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Mechanisms of tolerance to inhalant allergens: the relevance of a modified Th2 response to allergens from domestic animals

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Abstract Subjects can be “non-allergic” because (1) they are not exposed, (2) they fail to make an immune response, or (3) they make an immune response that does not include IgE antibodies (Ab). The recent observation that children raised in a house with a cat are *less* likely to become allergic to cat allergen than those who only get indirect exposure provides a model to investigate the factors controlling allergic responses. Many of these highly exposed children have made an IgG and IgG4 Ab response to Fel d 1 without IgE Ab, i.e., a “modified Th2 response”. In countries where cats are a major cause of asthma, the presence of a cat may *decrease* the risk of asthma. By contrast, in countries with high exposure to dust mites, cats can induce specific tolerance to Fel d 1 without influencing asthma or the IgE Ab response to dust mites. Using overlapping peptides to investigate T cell responses to Fel d 1 suggests that the structure of the molecule plays a special role in inducing the T cell responses that can “control” the immune response to cat allergens. This T cell response is characterized by high levels of IL-10 production, but this is not restricted to those who have made a modified Th2 response. The results suggest that there are major differences in the immune response to different allergens that profoundly affect their role in allergic disease. Dust mite and cockroach differ from cat (and rat) allergens not only in the quantity inhaled and the particles’ sizes but also in the biochemistry of the molecule.

Keywords Tolerance · Inhalant allergen · Modified Th2 response · Domestic animals

Introduction

Although allergic disease is common and apparently becoming more common, the majority of the population does not have symptoms related to pollens, dust mites, cat dander, or any of the well-defined allergens. For many years the most widely accepted explanation for non-responsiveness was a failure to recognize the antigen [15, 21], the argument being

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that foreign proteins delivered at low doses are not recognized or processed by antigen-presenting cells. This would imply that there would be no specific antibodies (Ab), no skin test response, and no allergen-specific T cells. Over the last 10 years several alternative explanations have been proposed, which have different theoretical backgrounds and different predictions. The first, which was a direct corollary of an early form of the cleanliness hypothesis, is that non-allergic individuals make a Th1 response to inhalant allergens. The proposal was that infections and other sources of stimulation to IL-12 production would deviate the immune response away from Th2 responses [3, 37]. In its simpler forms this hypothesis predicts that non-allergic but exposed individuals should have circulating T cells of the Th1 type [26]. However, it could also be suggested that if deviation to Th1 was a common event, some of the subjects should have delayed hypersensitivity skin tests and/or high titer IgG1 Ab. An alternative hypothesis is that high exposure to inhalant allergens, particularly those derived from domestic animals, can induce a modified form of the Th2 response. This form of “immunological tolerance” is characterized by negative skin tests, and the presence of allergen-specific IgG and IgG4 Ab in the circulation without IgE Ab [19, 23]. The observations specifically among individuals with high exposure to cat allergen that support the ‘modified Th2’ hypothesis are:

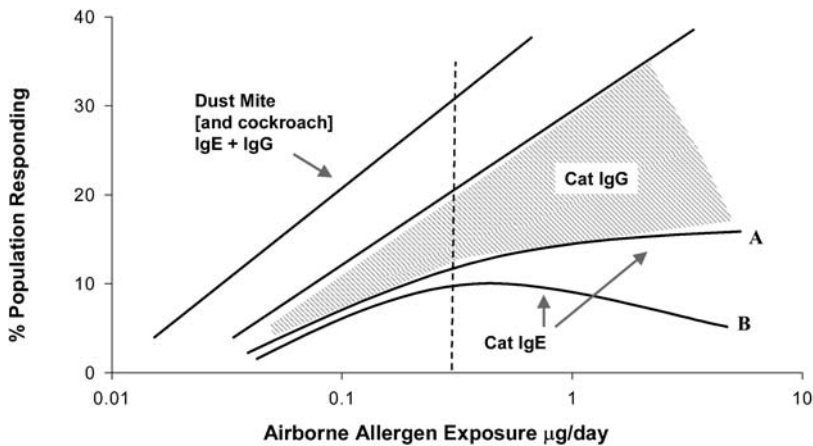
- The IgG4 isotype is dependent on IL-4 and is a normal part of the Th2 response.
- These non-allergic subjects do not have delayed hypersensitivity skin responses to cat or other common inhalant allergens.
- The peripheral blood T cell response to cat allergens *in vitro* is characterized by high levels of IL-10 and increased IL-5 compared to controls, while only small amounts of IFN- γ are produced when compared to other human responses (Reefer et al.; Evidence of a role for IL-10-mediated HLA-DR7-restricted T-cell dependent events in development of the Modified Th2 response to cat allergen, submitted).

Epidemiology

A major element of the argument that allergens play a causal role in allergic disease comes from studies showing an association with immediate hypersensitivity (IH) [18, 22, 32]. In case-control, population-based and prospective studies both rhinitis and asthma have been shown to correlate with IH as judged either by wheal and flare skin tests or serum IgE Ab. Interestingly, although the IH response includes other isotypes of Ab and T cells of the Th2 type, these other elements of the response have not been shown to correlate with asthma independently of IgE Ab. The overall evidence for a direct relationship between exposure and immune responses to allergens is good [7, 13, 22, 32]. For dust mite and cockroach, increasing exposure is related to increased sensitization. This can be seen within cross-sectional studies, but more strikingly by comparing results between studies in different countries. Thus, the overall prevalence of mite, cockroach, or pollen sensitization in a community is a direct function of exposure [39]. In a country where mite or cockroach exposure is high, a large proportion (i.e., 60–80%) of all the allergic individuals will be allergic to these allergens.

Although cats are well recognized as a source of allergens and as a cause of asthma, they play a relatively minor role compared to dust mite, cockroach, or pollen allergens [9, 31]. In addition, it was well known that many allergic individuals who live in houses with a cat deny symptoms from cat exposure. Until recently, this phenomenon was ignored. However, in 1997 some Swedish investigators suggested that children raised in a house

Contrast Between Exposure to Dust Mite or Cat Allergens and the Relevant Immune Responses



The dashed line indicates the approximate value of 20 µg Fel d 1/g floor dust or the presence of a cat.

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Fig. 1 Contrast between exposure to dust mite or cat allergens and the relevant immune response

with a cat were less likely to become allergic to cat dander [11]. At first it seemed possible that this effect was occurring because families with an allergic history were choosing to avoid animals. Indeed, choice of ownership may be a significant element in Sweden and the Netherlands. However, the same phenomenon has now been observed repeatedly in studies from the USA and UK, where choice about owning animals is not an important factor [6, 17, 33]. It is now clear that a large number of otherwise allergic children raised in a house with a cat do not become sensitized to cat allergens (Fig. 1). However, cat allergen is not restricted to homes with a cat. Indeed, cat allergen levels in many homes without a cat are higher than other allergens [5, 9, 14]. It is not surprising that some individuals become allergic to cats without living with an animal. Nonetheless, the scale of this phenomenon is remarkable. In a large cohort of school children in Sweden, 80% of the children who became allergic to cats had never lived in a house with a cat [19, 28]. In that study, owning a cat was a negative predictor of prevalent asthma and incident asthma, as well as being negatively associated with sensitization to cat allergen [19].

The finding that a significant number of otherwise allergic children living with a cat have not become allergic to cats provides an opportunity to evaluate the mechanisms by which these individuals become “tolerant”. The evidence from epidemiological studies suggests that most of these children have not made IgE Ab, and do not have positive skin tests. The serological evidence on allergen-specific Ab of other isotypes is complex because it depends on the techniques used [1, 20, 29]. Our evidence now from three separate studies suggests that a significant proportion of the exposed but not allergic subjects have IgG Ab to Fel d 1, and that this Ab includes IgG4 [19, 23, 25]. However, there are many exposed children who have no detectable Ab to Fel d 1. Thus, it remains possible that there is more than one mechanism for “tolerance” to cat allergens. The argument that the IgG Ab response to

cat allergens should be regarded as a “modified Th2 response” was originally based on two elements of the data: first, that the expression of the gene for IgG4 Ab is dependent on IL-4 (i.e., a Th2 cytokine) and second that the patients did not have delayed skin tests, high titer IgG1 Ab or any other feature that could be regarded as a firm marker of a Th1 response.

Circulating T cells

For some antigens, although perhaps not for allergens, the nature of a human T cell response can be evaluated using cell surface markers or the cytokine response to a specific allergen *in vitro*. However, the profile of human T cells is not straightforward. There are no reliable cell surface markers that can distinguish between Th1 and Th2 cells. T cells in culture respond to many different specific and non-specific stimuli that induce proliferation and cytokine production. Furthermore, cytokines can be released from monocytes, basophils and other cells in the circulation. The patterns of T cell cytokine production are also not simple; human T cells produce intracellular IL-4 but it does not accumulate in culture supernatants, presumably because it is taken up by IL-4 receptors on T cells; IFN- γ is produced in varying quantities by most human lymphocyte cultures; IL-5 is produced and secreted, but the quantities measured are often low, i.e., <20 pg/ml. Thus, in most situations the evaluation of T cell responses is based on the ratio of different cytokines produced *in vitro*. Furthermore, much of the published data is based on response to non-specific mitogens rather than specific allergens. Finally, evaluation of T cell responses to allergens *in vitro* is complicated because most extracts and even purified allergens are contaminated by endotoxin [8]. Several groups have reported cloning human allergen-specific T cells, from allergic and non-allergic individuals, with cytokine secretions patterns characteristic of Th2 and Th1 cells, respectively [27, 37]. However, cloning human T cells requires extensive *in vitro* manipulation, which creates major doubts about the relevance of the properties of the few cells that are cloned.

The problems of *in vitro* evaluation are further complicated because CD4⁺ T cells react to linear antigenic determinants (peptides) consisting of 14–24 amino acids. The response of subgroups of patients to different peptides within a molecule is different and the cytokines produced in response to one epitope may alter the response to other epitopes ([16, 38] and Reefer et al.; submitted). Thus, a thorough evaluation of allergen-specific T cell repertoire requires the use of peptides spanning the whole molecule. These peptides also have the advantage that they can be synthesized under endotoxin-free conditions.

There are several kinds of information that can be obtained from *in vitro* responses to peptides. First, the pattern of recognition of the molecule (i.e., which epitopes or regions of the molecule stimulate proliferation) can be determined. Second, the production of cytokines to different parts of the molecule can be determined. Thirdly, these responses can be compared between different groups of patients. The results with different allergens have not been the same. In some cases there are clear-cut differences between the *in vitro* response of allergic and non-allergic individuals. These include increased cytokines of the Th2 type (i.e., IL-5 and IL-13) in allergic individuals. However, in most studies the response of non-allergic individuals cannot be considered to be typical of a Th1 response.

Recent studies on the response to overlapping peptides of Fel d 1 suggest that the allergen molecule may play a larger role in the response than was previously realized (Reefer et al.; submitted). The N-terminal region of Fel d 1 polypeptide chain 2 contains epitopes that stimulate IL-10 and IFN- γ production. However, induction of IL-10 is not restricted to “tolerant” subjects. Indeed, the response to cat allergen peptides has many similarities

The Immune Response in Allergic Individuals and the Modified Th2 Response

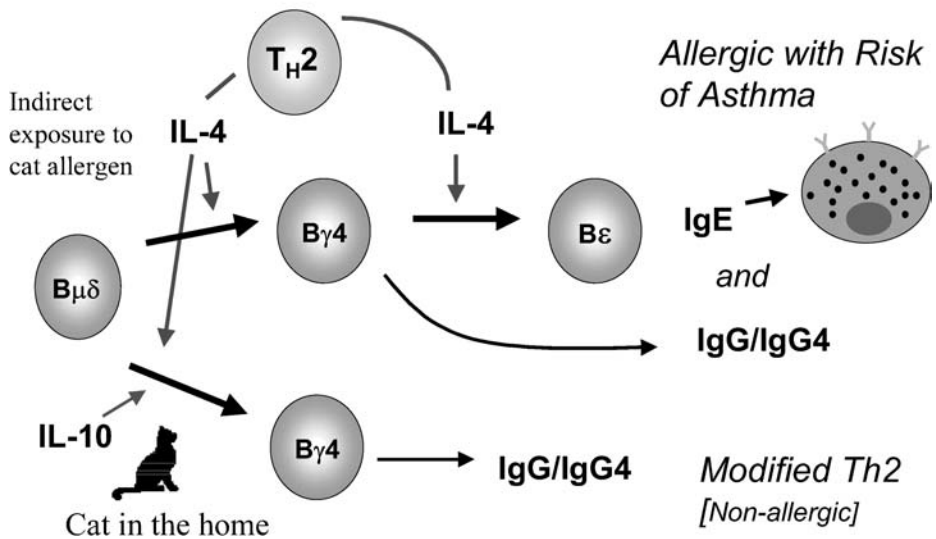


Fig. 2 The immune response in allergic individuals and the modified Th2 response

in allergic, non-allergic and “modified Th2” subjects (Fig. 2). The quantities of IL-10 produced and the ability to alter T cell response by blocking IL-10 strongly argue that the T cell response is playing a role in the response to cat allergen. However, the implication of the results is that the response to cat allergen is “controlled” in all groups of patients. More recent results suggest an association between the modified Th2 response and HLA-DR7, based on increased expression of the major DRB1 allele 0701. In DR7-positive subjects, IL-10 production to peptides of Fel d 1 in vitro is significantly enhanced. However, the fact that IL-10 levels are low in DR7-negative subjects with a modified response implies that there must be another mechanism of “tolerance” or control which is not mediated by IL-10. The important conclusion is that the structure of the major allergen from a particular source may influence both the tendency to make an IgE Ab response and the ability to control the response (Table 1) [24].

Whether other allergens can be as easily classified as dust mite and cat remains to be seen. An IgG4 Ab response without IgE Ab has been shown with high exposure to rat urinary allergen in animal handlers, with prolonged exposure to bee venom (either in bee keepers or with immunotherapy) and perhaps with other animals [1, 2, 29].

Serum IgE and IgG4 Ab and the implications for B cell and plasma cell development

The primary assessment of B cell function is the measurement of isotype-specific Ab in the circulation, or in secretions. For IgE Ab, there is widespread agreement about the tech-

Table 1 Summary of the structure and characteristics of the major allergen from the dust mite and cat and their ability to control the immune response (*Ab* antibodies)

	Class I: dust mite (Cockroach)	Class II: cat (Rat and dog)
Characteristics of major allergen	Der p 1 is a cysteine protease ^a	Fel d 1 is not an enzyme (but can selectively induce IL-10) (homology with CCSP ^b)
Particles	Faecal: 20–40 μ m in diameter	“Dander”: 2–20 μ m in diameter
Airborne	Only airborne transiently during and after disturbance	Allergen remains airborne
Exposure	≤ 10 ng/day	Up to 1 μ g/day ^c
Immune response	Increased prevalence and titer of IgE Ab with increased exposure	“Tolerance” becomes more common with increased exposure, up to 20% produce IgG/IgG4 without IgE

^a Can cleave CD23 and CD25 as well as disrupting tight junctions in epithelial cell layers

^b CCSP (Clara Cell secretory protein) or uteroglobin, which can down regulate responses in mouse lung

^c See [5]. Quality of cat allergen inhaled is such that it is possible to propose that swallowing could induce tolerance via the gastrointestinal tract

niques used, which are all derived from the RAST technique of Wide et al. [36]. By contrast, there are major differences in the techniques used for measuring IgG and IgG4 Ab that give different answers. The serum Ab measured in the circulation must reflect production by plasma cells, but cannot define the features of these cells or the sites of Ab production. In addition, the serum concentrations alone do not define the characteristics of B cell memory. There are two features of the IgE Ab response that we need to explain. The first is that Ab of the IgE isotype are not part of the response to most viruses, bacteria or routine immunization. The incidence of anaphylaxis following a booster injection with tetanus, diphtheria or pertussis is extremely low, i.e., <1 per 100,000 injections. This implies that the mechanisms for “preventing” IgE Ab responses are very successful. The second phenomenon is that increased exposure either naturally (the pollen season) or during immunotherapy produces very little increase in IgE Ab production [4, 10, 20]. This is in marked contrast to the increase in IgG or IgG4 Ab, and implies that there is a relative or complete failure to generate IgE B memory cells.

The primary site for immunoglobulin switch and for the generation of B cells bearing high-affinity Ab is within mature germinal centers. We have recently argued that the germinal center must be a poor site for the generation of IgE B cell memory (Aalberse and Platts-Mills, How do we avoid developing allergy: modifications of the Th2 response from a B Cell perspective; submitted). The evidence for this comes from (1) murine studies, in which two different knockout models that have very poor generation of germinal centers have normal or supernormal IgE Ab production; (2) evidence that the membrane form of IgE is defective in that part of the molecule that is necessary to protect B cells from apoptosis in the germinal center; and (3) the human evidence that IgE B cell memory is not generated effectively. Our view is that the primary route of production of IgE Ab to allergens is by direct Ig heavy chain switch from gamma to epsilon in B cells outside mature germinal centers, and that this leads to the production of long-lived IgE plasma cells. Taken together this model could explain why IgE responses can be remarkably long lived but with little ability to be boosted in response to increased exposure.

The further implication of the observations about the generation of B cell memory is that any signal that helps to generate mature germinal centers would tend to decrease or block IgE Ab production. Thus, all routine immunizations, e.g., DPT, and most natural infections would not be expected to induce persistent or “high” levels of IgE Ab production. The question is what feature of natural exposure to cat allergen or to injections of bee venom explains production of the IL-4-dependent IgG4 without IgE Ab. Many different immunization regimens will generate IL-12 and a consequent shift to Th1 cells. An IgG4 Ab response without IgE Ab requires conditions that control the production to IgE B cells without a shift to Th1. We should now ask what features of the T cell response to the cat allergen Fel d 1 explains this response. Obviously, our data argue that the N-terminal region of the Fel d 1 molecule specifically stimulates IL-10 and IFN- γ production. IL-10 is able to suppress IgE production and enhance IgG4 production by human tonsil lymphocytes *in vitro* [12]. Thus, we could argue that the distinct immune responses to Fel d 1 reflect a biological property of the molecule as well as the dose of exposure (Table 1). The conclusion that the cat allergen is special is supported by recent evidence that exposure to dog allergen is less effective as an allergen and also less effective as a “tolerogen”. At present it seems likely that the production of IgE is influenced not only by the properties of the helper T cells (including IL-10 production) but also by the environment in which the interaction with B cells occurs. However, it seems likely that there is more than one mechanism involved in controlling IgE Ab production.

Conclusion

The evidence we now have about cat and dust mite allergens establishes beyond doubt that they can induce different immune responses, and that the dose-response relationship between exposure and sensitization is not the same [24]. Furthermore, it is increasingly likely that this difference reflects the properties of the major allergens of each. For cat allergens, indirect exposure, i.e., from the neighbor’s cat or at school, is sufficient to sensitize. With a cat in the house, the prevalence of sensitization is not increased and fully 50% of the non-cat allergic children may have developed an alternative immune response, i.e., IgG/IgG4 Ab without IgE Ab. In a country where 50% of the houses have a cat, this form of tolerance may influence a large proportion of the population. From our recent studies, it seems clear that many of the individuals living in a house with a cat will have circulating T cells that respond to peptides of Fel d 1 and produce IL-10. The question is whether the responses to mite and cat are simply two out of many possible patterns of response, or that these responses define two patterns, which with minor alterations would provide a basis for classifying responses to allergens. There are, of course, major differences in exposure, e.g., seasonal pollens, versus domestic animals, versus mite or cockroach, which clearly influence these responses. Furthermore, endotoxin can influence the immune response to allergens both positively and negatively.

Coming back to the properties of the major allergens, it is well established that Der p 1 not only has homology with cysteine proteases but is an active enzyme. *In vitro*, this enzyme cleaves CD25 and CD23 off the surface of lymphocytes and can also disrupt tight junctions in epithelial cell layers [30, 34]. These properties of mite allergens may in some way prevent the induction of “tolerance” even at high dose. By contrast, the cat allergen Fel d 1 is not an enzyme, but it does have homology with Clara Cell secretory protein (CCSP), which is known to be tolerogenic in mice [35]. CCSP may be active in the lung,

so that in theory the cat allergen could interfere with the natural activity of CCSP, or have a similar effect of its own. Since the role of Fel d 1 in the cat is not known, it is still possible that the response to Fel d 1 is influenced by its biological role.

The mechanism by which cat allergen induces a “modified Th2” response is not clear. Certainly, the dose inhaled, i.e., up to 1 $\mu\text{g}/\text{day}$, must be important. It is also clear that exposure to Fel d 1 induces T cells or other cells capable of producing IL-10. Preliminary evidence suggests that at least some of the IL-10 is coming from CD4⁺ T cells and we assume that CD25⁺ T regulator cells (TR1) are involved. However, it is not clear how a particular peptide or group of peptides within the molecule favors this response. The fact that a similar response occurs in almost all individuals suggests that the immune response reflects the properties of Fel d 1 and that processing of the antigen is similar in all groups.

While there are many unanswered questions, the evidence is sufficient to say that the properties of allergens in general need to be reassessed. Allergens by definition induce IgE Ab responses in humans. This implies that they can activate T helper cells without inducing the generation of Th1 cells (which would not help IgE) or the formation of mature germinal centers that would favor apoptosis of B cells expressing IgE. We would now add that cat and rat allergens can also produce an IgG4 response without IgE Ab. In the past, we had assumed that the primary characteristics of allergens were physical, i.e., low dose, soluble proteins, that were good antigens and were delivered without an adjuvant that would induce IL-12. Today, the amino acid sequence of a wide range of allergens is known, but understanding why they produce different responses has become a major challenge. The importance of this lies not only in understanding the differences, but also because it may help to define the characteristics of an effective vaccine designed to induce a tolerant response comparable to that induced by high exposure to cat allergen.

References

1. Aalberse RC, Gaag R van der, Leeuwen J van (1983) Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. *J Immunol* 130:722
2. Akdis CA, Blesken T, Akdis M, et al (1998) Role of interleukin-10 in specific immunotherapy. *J Clin Invest* 102:98
3. Busse WW, Lemanske RF (2001) Asthma. *N Engl J Med* 344:350
4. Creticos PS, Norman PS (1987) Immunotherapy with allergens. *JAMA* 258:2874
5. Custis NJ, Woodfolk JA, Vaughan JW, et al (2003) Quantitative measurements of airborne allergens from dust mites, dogs, and cats using an ion charging device. *Clin Exp Allergy* (in press)
6. Custovic A, Hallam CL, Simpson BM, et al (2001) Decreased prevalence of sensitization to cats with high exposure to cat allergen. *J Allergy Clin Immunol* 108:537
7. Eggleston PA, Rosenstreich D, Lynn H, et al (1998) Relationship of indoor allergen exposure to skin test sensitivity in inner-city children with asthma. *J Allergy Clin Immunol* 102:563
8. Eisenbarth SC, Piggott DA, Huleatt JW, et al (2002) Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 196:1645
9. Gelber LE, Seltzer LH, Bouzoukis JK, et al (1993) Sensitization and exposure to indoor allergens as risk factors for asthma among patients presenting to hospital. *Am Rev Respir Dis* 147:573
10. Gleich GJ, Zimmermann EM, Henderson LL, et al (1982) Effect of immunotherapy on immunoglobulin E and immunoglobulin G antibodies to ragweed antigens: a six-year prospective study. *J Allergy Clin Immunol* 70:261
11. Hesselmar B, Aberg N, Eriksson B, et al (1999) Does early exposure to cat or dog protect against later allergy development? *Clin Exp Allergy* 29:611
12. Jeannin P, Lecoanet S, Delneste Y, et al (1998) IgE versus IgG4 production can be differentially regulated by IL-10. *J Immunol* 160:3555
13. Lau S, Illi S, Sommerfeld C (2000) Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. *Lancet* 356:1392

14. Luczynska CM, Arruda LK, Platts-Mills TAE, et al (1989) A two-site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp. allergens, Der p 1 and Der f 1. *J Immunol Methods* 118:227
15. Marsh DG (1976) Atopic allergy; a model for studying the genetics of immune response in man. *Birth Defects Orig Artic Ser* 12:223
16. Oldfield WL, Larche M, Kay AAB (2002) Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomized controlled trial. *Lancet* 360:47
17. Ownby DR, Johnson CC, Peterson EL (2002) Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. *JAMA* 288:963
18. Peat JK, Tovey E, Toelle BG, et al (1996) House dust mite allergens. A major risk factor for childhood asthma in Australia. *Am J Respir Crit Care Med* 153:141
19. Perzanowski MS, Ronmark E, Platts-Mills TAE, et al (2002) Effect of cat and dog ownership on sensitization and development of asthma among preteenage children. *Am J Respir Crit Car Med* 166:696
20. Platts-Mills TAE (1979) Local production of IgG, IgA and IgE antibodies in grass pollen hay fever. *J Immunol* 122:2218
21. Platts-Mills TAE (1982) Type I or immediate hypersensitivity. Hay fever and asthma. In: Lachmann PJ, Peters DK (eds) *Clinical aspects of Immunology*, vol 1. Blackwell, Oxford, p 579
22. Platts-Mills TAE, Vervloet D, Thomas WR, et al (1997) Indoor allergens and asthma: report of the Third International Workshop. *J Allergy Clin Immunol* 100:S2
23. Platts-Mills TAE, Vaughan JV, Squillace S, et al (2001) Sensitization, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet* 357:752
24. Platts-Mills TAE, Perzanowski M, Woodfolk JA, et al (2002) Relevance of early or current pet ownership to the prevalence of allergic disease. *Clin Exp Allergy* 32:335
25. Platts-Mills TAE, Erwin EA, Allison AB, et al (2003) The relevance of maternal immune responses to inhalant allergens to maternal symptoms, passive transfer to the infant, and development of antibodies in the first two years of life. *J Allergy Clin Immunol* 111:123
26. Prescott SL, Macaubas C, Smallacombe T, et al (1999) Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 353:196
27. Romagnani S (2001) T-cell responses in allergy and asthma. *Curr Opin Allergy Clin Immunol* 1:73
28. Ronmark E, Jonsson E, Platts-Mills TAE, et al (2001) Incidence and remission of asthma in schoolchildren: report from the obstructive lung disease in northern Sweden studies. *Pediatrics* 107:E37
29. Rowntree S, Platts-Mills TAE, Cogswell JJ, et al (1987) A subclass IgG4-specific antigen-binding radioimmunoassay (RIA): comparison between IgG and IgG4 antibodies to food and inhaled antigens in adult atopic dermatitis after desensitization treatment and during development of antibody responses in children. *J Allergy Clin Immunol* 80:622
30. Schulz O, Sewell HF, Shakib F (1998) Proteolytic cleavage of CD25, the α subunit of the human T cell Interleukin 2 receptor, by Der p 1, a major mite allergen with cysteine protease activity. *J Exp Med* 187:271
31. Sears MR, Hervison GP, Holdaway MD, et al (1989) The relative risks of sensitivity to grass pollen, house dust mite, and cat dander in the development of childhood asthma. *Clin Exp Allergy* 19:419
32. Sporik R, Holgate ST, Platts-Mills TAE, et al (1990) Exposure to house-dust mite allergen (Der p 1) and the development of asthma in childhood. *N Engl J Med* 323:502
33. Sporik R, Squillace SP, Ingram JM, et al (1999) Mite, cat, and cockroach exposure, allergen sensitization and asthma in children. A case-control study of three schools. *Thorax* 54:675
34. Wan H, Winton HL, Soeller C, et al (1999) Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. *J Clin Invest* 104:3
35. Wang SZ, Rosenberger CL, Espindola TM, et al (2001) CCSP modulates airway dysfunction and host responses in a Ova-challenged mouse model. *Am J Physiol Lung Cell Mol Physiol* 281:L1303
36. Wide L, Benich H, Johansson SG (1967) Diagnosis of allergy by an in-vitro test for allergen antibodies. *Lancet* II:1105
37. Wierenga EA, Snoek M, Jansen HM, et al (1991) Human atopen-specific types 1 and 2 T helper cell clones. *J Immunol* 147:2942
38. Woodfolk JA, Platts-Mills TAE (2001) Diversity of the human allergen-specific T cell repertoire associated with distinct skin test reactions: delayed-type hypersensitivity-associated major epitopes induce Th1- and Th2-dominated responses. *J Immunol* 167:5412
39. Woodfolk JA, Platts-Mills TAE (2002) The immune response to intrinsic and extrinsic allergens. Determinants of allergic disease. *Int Arch Allergy Immunol* 129:277