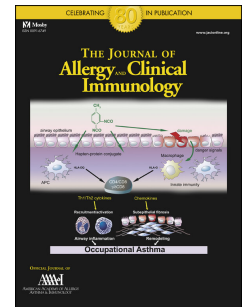


Accepted Manuscript

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PII: S0091-6749(16)30193-2

DOI: [10.1016/j.jaci.2016.04.010](https://doi.org/10.1016/j.jaci.2016.04.010)

Reference: YMAI 12084

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 12 April 2016

Revised Date: 22 April 2016

Accepted Date: 22 April 2016

Please cite this article as: Platts-Mills TA, Schuyler AJ, Erwin EA, Commins SP, Woodfolk JA, IgE in the Diagnosis and Treatment of Allergic Disease, *Journal of Allergy and Clinical Immunology* (2016), doi: 10.1016/j.jaci.2016.04.010.

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IgE in the Diagnosis and Treatment of Allergic Disease:

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Funded by AI-20565

Abstract

Traditionally the concept of allergy implied an abnormal response to an otherwise benign agent (e.g., pollen or food), with an easily identifiable relationship between exposure and the disease. However, there are syndromes where the relationship between exposure to the relevant allergen and the “allergic” disease is not clear. In these cases the presence of specific IgE antibodies can play an important role in identifying the relevant allergen and provide a guide to therapy. Good examples include: chronic asthma and exposure to perennial indoor allergens, and asthma related to infection with fungi. Finally, we are increasingly aware of forms of food allergy where the relationship between exposure and the disease is delayed by 3-6 hours or longer. Three forms of food allergy are now well-recognized, which have distinct clinical features. These are i) anaphylactic sensitivity to peanut; ii) eosinophilic esophagitis (EoE) related to cow’s milk; and iii) delayed anaphylaxis to red meat. In these syndromes the immunology of the responses is dramatically different. Peanut and alpha-gal are characterized by high or very high titer IgE antibodies for Ara h 2 and galactose-alpha-1,3-galactose, respectively. By contrast, EoE is characterized by low levels of IgE specific for milk proteins with high or very high titer IgG4 to the same proteins. The recent finding is that patients with alpha-gal syndrome do not have detectable IgG4 to the oligosaccharide. Thus, the serum results not only identify relevant antigens, but also provide a guide to the nature of the immune response.

JACI: IgE in the Diagnosis and Treatment of Allergic Disease:

Introduction:

The first awareness of immediate hypersensitivity came from the separate investigations of seasonal hay fever over the period 1870-1910 by Blackley, Wymann, Noon and Von Pirquet (see Ref ¹). At that point no evidence of an immune response to pollen could be detected in the serum of these patients ².

However in 1921 Prausnitz showed that serum of allergic subjects could transfer sensitivity locally in the skin ³. This P-K test was used widely between 1921 and 1960, and played a major role in the discovery of IgE. Once IgE was established, it was possible to investigate many different aspects of allergic disease both in terms of diagnosis and in understanding the relevant treatment. Thus, the discovery of IgE completely changed the investigation of these diseases because measurements were of a specific molecule measured in fully defined units; (first international units and then absolute quantitation) ie 2.4 ng/unit. The initial purification of P-K activity was primarily related to ragweed pollen ⁴. Interestingly, Ishizaka did not consider that the establishment of a new isotype of immunoglobulin, IgE, changed the argument about the causal role of pollen in hay fever. By contrast, measurement of IgE has now played a major role in the diagnosis of many allergic diseases other than hay fever. However, it has to be admitted that it is difficult to replicate the simple elegance of the evidence about the role of airborne pollen grains in hay fever.

Early in the 20th century Coca became editor of the Journal of Immunology and proposed the concept of atopy, or the hereditary tendency to become allergic to allergens in the environment. He included asthma, food allergy and eczema as well as hay fever amongst the atopic diseases. Furthermore, he clearly implied that the sensitizing allergens had a causal role in these diseases ⁵. For the main forms of food allergy recognized at that time e.g. fish, nuts, egg or milk, the rapid development of symptoms

including hives or angioedema after exposure left little doubt about causality. By contrast, for chronic asthma in patients, who are sensitized to one or more of the perennial allergens, the evidence of a relationship between allergens and disease is more difficult to establish. This is particularly true for allergens such as dust mite or cockroach where the allergens only become airborne during disturbance^{6, 7}. We would like to focus on the evidence for causality and the role of IgE in relation to three conditions:

- i. Chronic asthma in patients who are sensitized to one or more perennial allergens.
- ii. Allergic symptoms in patients who are colonized with fungi in their lungs, sinuses, or nails.
- iii. The different forms of food allergy including those newly recognized syndromes where the relationship between exposure and response is not “immediate.”

Chronic Asthma in patients with sensitization to one or more perennial allergens

The opinion about the relevance of perennial allergen exposure to chronic asthma has differed from enthusiastic acceptance, to denial or worst of all complete indifference^{8,9}. In part this reflects the failure of some important avoidance studies, and the relentless pressure for, treatment with pharmaceutical products. A more important cause is that the relationship between exposure and symptoms is not simple. Indeed for mite, cockroach, “domestic” mice, and *Alternaria* the patients often have very little sense that they are allergic, and do not understand the role these allergens play in their asthma¹⁰. For cat and dog, it is not unusual for the allergic patients to report that they develop acute symptoms on entering a house with a relevant animal. However, in many communities the majority of the cat allergic patients do not live in a house with a cat and nonetheless sensitization is strongly associated with asthma^{11,12}. In some studies, there is clear evidence that the children raised in a house with a cat are less likely to be sensitized¹². However, for cat the levels of this allergen in schools and homes without a cat increases with the prevalence of domestic cats in the community¹³.

Statements of this kind are possible because the source of these allergens can be identified (i.e. four legged animals) and because of the availability of sensitive assays for relevant allergens in dust or airborne¹⁴. For cockroaches and mice, visual identification is possible but is less reliable than measurement of specific allergens. For dust mites, there is no way of evaluating their presence or concentration in a house without microscopy or the use of an immunoassay^{6, 15}.

There are several major variables that have been used to understand the relationship of allergen exposure to IgE antibodies and asthma:

- I. Many studies have been carried out in areas where dust mites flourish in all homes; e.g. UK, New Zealand, Australia, Costa Rica, and the South East or North West of the USA¹⁶⁻¹⁹.
- II. Studies in areas where, because of the cold temperatures and low humidity, dust mites, cockroaches and *Alternaria* are either absent or at very low levels in the homes^{12, 20, 21}
- III. There are groups within the USA with dramatically different attitudes to animals such that a large section of the population have much lower exposure and sensitization^{19, 22}.
- IV. Pollens and fungal spores such as *Alternaria* have major seasonal and geographic differences in their outdoor particle counts. In areas where most or all homes now have air conditioning, overall exposure to outdoor pollens particularly ragweed is not as high as it was in the 1950's. Since the 1950's overall exposure to pollen allergens, particularly ragweed, has decreased considerably because of widespread use of home air-conditioning which started during the 1960's.

Diagnostic Relevance of IgE antibodies to Asthma

In almost all clinical studies, IgE antibodies or skin tests are carried out with extracts derived from the relevant allergens and these include multiple proteins. The relevance of the results can be

assessed by the prevalence of positive tests; the titer of IgE ab or the size of wheals; or alternatively by tests using specific proteins^{13, 23}. Tests for sensitization to proteins can use purified or recombinant allergens but these tests are carried out almost exclusively with *in vitro* assays.

There are many situations where the significance of sensitization to certain specific proteins is different from that for the whole extract. A simple example is the tropomyosin Der p 10 that cross reacts with both cockroach and shrimp and may not be a relevant inhalant allergen²³. A more complex situation has developed with cat extracts. The major inhalant allergen was purified by Ohman in 1974 as Cat 1, which subsequently became Fel d 1²⁴. More recently, Fel d 4 has been recognized as a second major inhalant allergen²⁵. However, extracts of cat can give positive skin tests or IgE assays because of IgE to two allergens that are not relevant to inhalant allergy. The first of these is Fel d 2 or cat albumin which cross reacts with pork albumin giving rise to the pork cat syndrome^{26, 27}. The second is galactose alpha-1, 3-galactose (alpha-gal) which has emerged as a major allergen related to red meat and is the main epitope on cat IgA²⁸⁻³⁰. A major cause of confusion comes from the difference between pelt extracts, [that contain cat albumin and many proteins carrying alpha-gal] and dander extracts which may only contain significant quantities of Fel d 1.

Understanding the role of Allergens in Asthma and the relevance of IgE antibodies

We would like to focus on three observations about chronic allergic asthma. Firstly, that the strongest association is with sensitization to common perennial inhalant allergens. In particular, this is true for high titer IgE antibodies to those allergens that are prevalent in the patient's community. Secondly, that the relevant allergens such as dust mite, cat, dog or cockroach have been shown to become airborne on particles of 5-35 microns in diameter [Figure 1 and Table I]. Thirdly, that many or most acute episodes of wheezing cannot be related

to increased or even high level exposure to allergens. The implication of this last observation is that exacerbations are most likely occurring as a result of viral or other triggers in the context of underlying inflammation of the lungs and the associated bronchial hyper-reactivity (BHR)^{18, 31, 32}.

In a simplistic view, the fact that bronchial provocation of an allergic individual with nebulized allergen extract can induce both a fall in FEV1 and eosinophil rich inflammation of the lungs could be taken as evidence that allergen exposure is causally related to asthma. However, the contrast between natural exposure of the lungs to particles such as mite faeces, and a bronchial challenge with nebulized allergen could hardly be more extreme^{33, 34} [Figure 2]. Exposure to dust mite allergen is thought to consist of 5-100 fecal particles entering the lungs per day. Nebulized allergen is estimated to be 10 million droplets inhaled over 2-5 minutes, where each droplet is ~2 microns in diameter. Mite faeces have been estimated to contain 0.2 ng of Der p 1 while the comparable figure for nebulized droplets is $<10^{-7}$ ng [Table 1].

We assume that the deposition of a particle on the bronchial wall represents sufficient local allergen not only to activate mast cells but also to recruit eosinophils, basophils, and lymphocytes. What is not clear is how long such an inflammatory focus would last. Our view is that some consequences of the local inflammation could persist for months or longer. These include persistence of changes in the vasculature that encourage local arrival of cells and persistence of allergen specific T cells. It seems likely that allergen specific T cells as well as mast cells could contribute to the long term persistence of BHR. Taken together the results could explain how IgE antibodies are relevant to the process but that in addition locally accumulating T cells could be a target for peptide immunotherapy³⁵.

Sensitization to fungi colonizing the skin, nails, or lungs as a risk factor for allergic diseases

There are literally hundreds of pollinating plants or fungi, that produce spores, which can act as a source of inhalant allergens. By contrast, there are only a limited number of fungi that induce the production of specific IgE, by growing on patients. Fungi can be found growing on the skin, on mucosal surfaces, in the sinuses or in the lungs. In many cases, these fungi have specific properties that facilitate their growth. Thus, dermatophytes have a specific ability to grow on keratinized surfaces (ie skin and nails). By contrast, *Aspergillus* species are able to grow both at the temperature of the soil and also at 37° C in the lungs or sinuses. Although the significance of fungal sensitization was recognized in the 1930's, acceptance of the clinical importance fluctuated until allergic broncho pulmonary aspergillosis (ABPA) was established by Hinson, Patterson, Pepys and others^{36, 37}. With the discovery of IgE, it became possible to define the diseases better and to show that many of these patients had IgE antibodies specific for fungal allergens. Although the primary focus of studies on fungal sensitization related to the lungs it was recognized years ago that sensitization to both Dermatophytes and *Candida* can also be a cause of urticaria and angioedema³⁸. Diagnostic criteria have been suggested for some fungal diseases; however, even with ABPA a large proportion of cases who are growing either *Aspergillus* or other fungi in their lungs do not fulfill criteria for ABPA or ABPM. These cases can be described as *forme fruste* allergic broncho pulmonary mycosis (ABPM).

There is an important distinction between cases where the fungus is growing at the site of the inflammatory reaction e.g. *Aspergillus* or other fungi growing in the lungs or sinuses; and cases where fungi growing on the feet appear to be the cause of an inflammatory response elsewhere in the body³⁹⁻⁴¹. The latter form can be described as an "ID" reaction i.e. comparable to those skin lesions which were associated with pulmonary TB but did not contain organisms that could infect a guinea pig. Historically, *Candida* and *Trichophyton* were well recognized by dermatologists as

causes of ID reactions in the hands or skin^{38, 42}. We would like to present three cases that illustrate these differences. **[Box 1]**.

Over the last 30 years, the field of fungal sensitivity has changed progressively because of the availability and widespread use of new oral anti-fungal drugs. In the early 1980's, the only oral drugs that were available were griseofulvin and ketoconazole. Both of these drugs had severe side effects. The first new drug to arrive was fluconazole, and then came itraconazole, terbinafine and voriconazole⁴³⁻⁴⁵. This means that there are not only better drugs that can be used to treat fungal infection associated with allergic disease, but that in addition there may be less cases of severe onychomycosis because of successful treatment in primary care. On the other hand, the use of antibiotics and steroids encourages growth of fungi particularly the yeasts. It is well recognized that many women need to take two or three doses of fluconazole when they are treated with antibiotics. It is a different question whether high dose inhaled steroids can enhance yeast growth in the lungs. Our view is that, all severe asthmatics who produce sputum should be monitored for growth of aspergillus, yeasts or other fungi in the lungs **[See Case I Box 1]**⁴⁶.

With all these fungal diseases, it is an open question whether it is necessary to obtain cultures before treatment. There is good evidence that patients with ABPA who are colonized with an *Aspergillus* strain that is sensitive to itraconazole respond better to treatment with that drug⁴⁴. The *in vitro* tests of sensitivity of cultured fungi to anti-fungals are improving and have become more readily available. We would argue that identifying the species improves the understanding of the condition and can contribute to the success of treatment. On the other hand, Denning et al demonstrated that itraconazole could improve the outcome for patients with asthma, who have skin sensitization to fungi, without establishing the presence of a colonizing organism^{45, 46}.

The role of IgE assays in the diagnosis of different forms of food allergy: peanut anaphylaxis, eosinophilic esophagitis (EoE), and delayed anaphylaxis to red meat (the alpha-gal syndrome).

Over the last 20 years, both the scientific investigation of allergic disease and the practice of allergy have moved progressively towards food allergy. In our practice, in central Virginia, this has included major increases in three different forms of allergic reactions to food. The thing that is truly interesting about this is that these three forms of “allergy” appear to be distinct both in their clinical presentation and in the underlying immune response [Table 2]. Peanut allergy is taken as the model for immediate and often anaphylactic reactions to food in children. This condition has been well recognized for many years but has increased rapidly during the period 1990-2010⁴⁷. Initially it was possible to argue that there was increased awareness of the disease but the prevalence of high titer IgE ab in random cohorts of children has increased⁴⁸.

The second form of food allergy is, eosinophilic esophagitis (EoE) which was hardly recognized at all before 1990 and has become progressively more common over the last twenty years so that today most academic centers in the USA have an EoE clinic. The disease is characterized by chronic inflammation of the esophagus which gets better with a diet including avoidance of cow’s milk⁴⁹⁻⁵¹. Although many of the children have detectable IgE ab to cow’s milk, wheat, egg or soy proteins, the titers are consistently low [Figure 3]⁵². Recent studies have shown that almost all children with EoE have high or very high titer IgG4 antibodies to cow’s milk proteins^{53, 54}. Clinically, patients with EoE continue eating cow’s milk proteins because they are not aware of being allergic. Thus, EoE does not have the features of an IgE mediated disease and also does not improve during treatment with omalizumab. If EoE is a T cell mediated disease, we should be able to focus on T cells that can recruit eosinophils, give rise to low levels of IgE and strongly enhance IgG4. In addition, these findings have major implications for the correct treatment.

The third group of patients presents with anaphylaxis or urticaria to red meat and is most common in adults^{29, 55, 56}. Indeed the typical case is a man over 50 years old who develops episodes of hives, angioedema, or anaphylaxis which start several hours after a meal which includes red meat. On skin testing, they have negative or weakly positive prick tests to a commercial beef extract, but strongly positive IgE assays for the oligosaccharide alpha-gal^{28, 29, 56, 57}. Similar syndromes have now been described in many countries including Australia, Sweden, France, Germany and Japan^{56, 58-60}. In each of these countries, the reported reactions to meat have been delayed for several hours after eating; IgE ab is directed at the same oligosaccharide; and there is reasonable or good evidence that tick bites are the primary cause of the sensitization^{58, 60, 61}. In our practice, the presence of alpha-gal specific IgE that represents more than 1% of the total IgE; a convincing report of bites from ticks [or chiggers]; and a history of urticaria or anaphylaxis starting 2 to 6 hours after consuming red meat provides a high level of diagnostic certainty.

The evidence about these three forms of food allergy has provided a stronger basis for evaluating reactions to food in general and several other syndromes have become much clearer. A simple example is the pork cat syndrome, which was first recognized in Europe^{26, 27}. In evaluating cases of reactions attributed to red meat, we found patients who were negative to alpha-gal but positive to pork with weak results for beef. In these cases, the pork response was explained by IgE specific for pork albumin. Absorption studies provided evidence that cat albumin was responsible for the initial sensitization and that the food reactions are due to cross reactivity²⁷. Patients with IgE to alpha-gal can have delayed reactions to foods containing gelatin: such as marshmallows or jelly babies⁶². This occurs because gelatin made from mammals can carry alpha-gal; however, in addition there are patients who are allergic to the protein epitopes on gelatin. The gelatins cross react extensively, and this has given rise to anaphylactic reactions to vaccines containing gelatin⁶³. Most recently, we have seen a case reporting delayed reactions to mammalian products including

cow's milk and beef; who was negative to alpha-gal. In this case, the dominant assay result was to milk and the specific protein was BSA⁶⁴. What matters here is that testing IgE to specific proteins and/or to the oligosaccharide alpha-gal makes it possible to identify the relevant allergens in many cases.

Relevance of the Route of Sensitization to the specificity and titer of IgE ab responses

For many years, there have been questions about whether sensitization to allergens could occur via routes other than inhaled or oral. The obvious example was atopic dermatitis (AD) where the skin is already damaged and IgE antibodies to inhalant allergens can reach high levels^{65, 66}. Furthermore, patients with severe AD often develop IgE specific for foods, bacterial antigens, and also to fungi, particularly yeasts such as *Pityrosporum ovale*^{67, 68}. During the last fifteen years, the evidence for sensitization through the skin has become much clearer. In relation to peanut allergy, it is well established that sensitization correlates with eczema and that the use of topical treatments that incorporate peanut proteins increases the risk of sensitization. The success of early introduction of oral peanut in decreasing sensitization has added further evidence^{69, 70}. Perhaps equally striking is the outbreak of wheat sensitization among women in Japan who use a mildly abrasive face preparation that contains wheat proteins⁷¹⁻⁷³.

Given that the oral route can be tolerogenic and that there are obvious advantages to not becoming allergic to food, the question arises whether the skin route can break tolerance induced by the oral route. This question became more complicated with the discovery that sensitization to alpha-gal was common in large areas of the United States^{28, 55}. It is now clear that tick bites are the dominant cause of this novel form of allergy in the USA, and that this syndrome provides another example of high titer IgE production, where the skin is the route of sensitization^{58, 61}. Since we all make IgM and IgG2 specific to alpha-gal as part of an innate response to gut bacteria it could be argued that

the IgE response to alpha-gal must have broken tolerance. However, we have remarkably little understanding of either the immunology of IgE responses to oligosaccharides, or the relevance of T cells in the response to tick bites. Initial studies in collaboration with Dr. Rispens in Amsterdam suggested that the response to alpha-gal does not include IgG4⁷⁴. Recently, we have extended those observations to confirm that neither the natural responses to this oligosaccharide or the IgE dominated response includes IgG4⁵⁴. There is excellent evidence that oral desensitization of children with immediate hypersensitivity to milk or peanut can increase or induce IgG4 antibodies^{69, 70, 75, 76}. Our current view is that the skin is an excellent route for inducing IgE to schistosomes, ticks, and peanut, etc , but in general is a poor route for inducing or boosting IgG4 antibodies. The exception may be multiple bee stings. The early data on IgG4 argued that this response required prolonged exposure. We would add that the oral route may be the best route for boosting high levels of IgG4 in an already sensitized individual, and that the esophagus may be an important route for this response [Figure 4].

Conclusions

The three areas we have focused on here were chosen because they illustrate the difficulty of understanding the role of allergens in diseases, where the relationship between exposure and the disease is not obvious to the patient. In these studies, the ability to measure IgE to allergen extracts, purified proteins and novel allergens has been central to understanding the conditions. Our first experience with this process came from investigating the epidemic of asthma in children in the UK at a time when it was already clear that many of these children were allergic to dust mites. The proof that Der p 1 was a major target for IgE was an essential part of the definition of this protein as a major mite allergen⁷⁷. In turn that was essential to proposing assays of Der p 1 in dust as a surrogate for microscopic counting of dust mites^{6, 15}.

Although sensitization to yeasts and dermatophytes was well known before the discovery of IgE, the level of scientific or clinical acceptance was “meager”. Purification of *Aspergillus* allergens allowed measurements of IgG and IgE antibodies. Sensitization to *Trichophyton* provides another example of the overall problem because very few of the patients have considered the possible relevance of the fungal infection on their feet to their disease. Purification of the relevant proteins was an important part of understanding the immune response that starts in the skin⁴¹. Clinically, the availability of the newer anti-fungals has completely changed the equation. We would stress that these cases continue to appear and that without considering fungal sensitization and using oral anti-fungal treatment they will continue to be treated with high doses of steroids.

The two new syndromes of food allergy illustrate well the problems with diagnosis and treatment of allergic diseases where allergen exposure does not give rise to immediate symptoms.

- I. With EoE the clearest guide to the relevance of foods comes from the response to diet and particularly from the exacerbations that can occur after reintroduction of specific foods. However, the esophageal symptoms don't reappear for 24 hours or more. Skin tests are generally not convincing in defining the important sensitivity. Using serum IgE assays, the presence of IgE to cow's milk proteins is clear⁵². Nonetheless, it seems unlikely that IgE antibodies play a direct role in the disease. The most recent findings about IgG4 antibodies to milk proteins add further evidence about the relevance of these proteins
- II. During the first years of the 21st century, many of the patients with the alpha-gal syndrome presented to allergists, and were consistently advised that their symptoms were not due to food allergy. This was because.
 - a) The onset of allergic reactions to meat in adult life was considered to be rare.

b) The prick skin tests to meat showed 2-3 mm wheals at best [Intradermal skin tests are positive].

c) The patients reported long delays after eating red meat before the symptoms started.

Without a specific assay for IgE antibodies to alpha-gal it would have been very difficult to connect the dots⁷⁸. Equally, establishing that the IgE binding to meat extracts was IgE specific for an oligosaccharide provided a further enigma until it became clear that sensitization was occurring through the skin.

Today it is clear that the skin is an important route for IgE responses; that a large proportion of allergic disease involves conditions where the relationship of exposure to disease is not obvious, and that the ability to measure IgE and IgG4 antibodies to specific proteins can provide an important part of the diagnosis and management [Table 3].

Legends to Figures

Fig 1 Allergens become airborne and are inhaled in the form of particles which range from 5-30 microns in diameter (see Table I). The number of allergen molecules free in the air is essentially zero and none of the source materials are inhaled.

Fig 2 Contrast between natural exposure to airborne allergen and bronchial provocation with nebulized extract. Natural exposure is to relatively large particles which contain a wide range of allergens and adjuvants.

Fig 3 IgE antibodies in patients with Eosinophilic Esophagitis (EoE) compared to two anaphylaxis syndromes; peanut and alpha-gal. Although IgE to milk, wheat, egg and soy are common in children with EoE the titers are generally low or very low.

Fig 4 Contrast between three body surfaces that are relevant to the induction and maintenance of immune responses for food allergens. i) Skin; keratinized squamous epithelium ii) esophagus; non keratinized squamous epithelium and iii) columnar epithelium in the intestines. We recognize that the lymphoid tissues draining the upper airways and mouth are also important for response to food allergens.

Box I

Three cases with severe allergic disease; documented fungal infection; sensitization; and response to anti-fungal treatment.

- 43 yr F: repeated severe attacks of asthma; Candida colonization of sinuses and lungs and full response to Fluconazole. IgE to Candida albicans 13.3 IU/ml.
- 62 yr M: judge with almost continuous facial angioedema taking 50 mg prednisone per day: severe fungal infection of feet for “many years”. Skin test Trichophyton ++ IgE ab 5.0 IU/ml. Trichophyton grown from nail scrapings. No response to three months fluconazole full response to itraconazole over one year.
- 56 yr M: diving instructor. Increasingly severe late onset asthma. Continuous obstruction with FEV1 45% predicted despite 40mg prednisone per day. Culture of BAL grew Aspergillus and Nocardia Successful treatment with Voriconazole and Augmentin for one year.

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Table 1. Sizes of particles carrying some airborne allergens associated with asthma

	Diameter (μm) ^a	Allergen Concentration	<u>Number of Particles</u>		Allergen per Particle or Droplet
			To Deliver 20 ng	Entering the lung	
Pollen grains	~20	~20 mg/ml ^b	~100	~5-100/day	0.1-0.3 ng
Mite fecal Pellets	15-35	~20 mg/ml	~100	~5-100/day	0.1-0.3 ng
Alternaria Spores	14x10	?	?	?	--
Rat allergen	7	1 mg/ml ^b	~27,000	~10,000/hr	~0.001 ng
Nebulized Allergen	2	1 $\mu\text{g}/\text{ml}$ ^c	>10 ⁸	~10 ⁸ /2 min	~10 ⁻⁷ ng

^aSizes determined from (1) direct microscopy, (2) distribution on Cascade impactor, (3) theoretical behavior of Wright's nebulizer.

^bConcentration of allergen in mite feces calculated from quantity and diameter; concentration of rat allergen is that measured in whole rat urine

^cConcentration of mite antigen P₁ or ragweed antigen E commonly used for bronchial provocation.

Table 2. Clinical and immunological differences between three common forms of allergic disease

	Peanut Anaphylaxis	Eosinophilic Esophagitis	Delayed Anaphylaxis to Mammalian Meat (Alpha-gal Syndrome)
Relevant Allergens	Ara h 1, Ara h 2 (Peanut)	Bos d 4, Bos d 5 (Cow's Milk) Wheat Proteins	Galactose-alpha-1,3-galactose [†]
Route of Sensitization	Skin	Esophagus (?) Skin unlikely	Skin (Tick bites)
Risk Factors	Eczema	Unknown	Outdoor Activity Lipitor (?)
Serum IgE Ab	High titer IgE to peanut proteins (5-500 IU/mL)	Low titer or negative IgE to cow's milk proteins [§] (<0.1-2 IU/mL)	High titer IgE to alpha-gal (Often 10-30% of total IgE)
Treatment	Diet free of peanut Oral Immunotherapy (OIT)	Diet free of cow's milk with swallowed cromolyn qid [°]	Diet free of mammalian products
Prevention	Prevent eczema Feed peanut from 3 months	Not known	Avoid tick bites
Timing of Reaction to Foods	15-60 minutes to local and generalized swelling*	12-24 hours	3-6 hours after eating red meat
[†] Chung et al., NEJM 2008; (28) Commins et al., JACI 2009 (29) [§] Erwin et al., JACI Pract 2015 (52) [°] Erwin et al., JACI 2016 (JACI, in press, 2016) [*] Du Toit et al., NEJM 2015 (69)			

Table 3. Relevance of Specific IgE antibodies to the treatment of Allergic Disease

Disease	IgE Assay & Skin Test	Role of IgE	Treatment [°]	Immunotherapy [¥]
Hay Fever	Seasonal Allergens	Primary	Avoidance [‡]	SCIT
Asthma	Indoor & Outdoor Allergens	Central	a) Avoidance [‡] b) Omalizumab [‡]	SCIT
Late Onset & Severe Asthma	AERD	Not Clear		
	Vocal Chord	Not Relevant	Voice Therapy, etc. [‡]	---
	Indoor Allergens	Central	a) Avoidance [‡]	SCIT
	Fungal	Mixed	b) Omalizumab [‡]	?
Food Allergy Immediate Reactions	Peanut, etc.	Primary	Avoidance	OIT
Eosinophilic Esophagitis	Cow's Milk, Wheat...	Probably none	Avoidance [‡]	SCIT?
Alpha-gal Syndrome	Alpha-gal, beef...	Primary	Avoidance [‡]	---

[°]Anti-histamines, local steroids, etc. are asked for all these disease.

[‡]Dependent on identifying specific allergen or allergens

[¥]Subcutaneous Immunotherapy (SCIT) or Oral immunotherapy (OIT)

References

1. Platts-Mills T. The Allergy Epidemics: 1870-2010. *JACI* 2015; 136:3-13.
2. Von Pirquet C. In P.G. H. Gell et al (eds). *Clinical Aspects of Immunology*. Blackwell Scientific Publications, Oxford 1906.
3. Prausnitz C, Kustner H. Studien uber die ueberempfindlichkeit. *Zentralbl. Bakteriol. [Orig. A]* 1921; 86:160.
4. Ishizaka K, Ishizaka T, Hornbrook M. Physicochemical properties of reaginic antibody. V. Correlation of reaginic activity with gamma-E-globulin antibody. *J Immunol* 1966; 97:840-53.
5. Coca A. Specific sensitiveness as a cause of symptoms in disease. *Bull N Y Acad Med* 1930; 6:593-604.
6. Tovey ER, Chapman MD, Wells CW, Platts-Mills TA. The distribution of dust mite allergen in the houses of patients with asthma. *Am Rev Respir Dis* 1981; 124:630-5.
7. de Blay F, Sanchez J, Hedelin G, Perez-Infante A, Verot A, Chapman MD, et al. Dust and airborne exposure to allergens derived from cockroach (*Blattella germanica*) in low-cost public housing in Strasbourg (France). *JACI* 1997; 99 (1 Pt 1):107-12.
8. Murray CS, Poletti G, Kebabdz T, Morris J, Woodcock A, Johnston SL, et al. Study of modifiable risk factors for asthma exacerbations: virus infection and allergen exposure increase the risk of asthma hospital admissions in children. *Thorax* 2006; 61:376-82.
9. von Hertzen LH, Haatela T. House dust mites in atopic diseases: accused for 45 years but not guilty? *American Journal of Respiratory and Critical Care Medicine/AJRCCM* 2009; 180:113-9.
10. Platts-Mills T, Erwin E, Heymann P, Woodfolk J. The evidence for a causal role of dust mites in asthma. *Am J Respir Crit Care Med* 2009; 180:109-13.

11. Platts-Mills T, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet* 2001; 357:752-6.
12. Perzanowski MS, Ronmark E, Platts-Mills TA, Lundback B. Effect of cat and dog ownership on sensitization and development of asthma among preteenage children. *Am J Respir Crit Care Med* 2002; 166:696-702.
13. Konradsen JR, Fujisawa T, van Hage M, Hedlin G, Hilger C, Kleine-Tebbe J, et al. Allergy to furry animals: New insights, diagnostic approaches, and challenges. *JACI* 2015; 135:616-25.
14. Leaderer BP, Belanger K, Triche E, Holford T, Gold DR, Kim Y, et al. Dust mite, cockroach, cat, and dog allergen concentrations in homes of asthmatic children in the northeastern United States: impact of socioeconomic factors and population density. *Environ Health Perspect* 2002; 110:419-25.
15. Luczynska CM, Arruda LK, Platts-Mills TA, Miller JD, Lopez M, Chapman MD. A two-site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp. allergens, Der p I and Der f I. *J Immunol Methods* 1989; 118:227-35.
16. Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. A prospective study. *N Engl J Med* 1990; 323:502-7.
17. Erwin EA, Wickens K, Custis NJ, Siebers R, Woodfolk J, Barry D, et al. Cat and dust mite sensitivity and tolerance in relation to wheezing among children raised with high exposure to both allergens. *J Allergy Clin Immunol* 2005; 115:74-9.
18. Soto-Quiros M, Avila L, Platts-Mills T, Hunt J, Erdman D, Carper H, et al. High titers of IgE antibody to dust mite allergen and risk for wheezing among asthmatic children infected with rhinovirus. *JACI* 2012; 129:1499-505.

19. Gruchalla R, Pongracic J, Plaut M, Evans Rr, Visness C, Walter M, et al. Inner City Asthma Study: relationships among sensitivity, allergen exposure, and asthma morbidity. *JACI* 2005; 115:478-85.
20. Ronmark E, Bjerg A, Perzanowski M, Platts-Mills T, Lundback B. Major increase in allergic sensitization in schoolchildren from 1996 to 2006 in northern Sweden. *J Allergy Clin Immunol* 2009; 124:357-63, 63 e1-15.
21. Erwin EA, Ronmark E, Wickens K, Perzanowski MS, Barry D, Lundback B. Contribution of dust mite and cat specific IgE to total IgE: relevance to asthma prevalence. *J Allergy Clin Immunol* 2007; 119:359-65.
22. Busse WW, Morgan WJ, Gergen PJ, Mitchell HE, Gern JE, Liu AH, et al. Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children. *N Engl J Med* 2011; 364:1005-15.
23. Bronnert M, Mancini J, Birnbaum J, Agabriel C, Labeuf V, Porri F, et al. Component-resolved diagnosis with commercially available D. pteronyssinus Der p 1, Der p 2 and Der p 10: relevant markers for house dust mite allergy. *Clin Exp Allergy* 2012; 42:1406-15.
24. Ohman JL, Lowell FC, Bloch KJ. Allergens of mammalian origin: characterization of allergen extracted from cat pelts. *J Allergy Clin Immunol* 1973; 52:231-41.
25. Hales B, Chai L, Hazell L, Elliot C, Stone S, O'Neil S, et al. IgE and IgG binding patterns and T-cell recognition of Fel d 1 and non-Fel d 1 cat allergens. *JACI Pract.* 2013; 1:656-65.
26. Hilger C, Kohnen M, Grigioni F, Lehnert C, Hentges F. Allergic cross-reactions between cat and pig serum albumin. Study at the protein and DNA levels. *Allergy* 1997; 52:179-87.
27. Posthumus J, James HR, Lane CJ, Matos LA, Platts-Mills TA, Commins SP. Initial description of pork-cat syndrome in the United States. *J Allergy Clin Immunol* 2013; 131:923-5.
28. Chung CH, Mirakhur B, Chan E, Le QT, Berlin J, Morse M, et al. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. *N Engl J Med* 2008; 358:1109-17.

29. Commins SP, Satinover SM, Hosen J, Mozena J, Borish L, Lewis BD, et al. Delayed anaphylaxis, angioedema, or urticaria after consumption of red meat in patients with IgE antibodies specific for galactose-alpha-1,3-galactose. *J Allergy Clin Immunol* 2009; 123:426-33.
30. Gronlund H, Adedoyin J, Commins SP, Platts-Mills TA, van Hage M. The carbohydrate galactose-alpha-1,3-galactose is a major IgE-binding epitope on cat IgA. *J Allergy Clin Immunol* 2009; 123:1189-91.
31. Heymann PW, Platts-Mills TA, Johnston SL. Role of viral infections, atopy and antiviral immunity in the etiology of wheezing exacerbations among children and young adults. *Pediatr Infect Dis J* 2005; 24:S217-22, discussion S20-1.
32. Zambrano JC, Carper HT, Rakes GP, Patrie J, Murphy DD, Platts-Mills TA, et al. Experimental rhinovirus challenges in adults with mild asthma: response to infection in relation to IgE. *J Allergy Clin Immunol* 2003; 111:1008-16.
33. Platts-Mills TA, Woodfolk JA. Allergens and their role in the allergic immune response. *Immunol Rev* 2011; 242:51-68.
34. Task Group on Lung Dynamics. *Health Physics* 1966; 12:173.
35. Larche M. Mechanisms of peptide immunotherapy in allergic airways disease. *Ann Am Thorac Soc* 2014.
36. Hinson K, Moon A, Plummer N. Broncho-pulmonary aspergillosis; a review and a report of eight new cases. *Thorax* 1952; 7:317-33.
37. Forman S, Fink J, Moore V, Wang J, Patterson R. Humoral and cellular immune responses in *Aspergillus fumigatus* pulmonary disease. *JACI* 1978; 62:131-6.
38. Champion R, Roberts S, Carpenter R, Roger J. Urticaria and angio-oedema. A review of 554 patients. *Br J Dermatol* 1988; 88:588-97.

39. Ward GW, Jr., Karlsson G, Rose G, Platts-Mills TA. Trichophyton asthma: sensitisation of bronchi and upper airways to dermatophyte antigen. *Lancet* 1989; 1:859-62.
40. Wise F, Sulzberger MB. Urticaria and hay fever due to Trichophyton (Epidermophyton interdigital). *JAMA* 1930:1504-8.
41. Woodfolk JA, Wheatley LM, Piyasena RV, Benjamin DC, Platts-Mills TA. Trichophyton antigens associated with IgE antibodies and delayed type hypersensitivity. Sequence homology to two families of serine proteinases. *J Biol Chem* 1998; 273:29489-96.
42. Weary P, Guerrant J. Chronic urticaria in association with dermatophytosis. Response to the administration of griseofulvin. *Arch Dermatol.* 1967; 95:400-1.
43. Ward GW, Jr., Woodfolk JA, Hayden ML, Jackson S, Platts-Mills TA. Treatment of late-onset asthma with fluconazole. *J Allergy Clin Immunol* 1999; 104:541-6.
44. Stevens DA, Schwartz HJ, Lee JY, Moskovitz BL, Jerome DC, Catanzaro A, et al. A randomized trial of itraconazole in allergic bronchopulmonary aspergillosis. *N Engl J Med* 2000; 342:756-62.
45. Denning DW, O'Driscoll BR, Powell G, Chew F, Atherton GT, Vyas A, et al. Randomized controlled trial of oral antifungal treatment for severe asthma with fungal sensitization: The Fungal Asthma Sensitization Trial (FAST) study. *Am J Respir Crit Care Med* 2009; 179:11-8.
46. Kennedy JL, Heymann PW, Platts-Mills TA. The role of allergy in severe asthma. *Clin Exp Allergy* 2012; 42:659-69.
47. Flinterman A, Knol E, Lencer D, Bardina L, den Hartog Jager C, Lin J, et al. Peanut epitopes for IgE and IgG4 in peanut-sensitized children in relation to severity of peanut allergy. *J Allergy Clin Immunol* 2008; 121:737-43.
48. Bunyavanich S, Rifas-Shiman SL, Platts-Mills T, Workman L, Sordillo JE, Gillman M, et al. Peanut allergy prevalence among school-age children in a US cohort not selected for any disease. *J Allergy Clin Immunol* 2014; 134:753-5.

49. Simon D, Cianferoni A, Spergel J, Aceves S, Holbreich M, Venter C, et al. Eosinophilic esophagitis is characterized by a non-IgE-mediated food hypersensitivity. *Allergy* 2016; 10.1111/all:12846.
50. Kelly K, Lazenby A, Rowe P, Yardley J, Perman J, Sampson H. Eosinophilic esophagitis attributed to gastroesophageal reflux: improvement with an amino acid-based formula. *Gastroenterology* 1995; 109:1503-12.
51. Spergel JM, Brown-Whitehorn TF, Cianferoni A, Shuker M, Wang ML, Verma R, et al. Identification of causative foods in children with eosinophilic esophagitis treated with an elimination diet. *J Allergy Clin Immunol* 2012; 130:461-7 e5.
52. Erwin E, Tripathi A, Ogbogu P, Commins S, Slack M, Cho C, et al. IgE Antibody Detection and Component Analysis in Patients with Eosinophilic Esophagitis. *J Allergy Clin Immunol Pract* 2015; 15:00260-3.
53. Clayton F, Fang J, Gleich G, Lucendo A, Olalla J, Vinson L, et al. Eosinophilic esophagitis in adults is associated with IgG4 and not mediated by IgE. *Gastroenterology* 2014; 147:602-9.
54. Schuyler A, Tripathi A, Kruszewski P, Russo J, Workman L, Platts-Mills T, et al. Serum IgG4 antibodies in Pediatric subjects with eosinophilic esophagitis treated with cow's milk elimination diet or swallowed fluticasone: high levels of specific IgG4 to cow's milk components despite low to negative IgE antibodies. *JACI* 2016; AB232 Abstracts:756.
55. Commins SP, Kelly LA, Ronmark E, James HR, Pochan SL, Peters EJ, et al. Galactose- α -1,3-Galactose-Specific IgE Is Associated with Anaphylaxis but Not Asthma. *Am J Respir Crit Care Med* 2012; 185:723-30.
56. Hamsten C, Tran T, Starkhammar M, Brauner A, Commins SP, Platts-Mills TA, et al. Red meat allergy in Sweden: association with tick sensitization and B-negative blood groups. *JACI* 2013; 132:1431-4.

57. Jappe U. [Update on meat allergy. alpha-Gal: a new epitope, a new entity?]. *Hautarzt* 2012; 63:299-306.
58. Van Nunen SA, O'Connor KS, Clarke LR, Boyle RX, Fernando SL. An association between tick bite reactions and red meat allergy in humans. *Med J Aust* 2009; 190:510-1.
59. Mullins RJ, James H, Platts-Mills TA, Commins S. Relationship between red meat allergy and sensitization to gelatin and galactose-alpha-1,3-galactose. *J Allergy Clin Immunol* 2012; 129:1334-42 e1.
60. Chinuki Y, Ishiwata K, Yamaji K, Takahashi H, Morita E. *Haemaphysalis longicornis* tick bites are a possible cause of red meat allergy in Japan. *Allergy* 2016; 713.
61. Commins S, James H, Kelly E, Pochan S, Workman L, Perzanowski M, et al. The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose- α -1,3-galactose. *JACI* 2011; 127:1286-93.
62. Hilger C, Fischer J, Swiontek K, Hentges F, Lehnert C, Eberlein B, et al. Two galactose-alpha-1, 3-galactose carrying peptidases from pork kidney mediate anaphylactogenic responses in delayed meat allergy. *Allergy* 2016.
63. Agarwal N, Spalding C, Nassef M. Life-threatening intraoperative anaphylaxis to gelatin in Floseal during pediatric spinal surgery. *J Allergy Clin Immunol Pract* 2015; 3:110-1.
64. 64 Hr.
65. Glatz M, Buchner M, von Bartenwerffer W, Schmid-Grendelmeier P, Worm M, Hedderich J, et al. *Malassezia* spp. -specific immunoglobulin E level is a marker for severity of atopic dermatitis in adults. *Acta Derm Venereol* 2015; 95:191-6.
66. Sampson H. The evaluation and management of food allergy in atopic dermatitis. *Clin Dermatol* 2003; 21:183-92.

67. White T, Findley K, Dawson TJ, Scheynius A, Boekhout T, Cuomo C, et al. Fungi on the skin: dermatophytes and *Malassezia*. *Cold Spring Harb Perspect Med* 2014; 4:019802.
68. Kekki O, Scheynius A, Poikonen S, Koskinen A, Kautiainen H, Turjanmaa K. Sensitization to *Malassezia* in children with atopic dermatitis combined with food allergy. *Pediatr Allergy Immunol* 2013; 24:244-9.
69. Du Toit G, Roberts G, Sayre P, Bahnson H, Radulovic S, Santos A. Randomized trial of peanut consumption in infants at risk for peanut allergy. *N Engl J Med* 2015; 372:803-13.
70. Du Toit G, Sayre P, Roberts G, Sever M, Lawson K, Bahnson H, et al. Effect of Avoidance on Peanut Allergy after Early Peanut Consumption. *N Engl J Med* 2016:Epub ahead of print.
71. Yokooji T, Kurihara S, Murakami T, Chinuki Y, Takahashi H, Morita E. Characterization of causative allergens for wheat-dependent exercise-induced anaphylaxis sensitized with hydrolyzed wheat proteins in facial soap. *Allergol Int* 2013; 64:435-45.
72. Dohi M, Suko M, Sugiyama H, Yamashita N, Tadokoro K, Juji F, et al. Food-dependent, exercise-induced anaphylaxis: a study of 11 Japanese cases. *J Allergy Clin Immunol* 1991; 87:34-40.
73. Brockow K, Kneissl D, Valentini L, Zelger O, Grosber M, Kugler C, et al. Using a gluten oral food challenge protocol to improve diagnosis of wheat-dependent exercise-induced anaphylaxis. *J Allergy Clin Immunol* 2014; 14:01197-X.
74. Rispens T, Derksen NI, Commins SP, Platts-Mills TA, Aalberse RC. IgE production to alpha-gal is accompanied by elevated levels of specific IgG1 antibodies and low amounts of IgE to blood group B. *PLoS One* 2013; 8:e55566.
75. Skripak JM, Nash SD, Rowley H, Brereton NH, Oh S, Hamilton RG, et al. A randomized, double-blind, placebo-controlled study of milk oral immunotherapy for cow's milk allergy. *J Allergy Clin Immunol* 2008; 122:1154-60.

76. Vickery B, Lin J, Kulis M, Fu Z, Steele P, Jones S, et al. Peanut oral immunotherapy modifies IgE and IgG4 responses to major peanut allergens. *JACI* 2013; 131:128-34.
77. Chapman MD, Platts-Mills TA. Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*-antigen P1. *J Immunol* 1980; 125:587-92.
78. Steinke J, Platts-Mills T, Commins S. The alpha-gal story: Lessons learned from connecting the dots. *JACI* 2015; 135:589-96.

Fig 1

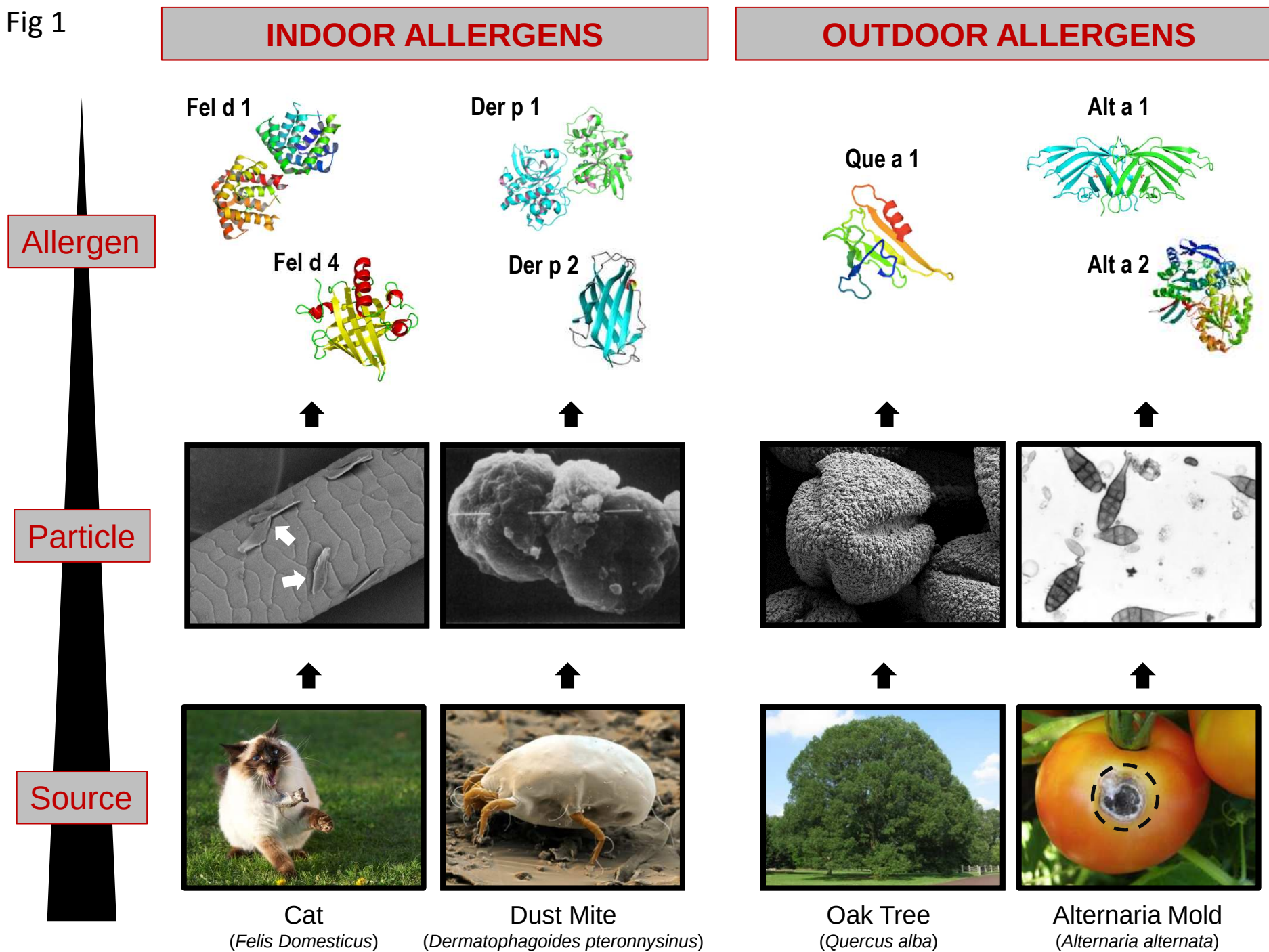
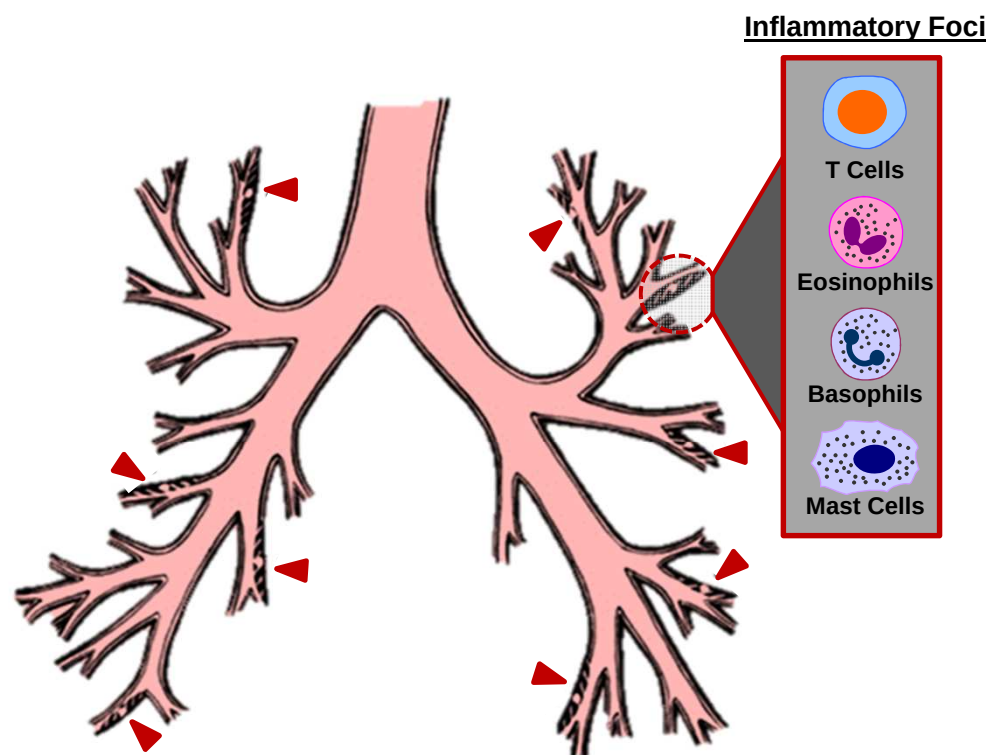


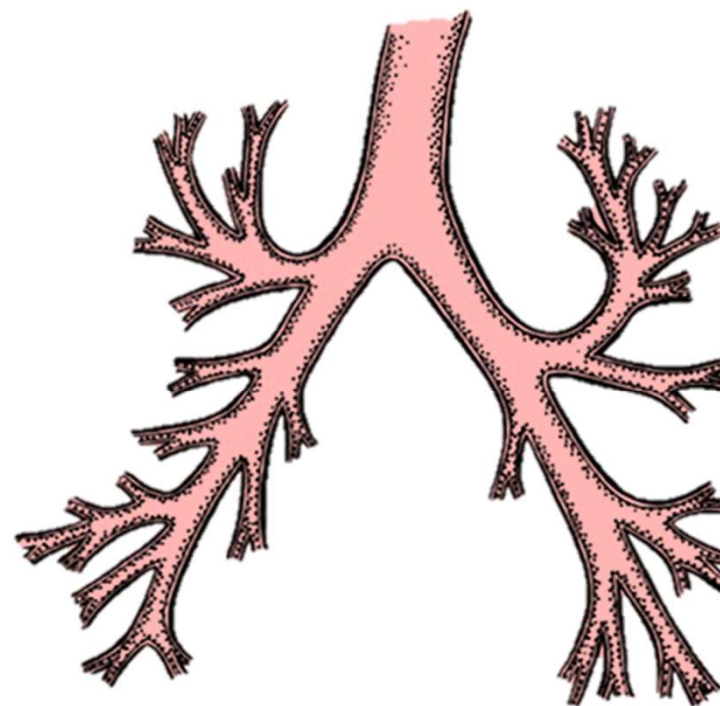
Fig 2

A. **Natural exposure** consist of a small number (10-200) of large particles per day



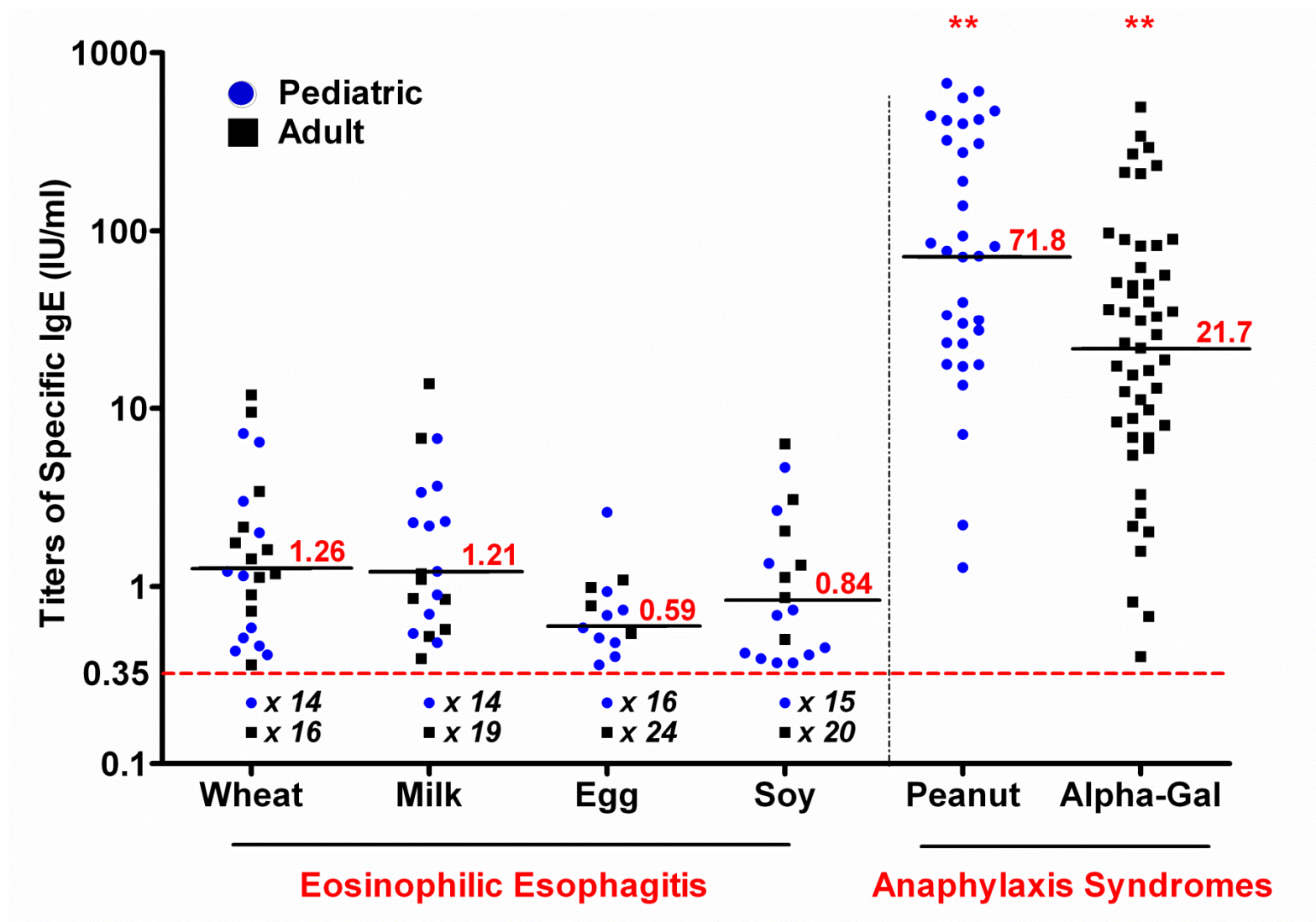
The concentration of allergen in the particles is sufficient to produce local inflammatory foci that can contribute to a prolonged increase in bronchial hyperreactivity.

B. **Bronchial challenge** with nebulized allergen extracts ($>10^7$ droplets in 2-5 min)



Challenge of an allergic patient can produce a fall in FEV1 and in some cases a transient increase in bronchial hyperreactivity

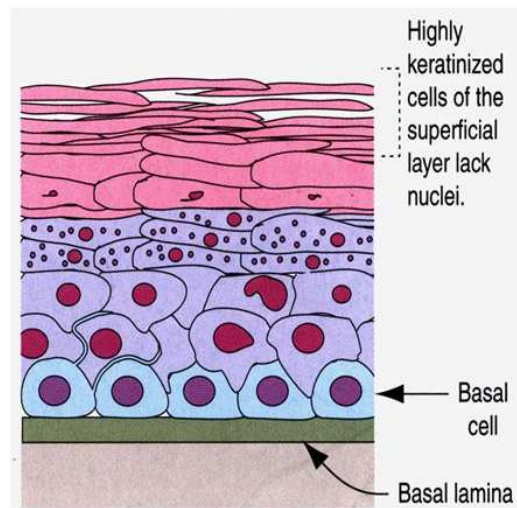
Fig 3



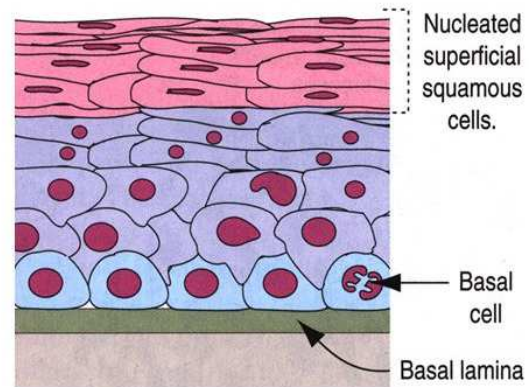
**p<0.001 compared to each of the EoE food allergens

Fig 4

Skin Epithelium (Keratinized)



Esophagus Epithelium (Non-Keratinized)



Gut Epithelium

