpha-1, 3-galactose<sup>5</sup>; and iii) wheat sensitization related to wheat dependent exercise induced anaphylaxis<sup>6,7</sup> (Table 2). It should also be noted that many of the patients with diseases shown in Table 1 do not describe an immediate response to exposure. In some cases, this may be because the inflammation in the tissues is induced by a T cell mechanism, but the apparent delay may also represent either a delayed inflammatory response following mast cell mediator release or the effects of chronic low dose exposure. There is also another allergic disease characterized by IgE to milk for which a diet avoiding cow's milk can be an effective treatment; however, the relevance of the IgE antibodies to the disease is not clear.<sup>8–10</sup> This disease is eosinophilic esophagitis, which presents a special challenge because the disease includes a progressive eosinophil-rich inflammation without any obvious immediate phase.

## Size of particles and proteins: relevance to sensitization

### Particles and the delivery of inhalant allergens

The purification of allergens started in the 1960's with ragweed and grass pollen, which were the major causes of hay fever in the USA and UK respectively.<sup>11–13</sup> At that time it was already recognized that exposure depended on both the nature of the proteins and the properties of the particles carrying them. It cannot be stressed too strongly that there is no such thing as airborne protein molecules—the saturated vapor pressure of molecules that are  $\geq 5$  kDa is close to zero. Thus the only relevant source of airborne allergens is on particles that are capable of becoming airborne (Fig. 1). For the outdoor exposures, it is possible both to count and identify pollen grains or fungal spores. However, this counting depends on the fact that these particles stay airborne. By contrast, the indoor environment is characterized by very low rates of air movement and particles as big as mite fecal particles or pollen grains fall rapidly. There are major differences between the particles on which dust mite and cat allergens become airborne.<sup>14–16</sup> The way in which small particles or flakes of dander remain airborne leads to very different calculations about the quantity of allergen that can be inhaled. However, it is worth remembering that a large proportion of the particles inhaled through the mouth will impact on the back of the throat, and are subsequently swallowed.

The best studied of the allergen particles are the fecal particles of dust mites. After the purification of the group I and group II proteins it rapidly became clear that the major form in which they become airborne was on these particles.<sup>17,18</sup> In addition, it was already suggested that Der p 1 was a digestive enzyme. With the cloning of Der p 1, it became clear that it had major homology with cysteine proteases.<sup>1,19</sup> A whole series of studies were carried out on the relevance of the enzymatic activity, and this was followed by the increasing realization that the fecal particles were a veritable treasure trove of toll-like receptor ligands, as well as foreign proteins (Table 3). The message is clearly that these particles have great potential to induce an IgE response, and also that it is very unlikely that the response can be attributed to one of these constituents alone.

**Table 1**  
Allergic diseases.

	Source	Mechanism/Exposure
<i>Immediate reactions:</i>		
Venom anaphylaxis	Bee, wasp or fire ant stings	IgE + injected
Penicillin anaphylaxis	Penicillins (oral or injected)	IgE + injected or swallowed
Peanut anaphylaxis (Other foods)	Peanut products (oral or skin)	IgE + swallowed
<i>Immediate + Delayed or chronic reactions:</i>		
Seasonal allergic rhinitis	Pollen grains	IgE + inhaled
Allergic asthma	Dust mite, cockroach, cat, dog, <i>Alternaria</i>	IgE + inhaled
<i>Non-immediate reactions:</i>		
Atopic dermatitis	Many allergens (both food and inhalant) + Infection of the skin	IgE and T cells + multiple routes of exposure
Poison ivy	Chemicals in the plant	T cells + contact
Delayed anaphylaxis to red meat	Tick bites give sensitization to alpha-gal	IgE + oral mammalian meat

**Table 2**  
Alternate routes of exposure.

Source	Route	IgE response	Syndrome
Tick bites	Skin	IgE to alpha-gal	Delayed anaphylaxis to red meat
Peanuts (e.g., peanut butter)	Skin	IgE to Ara h 1 and Ara h 2	Immediate reactions to oral peanut
Wheat in soap	Skin of face	IgE to wheat	Wheat dependent exercise induced anaphylaxis

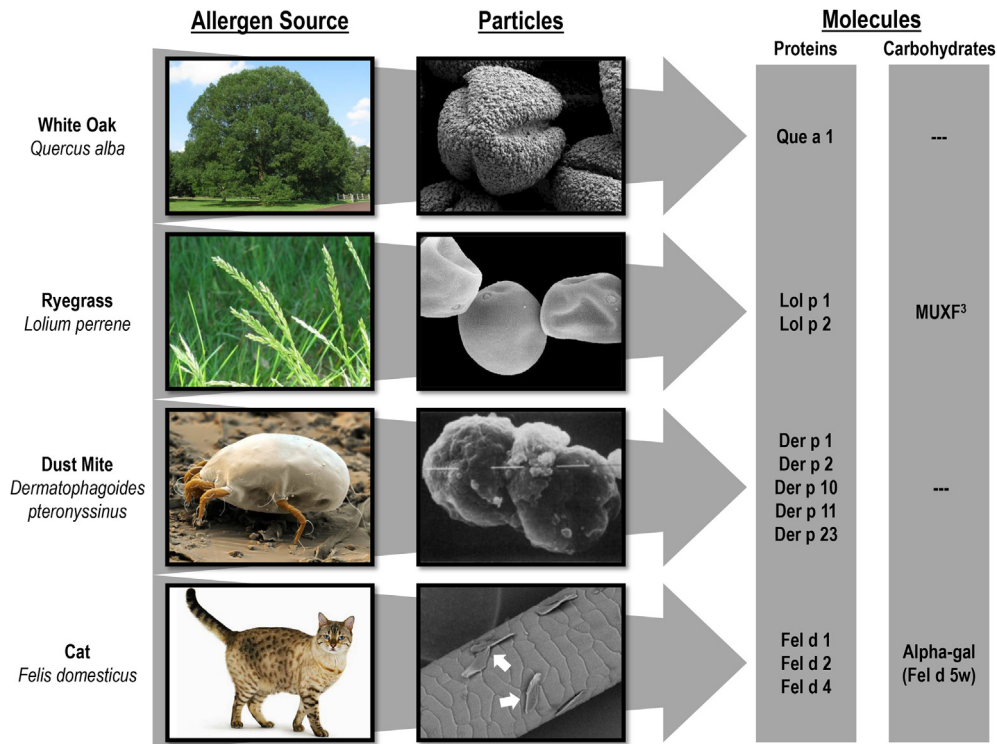


Fig. 1. White oak, ryegrass, dust mite, and cat allergens including the relevant particles and molecules.

Cat dander particles appear to be “sticky” and can be found on walls and clothing, as well as distributed widely in buildings which do not have an animal present. Again, this has nothing to do with the properties of Fel d 1. However, it is clear that many children, who have never lived in a house with a cat, become sensitized to Fel d 1. This raises the question about how much community exposure is influenced by the prevalence of cat ownership in the community.<sup>20,21</sup> Clearly, there must be a difference between a community such as the low income area of Atlanta where less than 5% of homes have a cat and New Zealand where over 50% of homes have a cat. There is good evidence that children who live in a house with a cat are less likely to become sensitized. Indeed, a major birth cohort in Detroit reported that children who had a cat at home during the first year of life were still less likely to be sensitized to cat allergens at the age of 18 years.<sup>22</sup> In the OLIN cohort in Norbotten, where ~25% of the homes have a cat, it appears that 80% of the cat sensitized children at age 12 did not currently live in a house with a

cat.<sup>23</sup> At age 19, 77% of the subjects with high titer IgE to cat did not live in a house with a cat. With this model, it is not surprising that cat sensitization is actually less common in New Zealand where 50% of the homes have cats.<sup>24,25</sup>

Proteins and glycoproteins

Purified allergens were initially defined by their molecular weight and by specific antibodies. This was followed by the production of monoclonal antibodies, sequencing and x-ray crystallography. An important development came with the definition of the nomenclature and the establishment of an official IUIS subcommittee (see [www.allergen.org](http://www.allergen.org)).<sup>26</sup> Today, we know the full structure of many hundreds of allergens, and it is increasingly possible to assay IgE antibodies to specific proteins rather than to the extract.<sup>27</sup> For some allergen sources there is enough evidence to recommend assay of IgE to specific proteins in order to define the relevance of the IgE response. This is best recognized for peanut allergy, for cat, and for the new syndromes related to meat allergy.<sup>28,29</sup> In addition, there are important cross-reactivities such as those between the tropomyosins, the lipocalins, and the albumins that can only be correctly understood using component assays (Table 4A–C). It is important to remember that identifying component specific sensitivity is only practical using *in vitro* IgE assays.

Most allergens identified to date are water-soluble proteins with a molecular weight between 5 kDa and 50 kDa. While many of these proteins are glycosylated, in general, this glycosylation does not play a significant role in their allergenicity. It was traditionally considered that the basement membrane of the nose was only permeable to molecules <60 kDa. However, it is now clear that dendritic cells can sample proteins by extending their dendrites across the membrane, and thus, may be able to collect larger molecules. On the other hand, a recent study found that

Table 3 Immunostimulating effects of mite fecal particles: allergens and pathogen-associated molecular patterns [PAMPs].

I	The dust mite allergen Der p 1 is a cysteine protease: a) Increased permeability of the respiratory epithelium because of the enzymatic digestion of the tight junctions b) Cleavage of receptors on lymphocytes, such as IL-2 receptor (CD25) and the low affinity receptor for IgE (CD23) c) Digestion of other proteins as well as Der p 1 itself to create fragments with altered antigenicity
II	Der p 2 is a homolog of the adapter protein MD-2 and can facilitate LPS-mediated signaling through TLR4
III	Other PAMPs and their targets: a) Mite DNA – unmethylated DNA can act on TLR 9 b) Bacterial DNA – unmethylated DNA can act on TLR 9 c) Endotoxin – Ligand for TLR 4 d) Chitin – C-type lectin (or FIBCDI)

**Table 4A**  
Component analysis of IgE response to peanut and birch.

Allergen	Peanut anaphylaxis	Oral allergy syndrome
Peanut	<b>139</b>	<b>16.7</b>
<i>Ara h 1</i>	<b>20.5</b>	<0.35
<i>Ara h 2</i>	<b>90.3</b>	<0.35
<i>Ara h 3</i>	<b>16</b>	<0.35
<i>Ara h 8</i>	0.6	<b>44.6</b>
Birch pollen	0.8	<b>62</b>
<i>Bet v 1</i>	1.0	<b>85</b>

The bold numbers are the positive values.

sensitization to Der p 11, which has a molecular weight of ~100 kDa, but forms aggregates of even higher molecular weight, was strongly associated with atopic dermatitis.<sup>29</sup> This suggests that molecules of this size are more likely to give rise to sensitization than smaller molecules, if they can penetrate the skin.

### The use of component assays

#### Peanut sensitization (Table 4A)

Peanut allergy has dramatically increased in prevalence and the severity of the clinical reactions correlates broadly with high titer IgE to *Ara h 1* and *Ara h 2*.<sup>28</sup> In some climatic areas there is an important cross-reactivity that can confuse diagnosis. Specifically, the peanut protein *Ara h 8*, cross-reacts extensively with the birch pollen allergen *Bet v 1*, and readily gives rise to moderate levels of IgE antibody to peanut.<sup>30</sup> However, this IgE is generally associated with oral allergy syndrome and not anaphylaxis (See examples in Table 4A). Because *Ara h 8* is only a minor component of the peanut extract this may give rise to a confusing situation where the level of IgE to *Ara h 8* is higher than the level of IgE to peanut extract.

#### Red meat cross-reactivity (Table 4B)

The earliest descriptions of cross-reactivity leading to allergic reactions to meat relate to cow's milk. Those children who present with allergic reactions to beef are in general already known to be allergic to cow's milk.<sup>31</sup> More recently, another syndrome was recognized in which patients who had positive skin tests and/or serum IgE assays for cat extract, presented with severe allergic reactions to pork. In these cases, the cross-reactivity relates to cat albumin and pork serum albumin.<sup>32,33</sup> Interestingly, this sensitivity appears to relate primarily to exposure to a cat at home, but is not generally associated with asthma. The next syndrome also includes

**Table 4B**  
Four syndromes in which sensitivity to mammalian allergens may require component analysis.

Allergen	Cat-allergic asthma	Pork cat	Alpha-gal	Meat anaphylaxis*
Cat extract	<b>85</b>	<b>4.3</b>	<b>33</b>	<0.35
Fel d 1	<b>90</b>	<0.35	<0.35	<0.35
Cat albumin	<0.35	<b>6.2</b>	<0.35	<0.35
Cat IgA	ND	<0.35	<b>6.8</b>	<0.35
Alpha gal	<0.35	<0.35	<b>23</b>	<0.35
Milk protein	<0.35	<0.35	<b>14</b>	<b>16.3</b>
Bos d 5	<0.35	<0.35	<0.35	<b>8.4</b>
Pork	<0.35	<b>3.6</b>	<b>6.2</b>	<b>8.7</b>
Beef	<0.35	0.8	<b>7.3</b>	<b>6.2</b>
Asthma	<b>Yes</b>	No	No	No
<i>Allergic reactions:</i>				
To pork	No	<b>Yes</b>	<b>Yes</b>	±
To beef	No	No	<b>Yes</b>	<b>Yes</b>

The asterisk indicates that the patient is pediatric.  
The bold numbers are the positive values.

**Table 4C**  
Comparison of total IgE and specific IgE titers in three syndromes.

Allergen	Eosinophilic esophagitis <sup>†</sup>	Eosinophilic esophagitis <sup>†</sup>	Eosinophilic esophagitis	Peanut anaphylaxis <sup>†</sup>	Alpha-gal
Wheat	<b>0.85</b>	<b>0.41</b>	<b>1.18</b>	<b>2.04</b>	<0.35
Milk	<b>1.68</b>	<b>2.32</b>	<0.35	<b>3.62</b>	<0.35
Egg	<b>0.68</b>	<0.35	<0.35	<b>3.49</b>	<0.35
Soybean	<0.35	<b>0.39</b>	<0.35	<b>16.8</b>	<0.35
Peanut	<0.35	<0.35	<0.35	<b>473</b>	<0.35
Alpha-gal	<0.35	<0.35	<0.35	<0.35	<b>32.3</b>
Total IgE	<b>63.7</b>	<b>141</b>	<b>19.2</b>	<b>1284</b>	<b>180</b>

Each column represents an individual case.

The bold numbers are the positive values.

<sup>†</sup> Denotes pediatric case.

sensitivity to cat proteins, but in this case the relevant protein is cat IgA and the epitope is the oligosaccharide galactose alpha-1, 3-galactose (or alpha-gal). This oligosaccharide is present on many different mammalian proteins. Sensitization to alpha-gal is associated with two forms of anaphylaxis: i) immediate and often severe reactions to the monoclonal antibody cetuximab<sup>34</sup> and ii) urticarial or anaphylactic reactions to red meat that start 3–6 h after eating.<sup>35</sup> The nature of the response and the reasons for this IgE response to alpha-gal will be discussed later. Finally in Table 4B, we include a case of allergic asthma related to IgE to cat. In this case, the IgE is primarily directed at Fel d 1, which does not cross-react with any meat proteins.

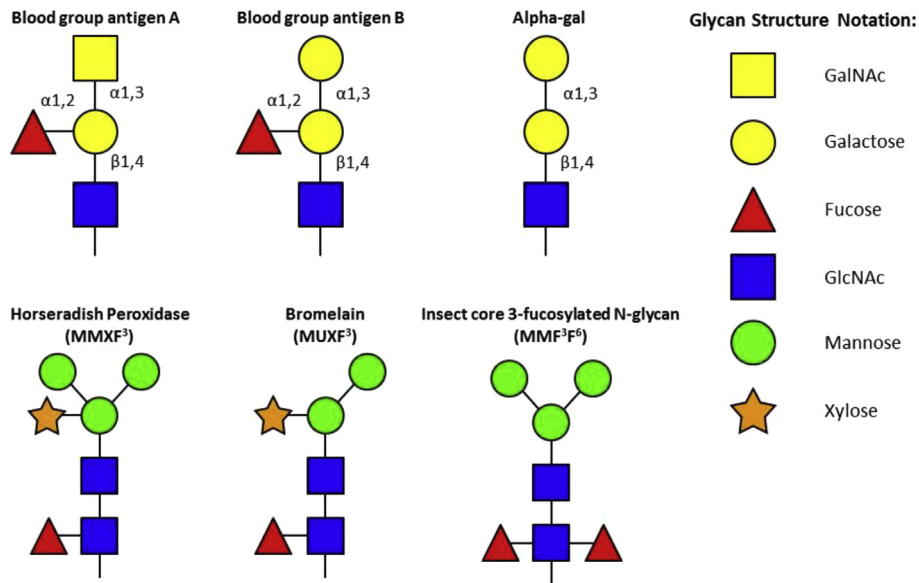
#### Oligosaccharide IgE responses and their relevance to allergic symptoms

There have been occasional reports of IgE responses to oligosaccharides such as dextran, but the cases were not common enough for an analysis of reasons why subjects became sensitized. Around 1990, it became clear that many patients who were allergic to pollen had a proportion of their IgE directed against glycosylation motifs on the pollen proteins (Fig. 2).<sup>36,37</sup> These IgE antibodies bind to many different proteins and can cause confusion in diagnosis. Furthermore, despite extensive investigation, no evidence could be found that IgE antibodies to these cross-reactive carbohydrate determinants (CCD's), contributed to symptoms.<sup>38</sup>

As we have already mentioned, there are also IgE antibodies to the oligosaccharide alpha-gal. These antibodies are present in individuals living in coastal New South Wales, Germany, Japan, France and Sweden, as well as a large area of the United States.<sup>39–43</sup> In America these IgE antibodies were known to be common in NC, TN, AK, MI, and VA but were also present in surrounding states.<sup>5</sup> This geographic localization initially led us to investigate the possible role of ticks, and to follow the response to tick bites. In the USA, the tick involved is *Amblyomma americanum* (commonly known as the Lone Star Tick). In Australia, *Ixodes holocyclus* is responsible for these responses, and in Europe the relevant tick is *Ixodes ricinus*.<sup>39,42</sup> In some cases, the response to these bites occurs within weeks and can dramatically increase the total IgE. In keeping with this, IgE antibodies to alpha-gal often make up a large proportion of the total IgE.<sup>5</sup>

In Japan and Singapore, another oligosaccharide-specific IgE response has been identified which may also be induced by a primary exposure through the skin. In Japan, subjects were identified because they had severe reactions to a soft drink that had been fortified with the prebiotic galacto-oligosaccharide (GOS).<sup>43</sup> However, the subjects all worked as oyster shuckers and had been exposed to a specific sea squirt.<sup>43</sup> Thus, in keeping with the role of tick bites, it is likely that sensitization in this case went through the skin. Sensitization to GOS has also been recognized in





**Fig. 2.** Glycosylation motifs of mammalian blood group antigens as well as common insect- and plant-related CCDs. In contrast to insect- and plant-related CCDs, mammalian glycoproteins do not contain alpha-1,3-fucose or xylose moieties.

Singapore. In this case, it was baby food fortified with GOS that identified the cases, but the cause of the sensitization in that area is not clear.<sup>44</sup>

**The sequence of allergic diseases in “post-hygiene” countries (Table 5)**

The primary measures of hygiene are clean water, control of helminth infections, shoes and probably some limited separation from farm animals.<sup>11</sup> The major elements of these changes were achieved in London, Berlin, Munich, New York and Chicago by 1920 at the latest.<sup>45</sup> In keeping with that, there were dramatic declines in infection disease mortality, particularly for cholera and typhoid.<sup>46</sup> In the UK, Northern Germany, and New England there were also progressive rises in hay-fever related primarily to grass pollen in Europe and ragweed in the USA.<sup>11</sup> The important thing here is that there was only modest awareness of the importance of the role of house dust before the 1960's. Furthermore, the increase in pediatric asthma did not start until ~1960. Thus the second “epidemic” of allergic disease started long after allergic rhinitis was fully established.<sup>47</sup> The rise in asthma was observed primarily in children and this disease had been uncommon among children before that time. Pediatric asthma has now been recognized as a problem in many different countries but this is only in countries, which are “post-

hygiene”. Thus in the villages of Kenya, Papua New Guinea or Ecuador and the poor areas of the city of Kumasi in Uganda, allergic asthma in children is not yet a problem.<sup>48–50</sup> Initially, the increase in asthma had been observed in countries where dust mites were being recognized as the primary source of perennial/indoor allergens. Thus, the increase was seen in the UK, Australia, New Zealand and Japan, long before it became clear that the same increase but on a smaller scale had been occurring in countries such as Finland and Sweden where the primary allergens related to asthma were those from animal dander.<sup>11,51,52</sup> Equally, it was not until 1990 that it became clear that cockroach allergens were an important cause of sensitization among patients with asthma living in poverty in the USA.<sup>53–55</sup>

The common feature of pediatric asthma in different communities is that sensitization is primarily to indoor allergens. This can be seen very clearly in countries which have become westernized relatively recently such as Singapore and Costa Rica.<sup>48,56</sup> The obvious reasons would be changes in housing conditions, more time spent indoors and dramatically decreased time spent outdoors. The move indoors was induced or made possible by the rise in indoor entertainment. This move has had multiple health consequences most of which have been harmful, with time spent in front of a screen being a major feature. So the primary contributors to asthma are a dramatic increase in the sensitization to indoor

**Table 5**  
Allergy epidemics of the last 100 years.

Date	Relevant changes	Epidemic
1870–1950	Clean water and helminth eradication	Seasonal allergy
1955–2000	Increase in dairy herds and in the pollen of grass and ragweed Onset of indoor lifestyle: <ul style="list-style-type: none"> <li>• Decreased physical activity with prolonged shallow breathing</li> <li>• Changes in houses</li> <li>• Increased sensitization to mite, cats, etc.</li> </ul>	Pediatric asthma
~1955	Changes in peanut products: <ul style="list-style-type: none"> <li>• Delay or oral exposure to peanut</li> <li>• Changes in the skin</li> <li>• Changes in vaccination policy</li> </ul>	Peanut allergy
~2005	Increase in tick bites secondary to the rising population of deer Free access of deer to suburban areas because of leash laws for dogs	Delayed anaphylaxis to red meat (Alpha-gal)

allergens and prolonged periods of time sitting still with inadequate expansion of the lungs<sup>11,57–59</sup> (Table 5).

The most recent “epidemic” is the rise in food allergy, most notably to peanuts. Here the important observations are now clear: first, that sensitization to peanuts can occur through the skin; and second, that avoidance of peanut exposure in early childhood is likely to make the situation worse.<sup>4,60,61</sup> However, the more relevant question here is whether regular washing of the skin, especially with detergents, has interfered with some element of the skin barrier. At present, there is good evidence that defects in the structural protein, filaggrin, can enhance sensitization through the skin, but this is most closely linked to eczema.<sup>62</sup> Further work is warranted to test whether repeated washing compromises skin barrier integrity in a similar fashion.

### Why do we make IgE?

In studies on children in tropical pre-hygiene villages or towns, the total serum IgE values are routinely over 400 IU/ml with mean values closer to 1000 IU/ml.<sup>48,49</sup> In addition, it is well recognized that helminth infections such as ascariasis, schistosomiasis, anisakiasis and echinococcosis give rise to IgE responses. Following the establishment of clean water and helminth eradication, the major documented IgE responses have been to inhalant allergens such as pollen, dust mite and cat.<sup>1,11,25</sup> Because of this, much of the analysis of IgE responses, including the genetics, has related to proteins derived from these sources. The primary conclusion is that proteins on particles inhaled through the nose or mouth can induce IgE responses in genetically predisposed individuals. For dust mite and the pollens, high titer IgE responses are common in areas where these allergens are common. Interestingly, high titer IgE responses to cat allergens are less common and this is particularly true among children raised in a house with a cat. Indeed, in communities where 20–30% of the homes have a cat, three quarters of the children with high titer IgE to Fel d 1 do not live in a house with a cat.<sup>23</sup>

As discussed earlier, high titer IgE responses can be initiated through the skin. In our practice in Virginia, high titer IgE responses are common in relation to peanut and also to alpha-gal, both of which are considered to go through the skin. In keeping with this, IgE antibodies are considered to have a direct mechanistic role in the diseases. In complete contrast, children and adults with eosinophilic esophagitis have low or very low titers of IgE to cow's milk and wheat proteins. In this case, although cow's milk and wheat appear to be important causes of the disease, there is very little evidence that IgE antibodies are mechanistically involved in the disease (Table 4C).

The ability to make IgE responses depends on initial recognition of allergen in the context of its associated molecules and particles, leading ultimately to activation of dendritic cells (DCs) or other antigen presenting cells (APC). In turn, this leads to priming of T cells that are capable of providing help to B cells for IgE production. This latter event involves the switch of B cells from IgM to IgE, which is mediated by IL-4 produced by Th2 cells. This event can occur within an organized germinal center (GC), or else by direct switch outside a GC.

### Initial recognition of allergens and the activation or priming of dendritic cells and T cells

As has already been emphasized, we are not exposed to allergens as individual molecules. Thus the correct question is whether the overall content of a particle, or source, includes molecules that could have biological effect that would be expected to influence the IgE response. Several allergens have been shown to exert their

effects via binding to pattern recognition receptors capable of triggering Th2 responses, and we list three examples:

- i) Ara h 1 in its native form can induce Th2 skewing in naïve T cells, and this is dependent on glycosylation of the molecule, which facilitates binding to the C-type lectin receptor, DC-SIGN, on DCs.<sup>63</sup>
- ii) Der p 2 functions as an analog of MD2, acting to bind LPS to the toll-like receptor-4 molecule.<sup>64</sup>
- iii) Bermuda grass pollen binds DC-SIGN to initiate activation of DC's, resulting in the production of TNF-alpha.<sup>65</sup>

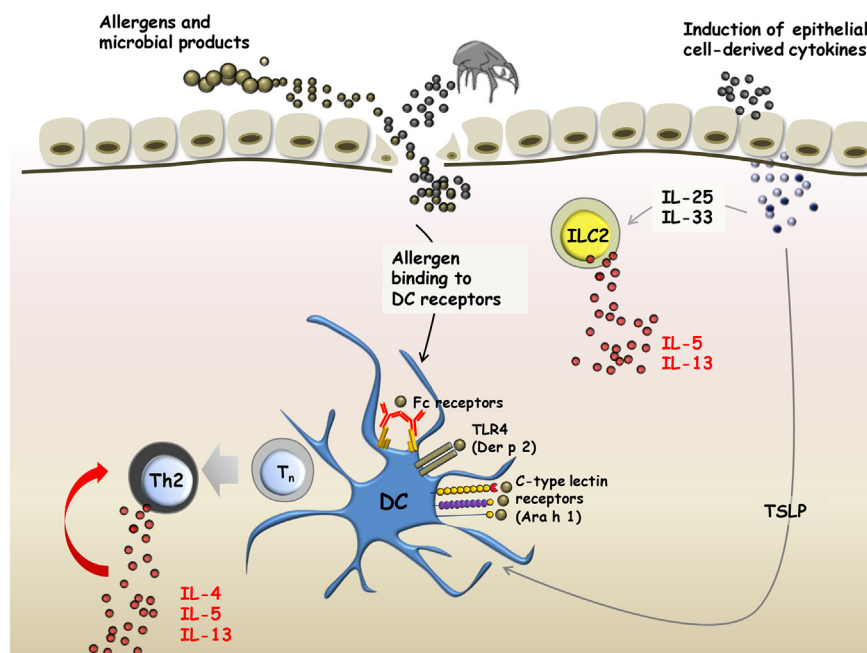
The evidence that different allergens can have significant pro-Th2 effects continues to accumulate. However, it is equally clear that an extremely diverse range of molecules are involved. Nonetheless, the dust mite remains one of the most impressive triggers of high titer IgE antibody responses. There are many studies where IgE to dust mites, including titers over 50 IU/ml (Class 5), are very strongly associated with asthma. This includes places as diverse as Singapore, Costa Rica, Taiwan, New Zealand and the UK, as well as Virginia in the USA.<sup>56,57,66,67</sup> The range of biological effects that can occur in response to the contents of mite feces is so diverse that attributing the overall phenomenon to one constituent seems foolish (See Table 3, Fig. 3).

### The role of epithelial cell-derived cytokines in Th2 responses

There is now extensive evidence that a broad range of allergens can act directly on epithelial cells to induce the production of a variety of Th2-promoting cytokines including TSLP, IL-25 and IL-33<sup>68</sup> (Fig. 3). Both in humans and in mice these molecules play a significant role in stimulating the activation of DCs and the development of Th2 cells from naïve T cells. More recently, these molecules have been implicated in the activation of innate lymphoid cells at epithelial surfaces that also serve to promote Th2 responses via production of the type 2 cytokines, IL-5 and IL-13.<sup>69</sup> In relation to TSLP, experiments in mice engineered to overexpress TSLP or else knockout its receptor, strongly support its role in allergic inflammation.<sup>70,71</sup> In humans, the evidence comes from the ability for TSLP-primed DCs to induce Th2 differentiation,<sup>72</sup> and increased levels of TSLP in the bronchial mucosa of asthmatics and in skin lesions of patients with atopic dermatitis.<sup>73,74</sup> Furthermore, there is *in vitro* evidence to indicate that optimal priming of DCs for Th2 responses occurs when TSLP and foreign antigen are combined, through a mechanism that involves antigen-mediated upregulation of the TSLP receptor.<sup>73,75</sup>

### The possible mechanisms influencing the response to exposure via the skin, gastrointestinal tract or nose

Priming of DCs in the skin is highly relevant for antigens such as bee stings, tick bites and schistosomes. The skin may also be relevant for peanut, dust mite and cat allergens particularly in patients with eczema and/or a defect in the barrier function of the skin. The evidence about tick bites inducing IgE to alpha-gal, and peanut allergy in children avoiding oral exposure, is in keeping with the skin being an excellent site for priming of DCs and induction of Th2 cells. However, there is also extensive evidence that the skin is rich in Th1 and Th17 cells as well as Th2 cells.<sup>76,77</sup> The obvious implication is that exposure through the skin is not inevitably a source of IgE rich responses. Indeed poison ivy, which is a very effective method of inducing a T cell response capable of recruiting eosinophils to the skin, does not include an IgE response. Similarly, the live viral vaccines applied to the skin, most obviously vaccinia itself, are remarkably effective at inducing long term immunity, but do not



**Fig. 3.** Role of allergens and epithelial cell-derived cytokines in induction of type 2 cytokines. Allergens trigger the release of TSLP, IL-25 and IL-33 from epithelial cells by binding to pattern recognition receptors. Allergens can also penetrate the subepithelial space through defects in the epithelial barrier. These defects may be structural (e.g., filaggrin deficiency) or else allergen-induced (eg. disrupted tight junctions arising from cleavage of occludin by Der p 1). Epithelial cell-derived cytokines induce IL-5 and IL-13 secretion by innate lymphoid cells, and, in conjunction with allergen, promote Th2 differentiation via dendritic cell-mediated pathways.

induce immediate hypersensitivity. Again, these aspects highlight the important contribution of the antigen itself and its associated molecules and particles.

The recent results on the “protective” effects of early oral exposure to peanut allergens confirmed that oral exposure can be, or normally is, a very effective method of inducing tolerance.<sup>61</sup> Oral tolerance was well established using dinitrochlorobenzene (DNCB), which can dramatically inhibit sensitization through the skin in mice.<sup>78</sup> Anecdotally the same thing is true for poison ivy in humans. The question then is, what is the mechanism for oral tolerance and is it similar for all food allergens? Sites have been identified in the oral cavity that have increased populations of T cells that preferentially express TGF- $\beta$ , IL-10, IFN- $\gamma$  and IL-17. The same sites have DCs expressing TLR-2 and TLR-4.<sup>79</sup> The implication is that sublingual immunotherapy can specifically induce T-cell-mediated tolerance. Consistent with this notion, sublingual grass pollen (Phl p 5) has been shown to induce “tolerogenic” properties in Langerhans cells of the oral mucosa.<sup>80</sup> Similarly, sublingual mite has been reported to increase circulating regulatory T cells.<sup>81</sup> At present the functional and phenotypic differences between DCs in the oral cavity or gut compared to those in the skin, are not well established.

There are two questions related to oral tolerance that may be highly relevant to the control of allergic disease.

- Firstly, is the tolerance to cat allergens that is common among children raised in a house with a cat, induced by exposure to cat allergen in the mouth or by swallowing?<sup>23,82</sup> Interestingly, students spending one or two years in a low allergen university setting, who had come from a house with a cat, had a highly significant fall in IgG antibodies to Fel d 1 with little change in their IgE antibodies to cat allergen.<sup>83</sup> Thus, progressive decline in IgG antibodies may be a marker of loss of tolerance during periods of decreased exposure.
- Secondly, it is well established that both allergen specific immunotherapy and seasonal increases in bee stings can induce

enhanced production of IL-10 by circulating T cells. This IL-10 production is thought to be an effective enhancer of IgG4 antibodies while suppressing the switch to IgE.<sup>84</sup> Whether oral exposure can achieve an equivalent degree of IL-10 production or tolerance it still not clear.

### Summary and conclusions

We now have a truly remarkable amount of information about the allergens, which are the target for IgE antibodies. Protein or glycoprotein allergens from hundreds of sources have been purified, cloned, sequenced and crystallized ([www.allergen.org](http://www.allergen.org)). In addition, the biological properties of these allergens have been studied extensively. There are several properties of these allergens that stand out:

- First, they are all foreign, for the pollens, fungi, insects and mites, we have been evolutionarily separate for 300 million years or more. Thus of all the sources only the mammals could be considered “close relatives”.
- Second, although almost all the allergens are water-soluble proteins of small to moderate size, the inhalant allergens are always inhaled on particles. Thus it is the physical properties of these particles that allow them to become and remain airborne, but it is the cumulative biological and immunological effects of the contents of the particles that make the IgE response possible.
- Third, for the main inhalant allergens the exposure that induces the immune response is similar to the exposure that causes symptoms. In the case of asthma, there is the added element that although exposure of the nose can induce an IgE response, the particles need to enter the lungs in order to cause the inflammation that underlies allergic asthma.
- Fourth, in recent years, it has become clear that there are many other routes for exposure, and the skin in particular may be an excellent route for inducing IgE responses. This is not only true

for insects, helminths, ticks and scabies mites that penetrate the skin, but also for allergens from peanut, wheat, cat and mite that penetrate the skin if the barrier function is disturbed. Same defects in barrier function are genetic, e.g. filaggrin, but in addition barriers can be disrupted by eczema or by excessive washing.

The increase in allergic disease over the last 100 years has not been unimodal. Initially, hay fever dominated our specialty and was epidemic by 1935. This increase related to the onset of hygiene, i.e., pathogen free water and helminth eradication, but also involved an increase in seasonal pollen. The rise in pediatric asthma started around 1960 and is best explained by the multiple effects of the change to an indoor life style. This included both increased exposure to and increased sensitization to the major perennial indoor allergens but also the disastrous effects of inactivity. Most recently many western countries have experienced a dramatic rise in food allergy, most obviously peanut. The primary causes of this are not yet clear. However, it is clear that avoidance of oral peanut exposure is not a method of preventing sensitization. What is increasingly clear is that all these epidemics relate to changes in human life-style.<sup>11</sup> It is also probable that we will continue to experience changes in allergic disease and equally inevitable that we will not successfully predict the consequences of future changes in human behavior.

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#### Conflict of interest

The authors have no conflict of interest to declare.

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