

T and B Cell Responses to HDM Allergens and Antigens

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Abstract

House dust mites provide well-characterized proteins to study human responses to inhaled antigens. Even in the absence of allergy they induce a high frequency of T cell precursors. The healthy response manifests by T cell proliferation and Th1 cytokines with little antibody. Responses of allergic people include Th1 and Th2 cytokines and IgE, IgG1, and IgG4 antibodies. Regulatory cells limit effector responses in healthy people. About half the IgE and IgG antibodies bind the group 1 and 2 allergens and 30% bind the group 4, 5, and 7 allergens. Although HLA independent, the recognition of the group 1 allergen shows an immunodominant region and a T cell receptor bias. The major allergens are not produced in higher amounts than many of the poorly non-allergenic proteins. The non-allergenic mite ferritin antigen shows high T cell proliferative responses with mixed cytokine production.

Key Words

IgE
IgG
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Allergen
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House dust mite

House Dust Mite Allergens

A major requirement for the mucosal immune system is to make harmless responses to innocuous inhaled antigens. House dust mites (HDM) are traditionally studied for their allergenicity, but they are also a well-characterized source of antigens that can be investigated for insights into the immunoregulatory processes. *Dermatophagoides pteronyssinus* is

the most widespread species while *D. farinae* predominates in regions with low relative humidity. Mixed populations occur, for example, in some regions of the US (1,2) and Japan (3). The allergens of the different species typically have 80–85% sequence identity and show antibody cross-reactivity (4). In general, most important sources of allergens have one or two specificities that dominate the response. Birch pollen allergy is the best example with

80% of the IgE antibody in 90% of subjects binding Bet v 1 with titers of about 50 ng/mL. Major allergens from other sources bind about 50% of the IgE antibody with titers of about 50 ng/mL except for those to the cat allergen Fel d 1, which are often low (5). Groups 1 and 2 are the major HDM allergens. Combined they typically bind about 50 ng/mL of IgE (6,7) constituting about 50% of IgE binding to all the HDM allergens regardless of whether the total titers are high or low (7).

The group 1 allergens are cysteine proteases (8) found in the gut and dung balls (9). Mice injected with enzymatically active Der p 1 produce more IgE antibody than mice injected with inactivated Der p 1 providing evidence for a Th2 promoting effect of the protease activity (10,11). The mechanism might be related to its ability to cleave a number of immunologically related (12) and protease-activated receptors (13). In vitro studies have shown that Der p 1 can increase the permeability of intercellular junctions (14), activate antigen-presenting cells (15), and induce the release of dendritic-cell-attracting chemokines from respiratory epithelium (16). The role of the protease activity, however, is not resolved. Most allergens are not proteases, and the mouse experiments were reliant on alum adjuvant to induce the Th2 responses. Indeed for dogs the most important HDM allergen is a chitinase (17–19). The group 2 allergens are proteins of the ML domain family, related to the Toll-like receptor 4 (TLR-4) co-receptor MD2. (20–22). It is possible that they could have a weak interaction with TLR4 and thus impart an adjuvant effect but there are significant differences in the structures (23).

Antibody Responses

There are now 22 denominated HDM allergens or IgE binding proteins. Some could drive the sensitization while others could be

bystander responses. Apart from the high IgE binding of Der p 1 and 2, Hales et al. have shown that three allergens, Der p 4, 5, and 7, each have about 10% of the anti-HDM titer and that the relative proportions of antibodies to these specificities were remarkable constant across a wide range of anti-HDM titers. Their average IgE binding of 10–15 ng/mL is higher than the responses of many people to major allergens of mites and other sources. Collectively, it would appear that the Der p 1, 2, 4, 5, and 7 allergens elicit 80% of the response. The IgE binding to the other allergens is low even when the prevalence is high. This has been directly demonstrated for the group 3, 8, 10, and 20 allergens (7) and, because the group 6 and 9 allergens bind even less IgE than Der p 3 (24,25), they are low. Only occasional people have IgE to the group 13 fatty-acid-binding-protein allergens (26) and IgE binding to the group 10 tropomyosin allergen has been shown to be low in Australia (7), Europe (27), USA (28), and Singapore (29). High reactivity has been found in Japan (30) and Africa (27); the geographical inconsistency suggests a cross reactivity, which is plausible because tropomyosin has a very highly conserved amino acid sequence with 70% and 80% identity to helminth and seafood allergens (31). The IgE binding for the group 16, 17 (32) are also low in comparison to other allergens.

IgG1 and IgG4 antibodies provide a comparison of Th1 and Th2 biased responses. Many reports have confirmed early studies showing that IgG antibodies to HDM extracts are predominantly found in sera with IgE antibody (33). Greatly increased IgG1 and IgG4 have been described for Der p 1 (7,34) and Der p 2 (7,35) as well as pan IgG anti-Der p 1 (36). Some studies, however, have reported that increased titers are confined to the IgG4 subclass (5,37). The reverse of this has also been reported (38), but the same group also reported the opposite result (39). A study that

found no difference in the IgG1 and IgG4 antibody titers of allergic and non-allergic children to Der p 2 is more difficult to reconcile (40). Overall the IgG1 and IgG4 antibody responses show that allergic people induce Th1 and Th2 responses (7,34) and both are higher than in non-allergic subjects.

A comparison of IgG responses to a range of purified HDM allergens showed that both IgG1 and IgG4 antibodies are associated with the ability of the allergens to induce IgE (7). Der p 1 and 2 frequently bound both subclasses, the mid potency Der p 4, 5, and 7 bound them to a lesser degree, and the weak allergens had little activity. Studies with Western blotting also failed to reveal consistent IgG-binding components other than the major allergens (41,42). A caveat of the antibody binding results is other important allergens may exist. One is paramyosin (group 11) (43,44) reported to bind IgE with high frequency in Taiwan and another is the group 14 apolipoprotein protein shown to bind IgE from sera of Japanese population with high frequency and potency (32,45,46). Both these allergens are labile in extracts and difficult to produce as recombinant proteins. Other potential major allergens are the group 15 and 18 chitinase allergens that are the most important allergens for dogs (17–19). The frequency of binding of recombinant chitinase polypeptides is 60–70% (17,19,47), which is high but quantitative measurements have yet to be accomplished.

T Cell Responses

T cell cloning has provided definitive evidence that non-allergic subjects make T cell responses to HDM allergens (48–50). Some reports have shown that PBMC from allergic and non-allergic subjects proliferate equally well to HDM extracts or major allergens (51,52) but most have shown that PBMC from

allergic subjects produce higher responses to HDM extracts (53–56). The increased proliferation has also been demonstrated for the major allergens Der p 1 (57), Der p 2 (57), Der f 1 (58), and Der f 2 (59). An early study (60) reported that Der p 1 could not stimulate proliferation of T cells from HDM-allergic asthmatics, but this is inconsistent with contemporary evidence including studies from the same laboratory (61). The precursor frequencies of T cells responding to defined allergens have not been examined, but extracts have been studied. Limiting dilution assays have reported frequencies of 0.05% in mite allergic and 0.01% for non-allergic subjects (62); 0.02% in both allergic and non-allergic (51); 0.01% for non-allergic and 0.04–0.09% for allergic subjects (63); and 0.2% and 0.8 % (64). Limiting dilution analyses of exotic antigens in naive people show frequencies of 0.001% (65–67) and the frequencies found after vaccinations are about 0.02% (67). By comparison it must be concluded that the anti-HDM precursor frequencies are high even in the non-allergic people. Similar high frequencies have been found for responses to birch and grass pollen allergens (68).

Anti-HDM reactive T cell clones provided some of the first evidence that Th1 and Th2 cytokine polarization occurred in humans (49,50). However, it is now known that HDM allergens induce similar quantities of IFN- γ from the PBMC of both allergic and non-allergic subjects (34,54,69–74). The cloning probably shows the strong in vitro polarizing activity of IL-4 produced by cells from allergic but not non-allergic subjects. Some observers have reported that the Th1 release can be induced by higher doses (75), but others have found that increased doses increase both Th1 and Th2 cytokines (71–74). The induction of IFN- γ has been demonstrated with purified Der p 1 and Der p 2 allergens as well as extracts (34,69–71,73,74,76).

Similar findings for IFN- γ release have also been found for pollen allergens (73,77), but it is possible that the IFN- γ is reduced in severe long-term allergy (69,78,79).

There is little definitive data on the T cell responses elicited by purified allergens other than the major groups 1 and 2. The stimulation of PBMC with Der p 5 and 7 was shown to induce lower Th2 cytokine responses than Der p 1 (80), and there was no relationship between the IgE antibody and cytokine induction. A report previous to this described that a preparation of Der p 7 isolated from mite extracts induced similar responses to Der p 1 (71), but this is now thought to be the result of impurities. Proliferative responses of PBMC to group 14 allergens have been shown to be similar to those induced by major allergens by two research groups (59,81). The group 14 allergens have been difficult to produce or purify in the quantities required for T cell studies, so the studies have been limited to stimulation by peptides. Studies with extracts have also shown that T cell responses to antigens other than groups 1 and 2 allergens can be high (82,83).

T Cell Regulatory Responses

Allergen-stimulated PBMC from allergic subjects have been reported to make higher levels of IL-10 than responses of non-allergic subjects but paradoxically with a negative correlation between the IL-10 responses and the size of skin prick test of the donors (34,74). Reports of IL-10 production from allergen-stimulated PBMC from other laboratories have similarly found increased IL-10 release for patients with HDM allergy (84,85) but different to the results with skin tests the release was greater in the more severe asthmatics (84). IL-10 is also produced by HDM-allergic patients after *in vivo* allergen challenge (86) being produced by both monocytes and T cells. Evi-

dence for a role of IL-10 in downregulating responses has been obtained by different types of analyses. Jutel et al. found that addition of anti-IL-10 receptor to cultures of PBMC from non-allergic subjects enhanced proliferative responses to Der p 1 (39). A follow up showed that cultures from healthy people had more IL-10-producing T-cells than cultures from allergic subjects when measured 12 h after stimulation with allergen (73). The IL-10-producing cells had poor proliferative activity in the absence of exogenous cytokines, so they may not be manifest in increased IL-10 production in the 5–6 d culture periods used by others. Indeed cytokines produced by the PBMC of allergic subjects could provide help for the growth of IL-10-producing cells measured at later time points.

Suppressive effects of CD4+CD25+ T cells have been demonstrated in *in vitro* cultures of PBMC stimulated with cat and pollen allergen extracts (87). There has not been a direct study on HDM, but Chang et al. made the observation that HDM stimulated more CD4+CD25+ cells from cultures from allergic than non-allergic subjects (54). Similarly studies of atopic dermatitis showed that HDM extract stimulated more of the regulatory cell transcription factor FOXP3 from PBMC from HDM allergic subjects than PBMC from non-allergic subjects (88). It is possible these effects are linked to increased IL-2 production by the higher responses of allergic subjects.

T Cell Phenotypes

The HDM extract-responsive T cells of allergic subjects are mainly in the memory CD45RO+ population (62). Bellinghausen et al. (52) showed that twice the proliferation could be induced with CD45RO+ compared to the CD45RA+ cells and that the proliferation was similar for cells from both allergic and non-allergic people. Th2 cytokines were

released from the CD45RO+ cells of atopic subjects while the IFN- γ was readily produced by CD45RA+ or CD45RO+ cells in PBMC from all subjects. The induction of the Th2 transcription factor GATA-3 by HDM extract has also been shown to be stronger in CD45RO+ cells (89).

The detection of allergen-responsive cells into populations with different chemokine receptors is a measure that reflects T cell polarization in vivo. The Th1-cell-attracting chemokines, CXCL10 (IP10) and CXCL9 (MIG), are induced by IFN- γ and Th2-cell-attracting chemokines CCL11 (eotaxin), CCL17 (TARC), and CCL22 (MDC) by IL-4. The Th1 cells that respond to the Th1 chemokines preferentially express CXCR3 and Th2 cells preferentially express CCR3 and CCR4 (90). Bronchial challenge of birch or HDM allergic patients with allergen extracts induces an infiltration of CCR4+ cells with few CXCR3-bearing Th1 cells (91,92). Airway challenge also induces the production of the Th2 ligands MDC and TARC (92–94) to attract these cells. The chemokine receptor profile of allergen-responsive PBMC has not been investigated for mite allergens. CCR4 has however been shown to be up regulated for grass pollen-allergic subjects (68,95). It could be detected on 40% of T cells responding to pollen stimulation compared to undetectable levels on the allergen responsive cells of healthy people (68).

The Th2 cytokine and GATA-3 responses of PBMC (55,89), and the immunohistochemistry of biopsies of allergic reactions (96), show the importance of CD4+ cells. CD8+ cells are nevertheless stimulated as demonstrated by O'Brien et al. with IL-2 supplemented cultures stimulated with Der p 1 (70). CD8+ cells from allergic subjects released IFN- γ and the amounts from cells of allergic subjects were higher, comparable to the release from unfrac-

tionated cultures. Th2 cytokine production could not be detected despite stimulating the cells under a variety of conditions. The Der p 1-specific CD8+ cells have also been detected by isolating T cells with an MHC class I binding peptide tetramer (97).

HLA and Epitopes

Several studies have indicated an involvement of genes in the HLA class II region, but while isolated studies have demonstrated positive and negative associations of particular class II alleles, none have been consistent between studies. Positive associations have been found for IgE anti-Der p 1 and 2 and DRB1 alleles in Korea (98), for DRB1, DQA1, and DPB1 alleles in Taiwan (99), and DRB1 alleles in non-indigenous Australians (100). High T cell responses reportedly had a positive association with a DQA1/DQ allele for responses to Der p 2 (101) and with a DPB1 allele to Der p 1 (102).

T cell cloning has shown a broad HLA restriction, including restriction by HLA-DQ and -DP alleles. Expressly Der p 1 has been shown to be presented by DRB1 (103,104) and DP (103), Der f 1 by DRB1, DRB3, and DQ (105), Der p 2 by DRB1 (106–108), and DQ (108,109) and unidentified allergens of *D. farinae* by DRB1, DRB3 and DRB5 (110, 111). As expected from the diverse HLA restriction, peptide mapping has revealed a range of T cell epitopes. There has however been an interesting pattern for Der p 1 with T cells preferentially responding to peptides from a domain-joining loop in the center of the molecule (103,104,112). Kirchner et al. confirmed the strong T cell stimulating activity of the peptide 101–131 and showed that the responding T cells had a biased use of V β 18.1+ V α 2,3+ T cell receptors (76). This TCR usage pattern had been previously noted (113). The immunodominance is not due to a

restricted MHC presentation because the region contains multiple T cell epitopes (103,112) and the responses are presented by multiple MHC class II molecules (103). This differs from responses that have MHC-based epitope immunodominance as found for Art v 1 from mugwort (114). The study of the responses of six subjects to Der f 1 found a more diverse pattern reported for Der p 1 (105). This argues against a special role for the immunodominant region but the number of patients was small and 3/6 did respond to the peptides in the domain-connecting region. Responses to Der p 2 show no immunodominant region (101,107,115).

Major mite allergens have highly polymorphic amino acid sequences (116,117) with variation in about 5% of their residues. Many regions also have both *D. pteronyssinus* and *D. farinae* that have a 20% amino acid sequence disparity in their homologous allergens. The sequence diversity possibly could aid the responsiveness by presenting a larger array of structures for MHC and TCR binding.

Developmental

Children develop aeroallergy over 3–5 yr. This contrasts with food allergy where IgE antibodies appear early and then often disappear. There is debate about whether the sensitization has antecedents *in utero*. Allergen can reportedly be detected in the amniotic fluid (118), and there are plausible transport mechanisms especially via IgG antibody. A less controversial observation is that cord blood of subjects who develop allergies contain more activated T and accessory cells (119) but the specificity of the activation is not known. A majority of reports show the *in vitro* proliferative responses of cord blood cells to allergens are higher in children who develop allergy (120) or in high-risk children (121,122). Some studies have produced con-

trary results but only used early time points to categorize disease so they may not be valid (123,124). Nevertheless, it cannot be concluded that the increased responsiveness reported is due to priming *in utero*. Indeed, no association has been found with the cord responses and HDM exposure (122,125). Thornton et al. found that allergen-responsive cord cells had a regulatory phenotype and apoptose in the absence of anti-apoptotic cytokines like IL-7 (126). The cells thus may not be the precursors of T cell memory, but, because the anti-apoptotic cytokines are readily found in the placenta, the results may reflect inadequacy of the culture system. Devereux et al. did find that 50% of the cells responding to allergen in the cord were of the memory CD45RO+ phenotype, indicating antigenic stimulation (127). The studies comparing the responses of cord blood and the development of allergy have only been examined with HDM extracts, so it is not known if the allergens are responsible for their observations. However, it has been reported that CBMC proliferate in response to Der p 1 (128) and Der p 1 and 2 (119).

The cytokine responses of allergen-stimulated CBMC show a Th2 bias for both allergic and non-allergic subjects with very little IFN- γ production (121,129). The early Th2 cytokine responses of children that become allergic are in fact lower than the non-allergic as are the IL-6 and IL-10 responses (129). During infancy the Th2 responses of the allergic infants increase while the Th1 responses of allergic and non-allergic subjects remain similar (129,130). By 6 yr the response of PBMC of children are similar to adults with Th1 and Th2 cytokines produced by the T cells of allergic children and only the Th1 cytokine for the non-allergic (34). These studies of infant development have only been conducted with HDM extracts so the responding specificities are unknown. It should be

noted that adult immigrants newly exposed to mite and other allergens develop allergies with a similar propensity and time course, so the neonatal immaturity is not essential (*131*).

Abundance of HDM Allergens

Dose responses are critical for immune responses. Air sampling shows that the quantities of the major allergen in inhalable air are very low in normal domestic life, 29 and 6 pg/m³, respectively, for groups 1 and 2 (*3*). Activities like bed making increase the levels 1000-fold. Most of the allergen is found on particles from 6 to 20 μ m that would be deposited in the upper regions of the airway (*132*). Assuming a 50% deposition and the inspiration of 10 m³ per day the inhalation in undisturbed air would be less than 0.1 pg per day. If the exposure was high for 8 h, for example, while sleeping, it would be 100 ng per day.

The group 1 and 2 allergens are abundant constituents of HDM extracts comprising 2–5% of the protein content (*133*). They are typically present at 20–100 μ g/mL. The relationship of these extracts to the respirable allergen is unknown. HDM allergens may be underrepresented in extracts because they are mainly secreted, poorly extractable, or labile, for example, to the hydrolytic enzymes of the gut. Lability is important for the group 11 (*134*) and 14 (*45*) allergens. The only other allergens to be measured are Der p 5 (*135*) and Der p 7 (*136*) and they have concentrations less than 0.1 μ g/mL. The trypsin activity of the group 3 allergens is also difficult to detect in extracts (*137*). 2-D gel electrophoresis shows that extracts contain at least 50 different compounds with strong protein staining, and these have no special association with the dominant IgE binding proteins (*138*). An analysis made directly from live mites (*139*) showed that Der p 1 and 2 were only the 30th and 40th most abundant proteins. The poor

allergens Der p 10, 13, and 20 and the non-allergenic ferritin were more abundant.

Non-allergenic Antigens

There is a dearth of information on immune responses to inhaled non-allergenic noninfectious antigens. The nature of these responses could provide insights into the events that lead to healthy immune responses. They would also provide an additional perspective to comparing the responses of allergic and non-allergic subjects because the differences will not be due to genetic or other constitutional peculiarities of allergic subjects, for example, immunological immaturity in infancy (*140*). Western blotting with HDM extracts has not shown stand-out bands of IgG reactivity (*41*), so there is little to indicate that non-allergenic antigens could be characterized by this approach. Ferritin heavy chain was identified as an immunogenic polypeptide that did not bind IgE by comparing the results of screening cDNA expression libraries with hyperimmune and allergic sera (*141*). It induces high proliferative responses of PBMC from allergic and non-allergic subjects with moderate levels of both Th1 and Th2 cytokines. The Th2 cytokine response to Der p 2 of PBMC from allergic subjects was expectedly higher than the Th2 response to ferritin, but interestingly the Th2 response to Der p 2 of PBMC from non-allergic subjects was lower than that of ferritin. Thus, using the response to ferritin as a reference, the Th2 response to Der p 2 appears to be actively downregulated in non-allergic people. The IgG responses to ferritin were low indicating that they do not manifest an effector function.

Conclusions

Harmless responses to inhalant antigens are critical for a healthy life. People who do

not develop HDM allergy have a high frequency of HDM allergen-responsive memory T cells, albeit lower than the allergic people, but their responses induce little antibody production or delayed hypersensitivity. A regulatory response is indicated consistent with reports of an early production of IL-10-producing T cells. The production of IL-10 and the FOXP3 transcription factor of regulatory T cells, however, has not been an obvious feature of in vivo allergic responses or responses of T cells expanded in vitro by allergen stimulation so the sequence of regulatory events needs to be further elucidated. The downregulation of the Th2 response of non-allergic subjects to Der p 2, in comparison to the responses to ferritin, provides in vivo evidence for an active regulation of the healthy response to the allergen. The majority of the anti-HDM response of allergic subjects, whether large or small, is directed to two major allergens. There is also an allergenic hierarchy for the allergens that bind less IgE. At least three other allergens induce lower but still significant responses in most HDM-allergic people, while the other allergens examined consistently induce very low responses. The hierarchy for IgE also applies to both the IgG1 and IgG4 subclass antibodies. The finding that responses of both subclasses associate with allergenicity matches the fact that T cells from allergic people, as well producing Th2 cytokines, pro-

duce IFN- γ to the same degree as T cells from nonallergic subjects. The reasons for the hierarchy are not known. Der p 1 has a particular region that contains highly stimulatory T cell epitopes associated with a biased T cell receptor usage but this has not been found for Der p 2, and there are no consistent associations with HLA class II alleles. It is not known if the T cells in non-allergic people are still preferentially directed to the major allergens. At face value the hierarchy is not due to the amount of allergen produced. Mites produce more of some of the less potent allergens and non-allergenic proteins than the major allergens, although information on antigen in inhaled air is lacking. It is proposed that as well as comparing responses of allergic and non-allergic people, comparisons between the responses to the allergenic and less or non-allergenic molecules will be important. The knowledge obtained from characterizing HDM allergens, and the use of recombinant or purified allergens and antigens for meaningful investigations, can now be used to elucidate the mechanism of healthy immune responses. Some of the key areas of research in HDM allergy, particularly the development of responses in infancy, have not been conducted with purified allergens and antigens so they cannot distinguish healthy and unhealthy responses that are made to different specificities.

References

1. Huss K, Adkinson NF, Jr, Eggleston PA, Dawson C, Van Natta ML, Hamilton RG: House dust mite and cockroach exposure are strong risk factors for positive allergy skin test responses in the Childhood Asthma Management Program. *J Allergy Clin Immunol* 2001;107:48–54.
2. Arlian LG, Bernstein D, Bernstein IL, et al: Prevalence of dust mites in the homes of people with asthma living in eight different geographic areas of the United States. *J Allergy Clin Immunol* 1992;90(3 Pt 1):292–300.
3. Sakaguchi M, Inouye S, Yasueda H, Irie T, Yoshizawa S, Shida T: Measurement of allergens associated with dust mite allergy. II. Concentrations of airborne mite allergens (Der I and Der II) in the house. *Int Arch Allergy Appl Immunol* 1989;90:190–193.
4. Lind P, Hansen OC, Horn N: The binding of mouse hybridoma and human IgE antibodies to the major fecal allergen, Der p I, of *Dermatophagoides pteronyssinus*. Relative binding site location and species specificity

- studied by solid-phase inhibition assays with radiolabeled antigen. *J Immunol* 1988;140:4256–4262.
5. Erwin EA, Wickens K, Custis NJ, 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;115:74–79.
6. Trombone AP, Tobias KR, Ferriani VP, et al: Use of a chimeric ELISA to investigate immunoglobulin E antibody responses to Der p 1 and Der p 2 in mite-allergic patients with asthma, wheezing and/or rhinitis. *Clin Exp Allergy* 2002;32:1323–1328.
7. Hales BJ, Martin AC, Pearce LJ, et al: IgE and IgG anti-house dust mite specificities in allergic disease. *J Allergy Clin Immunol* 2006;118:361–367.
8. Chua KY, Stewart GA, Thomas WR, et al: Sequence analysis of cDNA coding for a major house dust mite allergen, Der p 1. Homology with cysteine proteases. *J Exp Med* 1988;167:175–182.
9. Tovey ER, Chapman MD, Platts-Mills TA: Mite faeces are a major source of house dust allergens. *Nature* 1981;289:592–593.
10. Gough L, Sewell HF, Shakib F: The proteolytic activity of the major dust mite allergen Der p 1 enhances the IgE antibody response to a bystander antigen. *Clin Exp Allergy* 2001;31:1594–[???]1598.
11. Kikuchi Y, Takai T, Kuhara T, et al: Crucial commitment of proteolytic activity of a purified recombinant major house dust mite allergen Der p1 to sensitization toward IgE and IgG responses. *J Immunol* 2006;177:1609–1617.
12. Takai T, Kato T, Ota M, et al: Recombinant Der p 1 and Der f 1 with in vitro enzymatic activity to cleave human CD23, CD25 and alpha1-antitrypsin, and in vivo IgE-eliciting activity in mice. *Int Arch Allergy Immunol* 2005;137:194–200.
13. Asokanathan N, Graham PT, Stewart DJ, et al: House dust mite allergens induce proinflammatory cytokines from respiratory epithelial cells: the cysteine protease allergen, Der p 1, activates protease-activated receptor (PAR)-2 and inactivates PAR-1. *J Immunol* 2002;169:4572–4578.
14. Stewart GA, Robinson C: The immunobiology of allergenic peptidases. *Clin Exp Allergy* 2003;33:3–6.
15. Charbonnier AS, Hammad H, Gosset P, et al: Der p 1-pulsed myeloid and plasmacytoid dendritic cells from house dust mite-sensitized allergic patients dysregulate the T cell response. *J Leukoc Biol* 2003;73:91–99.
16. Pichavant M, Charbonnier AS, Taront S, et al: Asthmatic bronchial epithelium activated by the proteolytic allergen Der p 1 increases selective dendritic cell recruitment. *J Allergy Clin Immunol* 2005;115:771–778.
17. McCall C, Hunter S, Stedman K, et al: Characterization and cloning of a major high molecular weight house dust mite allergen (Der f 15) for dogs. *Vet Immunol Immunopathol* 2001;78:231–247.
18. Nuttall TJ, Pemberton AD, Lamb JR, Hill PB: Peripheral blood mononuclear cell responses to major and minor *Dermatophagoides* allergens in canine atopic dermatitis. *Vet Immunol Immunopathol* 2002;84:143–150.
19. Weber E, Hunter S, Stedman K, et al: Identification, characterization, and cloning of a complementary DNA encoding a 60-kd house dust mite allergen (Der f 18) for human beings and dogs. *J Allergy Clin Immunol* 2003;112:79–86.
20. Johannessen BR, Skov LK, et al: Structure of the house dust mite allergen Der f 2: implications for function and molecular basis of IgE cross-reactivity. *FEBS Lett* 2005;579:1208–1212.
21. Mueller GA, Benjamin DC, Rule GS: Tertiary structure of the major house dust mite allergen Der p 2: sequential and structural homologies. *Biochemistry* 1998;37:12707–12714.
22. Ichikawa S, Takai T, Inoue T, et al: NMR study on the major mite allergen Der f 2: its refined tertiary structure, epitopes for monoclonal antibodies and characteristics shared by ML protein group members. *J Biochem (Tokyo)* 2005;137:255–263.
23. Gruber A, Mancek M, Wagner H, Kirschning CJ, Jerala R: Structural model of MD-2 and functional role of its basic amino acid clusters involved in cellular lipopolysaccharide recognition. *J Biol Chem* 2004;279:28475–28482.
24. Yasueda H, Mita H, Akiyama K, et al: Allergens from *Dermatophagoides* mites with chymotryptic activity. *Clin Exp Allergy* 1993;23:384–390.
25. King C, Simpson RJ, Moritz RL, Reed GE, Thompson PJ, Stewart GA: The isolation and characterization of a novel collagenolytic serine protease allergen (Der p 9) from the dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1996;98:739–747.
26. Chan SL, Ong ST, Ong SY, Chew FT, Mok YK: Nuclear magnetic resonance structure-based epitope mapping and modulation of dust mite group 13 allergen as a hypoallergen. *J Immunol* 2006;176:4852–4860.
27. Westritschnig K, Sibanda E, Thomas W, et al: Analysis of the sensitization profile towards allergens in central Africa. *Clin Exp Allergy* 2003;33:22–27.
28. Satinover SM, Reefer AJ, Pomes A, Chapman MD, Platts-Mills TA, Woodfolk JA: Specific IgE and IgG antibody-binding patterns to recombinant cockroach allergens. *J Allergy Clin Immunol* 2005;115:803–809.
29. Yi FC, Cheong N, Shek PC, Wang DY, Chua KY, Lee BW: Identification of shared and unique immunoglobulin E epitopes of the highly conserved tropomyosins in *Blomia tropicalis* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 2002;32:1203–1210.
30. Aki T, Kodama T, Fujikawa A, et al: Immunochemical characterization of recombinant and native tropomyosins as a new allergen from the house dust mite, *Dermatophagoides farinae*. *J Allergy Clin Immunol* 1995;96:74–83.
31. Fernandes J, Reshef A, Patton L, Ayuso R, Reese G, Lehrer SB: Immunoglobulin E antibody reactivity to the major shrimp allergen, tropomyosin, in unexposed Orthodox Jews. *Clin Exp Allergy* 2003;33:956–961.
32. Kawamoto S, Aki T, Yamashita M, et al: Toward elucidating the full spectrum of mite allergens—state of the art. *J Biosci Bioeng* 2002;94:285–298.
33. Miyamoto T, Akiyama K, Ohta K, Urata C, Horiuchi Y: Studies in atopic asthma with emphasis on correlation

- among various tests and various antibodies. *J Allergy Clin Immunol* 1981;67:279–284.
34. Macaubas C, Sly PD, Burton P, et al: Regulation of T-helper cell responses to inhalant allergen during early childhood. *Clin Exp Allergy* 1999;29:1223–1231.
 35. Oshika E, Kuroki Y, Sakiyama Y, Matsumoto S: Measurement of IgG subclass antibodies to the group II antigen of *Dermatophagoides pteronyssinus* (Der p II) in sera from children with bronchial asthma. *Ann Allergy* 1992;69:427–432.
 36. Chapman MD, Platts-Mills TA: Measurement of IgG, IgA and IgE antibodies to *Dermatophagoides pteronyssinus* by antigen-binding assay, using a partially purified fraction of mite extract (F4P1). *Clin Exp Immunol* 1978;34:126–136.
 37. Tame A, Sakiyama Y, Kobayashi I, Terai I, Kobayashi K: Differences in titres of IgE, IgG4 and other IgG subclass anti-Der p 2 antibodies in allergic and non-allergic patients measured with recombinant allergen. *Clin Exp Allergy* 1996;26:43–49.
 38. Bahceci NN, Arikian C, Taylor A, et al: Impact of sublingual immunotherapy on specific antibody levels in asthmatic children allergic to house dust mites. *Int Arch Allergy Immunol* 2005;136:287–294.
 39. Jutel M, Akdis M, Budak F, et al: IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 2003;33:1205–1214.
 40. Smith AM, Yamaguchi H, Platts-Mills TA, Fu SM: Prevalence of IgG anti-Der p 2 antibodies in children from high and low antigen exposure groups: relationship of IgG and subclass antibody responses to exposure and allergic symptoms. *Clin Immunol Immunopathol* 1998;86:102–109.
 41. Noguchi E, Shibasaki M, Isoyama S, Takita H: Comparisons of IgE, IgG, and IgG4 responsiveness to *Dermatophagoides farinae* in children by immunoblotting. *Allergy* 1996;51:907–913.
 42. Lin KL, Wang SY, Hsieh KH: Analysis of house dust mite-specific IgE, IgG4, and IgG antibodies during immunotherapy in asthmatic children. *Ann Allergy* 1991;67:63–69.
 43. Tsai L, Sun Y, Chao P, et al: Sequence analysis and expression of a cDNA clone encoding a 98-kDa allergen in *Dermatophagoides farinae*. *Clin Exp Allergy* 1999;29:1606–1613.
 44. Tsai LC, Peng HJ, Lee CS, et al: Molecular cloning and characterization of full-length cDNAs encoding a novel high-molecular-weight *Dermatophagoides pteronyssinus* mite allergen, Der p 11. *Allergy* 2005;60:927–937.
 45. Fujikawa A, Uchida K, Yanagidani A, et al: Altered antigenicity of M-177, a 177-kDa allergen from the house dust mite *Dermatophagoides farinae*, in stored extract. *Clin Exp Allergy* 1998;28:1549–1558.
 46. Fujikawa A, Ishimaru N, Seto A, et al: Cloning and characterization of a new allergen, Mag 3, from the house dust mite, *Dermatophagoides farinae*: cross-reactivity with high-molecular-weight allergen. *Mol Immunol* 1996;33:311–319.
 47. O'Neil SE, Heinrich TK, Hales BJ, et al: The chitinase allergens Der p 15 and Der p 18 from *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 2006;36:831–839.
 48. O'Hehir RE, Bal V, Quint D, et al: An in vitro model of allergen-dependent IgE synthesis by human B lymphocytes: comparison of the response of an atopic and a non-atopic individual to *Dermatophagoides* spp. (house dust mite). *Immunology* 1989;66:499–504.
 49. Parronchi P, Macchia D, Piccinini MP, et al: Allergen- and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. *Proc Natl Acad Sci USA* 1991;88:4538–[??]4542.
 50. Wierenga EA, Snoek M, Jansen HM, Bos JD, van Lier RA, Kapsenberg ML: Human atopen-specific types 1 and 2 T helper cell clones. *J Immunol* 1991;147:2942–2949.
 51. Halvorsen R, Bosnes V, Thorsby E: T cell responses to a *Dermatophagoides farinae* allergen preparation in allergies and healthy controls. *Int Arch Allergy Appl Immunol* 1986;80:62–69.
 52. Bellinghausen I, Brand U, Knop J, Saloga J: Comparison of allergen-stimulated dendritic cells from atopic and nonatopic donors dissecting their effect on autologous naive and memory T helper cells of such donors. *J Allergy Clin Immunol* 2000;105:988–996.
 53. McHugh SM, Lachmann PJ, Ewan PW: Peripheral blood mononuclear cells from house dust mite allergic patients produce IL-2 in response to specific allergen challenge. *Clin Exp Allergy* 1993;23:137–144.
 54. Chang JH, Chan H, Quirce S, et al: In vitro T-lymphocyte response and house dust mite-induced bronchoconstriction. *J Allergy Clin Immunol* 1996;98:922–931.
 55. Till S, Dickason R, Huston D, et al: IL-5 secretion by allergen-stimulated CD4+ T cells in primary culture: relationship to expression of allergic disease. *J Allergy Clin Immunol* 1997;99:563–569.
 56. Nurse B, Puterman AS, Haus M, Berman D, Weinberg EG, Potter PC: PBMCs from both atopic asthmatic and nonatopic children show a TH2 cytokine response to house dust mite allergen. *J Allergy Clin Immunol* 2000;106:84–91.
 57. O'Brien RM, Thomas WR: Immune reactivity to Der p I and Der p II in house dust mite sensitive patients attending paediatric and adult allergy clinics. *Clin Exp Allergy* 1994;24:737–742.
 58. Cavaillon JM, Fitting C, Guinnee MT, Rassemont R, David B: Lymphocyte proliferative responses to the purified *Dermatophagoides farinae* major allergen in untreated and hyposensitized atopic patients. *Allergy* 1988;43:146–151.
 59. Fujii S, Ono K, Shigeta S, Jyo T, Yamashita U: Human T cell responses to recombinant mite antigens of *Dermatophagoides farinae*. *Clin Exp Immunol* 1997;108:284–288.
 60. Rawle FC, Mitchell EB, Platts-Mills TA: T cell responses to the major allergen from the house dust mite *Dermatophagoides pteronyssinus*, Antigen P1:

- comparison of patients with asthma, atopic dermatitis, and perennial rhinitis. *J Immunol* 1984;133:195–201.
61. Slunt JB, Taketomi EA, Platts-Mills TA: Human T-cell responses to *Trichophyton tonsurans*: inhibition using the serum free medium Aim V. *Clin Exp Allergy* 1997;27:1184–1192.
62. Richards D, Chapman MD, Sasama J, Lee TH, Kemeny DM: Immune memory in CD4+ CD45RA+ T cells. *Immunology* 1997;91:331–339.
63. Burastero SE, Fenoglio D, Crimi E, Brusasco V, Rossi GA: Frequency of allergen-specific T lymphocytes in blood and bronchial response to allergen in asthma. *J Allergy Clin Immunol* 1993;91:1075–1081.
64. Khirwadkar K, Schmitz M, Kabelitz D: Frequency analysis of allergen-reactive T-lymphocytes in individuals allergic against the house-dust mite *Dermatophagoides pteronyssinus*. *Int Arch Allergy Immunol* 1992;98:6–12.
65. Ford D, Burger D: Precursor frequency of antigen-specific T cells: effects of sensitization in vivo and in vitro. *Cell Immunol* 1983;79:334–344.
66. Gabaglia CR, Valle MT, Fenoglio D, Barcinski MA, Manca F: Cd4(+) T cell response to *Leishmania* spp. in non-infected individuals. *Hum Immunol* 2000;61:531–537.
67. Avanzini MA, Belloni C, Soncini R, et al: Increment of recombinant hepatitis B surface antigen-specific T-cell precursors after revaccination of slow responder children. *Vaccine* 2001;19:2819–2824.
68. Assing K, Nielsen CH, Poulsen LK: Immunological characteristics of subjects with asymptomatic skin sensitization to birch and grass pollen. *Clin Exp Allergy* 2006;36:283–292.
69. Byron KA, O'Brien RM, Varigos GA, Wootton AM: *Dermatophagoides pteronyssinus* II-induced interleukin-4 and interferon-gamma expression by freshly isolated lymphocytes of atopic individuals. *Clin Exp Allergy* 1994;24:878–883.
70. O'Brien RM, Xu H, Rolland JM, Byron KA, Thomas WR: Allergen-specific production of interferon-gamma by peripheral blood mononuclear cells and CD8 T cells in allergic disease and following immunotherapy. *Clin Exp Allergy* 2000;30:333–340.
71. Hales BJ, Shen H, Thomas WR: Cytokine responses to Der p 1 and Der p 7: house dust mite allergens with different IgE-binding activities. *Clin Exp Allergy* 2000;30:934–943.
72. Smart JM, Kemp AS: Increased Th1 and Th2 allergen-induced cytokine responses in children with atopic disease. *Clin Exp Allergy* 2002;32:796–802.
73. Akdis M, Verhagen J, Taylor A, et al: Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 2004;199:1567–1575.
74. Héaton T, Rowe J, Turner S, et al: An immunoepidemiological approach to asthma: identification of in-vitro T-cell response patterns associated with different wheezing phenotypes in children. *Lancet* 2005;365:142–149.
75. Gardner LM, Spyroglou L, O'Hehir RE, Rolland JM: Increased allergen concentration enhances IFN-gamma production by allergic donor T cells expressing a peripheral tissue trafficking phenotype. *Allergy* 2004;59:1308–1317.
76. Kircher MF, Haesler T, Nickel R, Lamb JR, Renz H, Beyer K: Vbeta.18.1(+) and V(alpha).2.3(+) T-cell subsets are associated with house dust mite allergy in human subjects. *J Allergy Clin Immunol* 2002;109:517–523.
77. Li Y, Simons FE, Jay FT, HayGlass KT: Allergen-driven limiting dilution analysis of human IL-4 and IFN-gamma production in allergic rhinitis and clinically tolerant individuals. *Int Immunol* 1996;8:897–904.
78. Looney RJ, Pudiak D, Rosenfeld SI: Cytokine production by mite-specific T cells from donors with mild atopic disease. *J Allergy Clin Immunol* 1994;93:476–483.
79. Leonard C, Tormey V, Burke C, Poulter LW: Allergen-induced cytokine production in atopic disease and its relationship to disease severity. *Am J Respir Cell Mol Biol* 1997;17:368–375.
80. Hales BJ, Chu NR, Bosco A, Smith W, Heinrich TK, Thomas WR: Determinants of house dust mite allergenicity. *Allergy Clinical Immunol Int* 2006;18:65–70.
81. Epton MJ, Dilworth RJ, Smith W, Thomas WR: Sensitisation to the lipid-binding apolipoprotein allergen Der p 14 and the peptide Mag-1. *Int Arch Allergy Immunol* 2001;124:57–60.
82. Kimura JY, Ohta N, Ishii A, Nagano T, Usui M: Functional characterization of lymphocyte response to fractionated house dust mite antigens (*Dermatophagoides pteronyssinus*) in atopic and non-atopic individuals. *Immunology* 1990;70:385–390.
83. Okano M, Nagano T, Ono T, Masuda Y, Ohta N: Clonal analysis of human T-cell responses to fractionated house dust mite antigens (*Dermatophagoides pteronyssinus*). *Int Arch Allergy Immunol* 1993;101:82–88.
84. Matsumoto K, Gauvreau GM, Rerecich T, Watson RM, Wood LJ, O'Byrne PM: IL-10 production in circulating T cells differs between allergen-induced isolated early and dual asthmatic responders. *J Allergy Clin Immunol* 2002;109:281–286.
85. Hales BJ, Hazell LA, Smith W, Thomas WR: Genetic variation of Der p 2 allergens: effects on T cell responses and immunoglobulin E binding. *Clin Exp Allergy* 2002;32:1461–1467.
86. Robinson DS, Tsicopoulos A, Meng Q, Durham S, Kay AB, Hamid Q: Increased interleukin-10 messenger RNA expression in atopic allergy and asthma. *Am J Respir Cell Mol Biol* 1996;14:113–117.
87. Ling EM, Smith T, Nguyen XD, et al: Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. *Lancet* 2004;363:608–615.
88. Taylor AL, Hale J, J. HB, Dunstan JA, R. TW, L. PS: FOXP3 mRNA expression at 6 months of age is not affected by giving probiotics from birth, but is higher

- in infants who develop atopic dermatitis. *Pediatr Allergy Immunol* 2006;in press.
89. Macaubas C, Lee PT, Smallacombe TB, et al: Reciprocal patterns of allergen-induced GATA-3 expression in peripheral blood mononuclear cells from atopics vs. non-atopics. *Clin Exp Allergy* 2002;32:97–106.
 90. Wardlaw AJ, Guillen C, Morgan A: Mechanisms of T cell migration to the lung. *Clin Exp Allergy* 2005;35:4–7.
 91. Kallinich T, Schmidt S, Hamelmann E, et al: Chemokine-receptor expression on T cells in lung compartments of challenged asthmatic patients. *Clin Exp Allergy* 2005;35:26–33.
 92. Panina-Bordignon P, Papi A, Mariani M, et al: The C-C chemokine receptors CCR4 and CCR8 identify airway T cells of allergen-challenged atopic asthmatics. *J Clin Invest* 2001;107:1357–1364.
 93. Liu L, Jarjour NN, Busse WW, Kelly EA: Enhanced generation of helper T type 1 and 2 chemokines in allergen-induced asthma. *Am J Respir Crit Care Med* 2004;169:1118–1124.
 94. Bochner BS, Hudson SA, Xiao HQ, Liu MC: Release of both CCR4-active and CXCR3-active chemokines during human allergic pulmonary late-phase reactions. *J Allergy Clin Immunol* 2003;112:930–934.
 95. Nouri-Aria KT, Wilson D, Francis JN, et al: CCR4 in human allergen-induced late responses in the skin and lung. *Eur J Immunol* 2002;32:1933–1938.
 96. Frew AJ, Kay AB: The pattern of human late-phase skin reactions to extracts of aeroallergens. *J Allergy Clin Immunol* 1988;81:1117–1121.
 97. Seneviratne SL, Jones L, King AS, et al: Allergen-specific CD8(+) T cells and atopic disease. *J Clin Invest* 2002;110:1283–1291.
 98. Kim YK, Oh SY, Oh HB, et al: Positive association between HLA-DRB1*07 and specific IgE responses to purified major allergens of *D. pteronyssinus* (Der p 1 and Der p 2). *Ann Allergy Asthma Immunol* 2002;88:170–174.
 99. Hu C, Hsu PN, Lin RH, Hsieh KH, Chua KY: HLA DPB1*0201 allele is negatively associated with immunoglobulin E responsiveness specific for house dust mite allergens in Taiwan. *Clin Exp Allergy* 2000;30:538–545.
 100. Moffatt MF, Schou C, Faux JA, et al: Association between quantitative traits underlying asthma and the HLA-DRB1 locus in a family-based population sample. *Eur J Hum Genet* 2001;9:341–346.
 101. O'Brien RM, Thomas WR, Nicholson I, Lamb JR, Tait BD: An immunogenetic analysis of the T-cell recognition of the major house dust mite allergen Der p 2: identification of high- and low-responder HLA-DQ alleles and localization of T-cell epitopes. *Immunology* 1995;86:176–182.
 102. O'Brien RM, Tait BD, Varney MD, Thomas WR: HLA-DPB1 alleles in house dust mite allergic patients. *Eur J Immunogenet* 1999;26:281–284.
 103. Higgins JA, Thorpe CJ, Hayball JD, O'Hehir RE, Lamb JR: Overlapping T-cell epitopes in the group I allergen of *Dermatophagoides* species restricted by HLA-DP and HLA-DR class II molecules. *J Allergy Clin Immunol* 1994;93:891–899.
 104. Yssel H, Johnson KE, Schneider PV, et al: T cell activation-inducing epitopes of the house dust mite allergen Der p 1. Proliferation and lymphokine production patterns by Der p 1-specific CD4+ T cell clones. *J Immunol* 1992;148:738–745.
 105. Matsuoka T, Kohrogi H, Ando M, Nishimura Y, Matsushita S: *Dermatophagoides farinae*-1-derived peptides and HLA molecules recognized by T cells from atopic individuals. *Int Arch Allergy Immunol* 1997;112:365–370.
 106. Tsitoura DC, Verhoef A, Gelder CM, O'Hehir RE, Lamb JR: Altered T cell ligands derived from a major house dust mite allergen enhance IFN-gamma but not IL-4 production by human CD4+ T cells. *J Immunol* 1996;157:2160–2165.
 107. Joost van Neerven R, van t'Hof W, Ringrose JH, et al: T cell epitopes of house dust mite major allergen Der p 11. *J Immunol* 1993;151:2326–2335.
 108. Verhoef A, Higgins JA, Thorpe CJ, et al: Clonal analysis of the atopic immune response to the group 2 allergen of *Dermatophagoides* spp.: identification of HLA-DR and -DQ restricted T cell epitopes. *Int Immunol* 1993;5:1589–1597.
 109. van Neerven RJ, van de Pol MM, Wierenga EA, Aalberse RC, Jansen HM, Kapsenberg ML: Peptide specificity and HLA restriction do not dictate lymphokine production by allergen-specific T-lymphocyte clones. *Immunology* 1994;82:351–356.
 110. O'Hehir RE, Eckels DD, Frew AJ, Kay AB, Lamb JR: MHC class II restriction specificity of cloned human T lymphocytes reactive with *Dermatophagoides farinae* (house dust mite). *Immunology* 1988;64:627–631.
 111. O'Hehir RE, Mach B, Berte C, et al: Direct evidence for a functional role of HLA-DRB1 and -DRB3 gene products in the recognition of *Dermatophagoides* spp. (house dust mite) by helper T lymphocytes. *Int Immunol* 1990;2:885–892.
 112. Hales BJ, Thomas WR: T-cell sensitization to epitopes from the house dust mites *Dermatophagoides pteronyssinus* and *Euroglyphus maynei*. *Clin Exp Allergy* 1997;27:868–875.
 113. Wedderburn LR, O'Hehir RE, Hewitt CR, Lamb JR, Owen MJ: In vivo clonal dominance and limited T-cell receptor usage in human CD4+ T-cell recognition of house dust mite allergens. *Proc Natl Acad Sci USA* 1993;90:8214–8218.
 114. Jahn-Schmid B, Fischer GF, Bohle B, et al: Antigen presentation of the immunodominant T-cell epitope of the major mugwort pollen allergen, Art v 1, is associated with the expression of HLA-DRB1*01. *J Allergy Clin Immunol* 2005;115:399–404.
 115. O'Hehir RE, Verhoef A, Panagiotopoulou E, et al: Analysis of human T cell responses to the group II allergen of *Dermatophagoides* species: localization of major antigenic sites. *J Allergy Clin Immunol* 1993;92:105–113.
 116. Smith WA, Hales BJ, Jamicki AG, Thomas WR: Allergens of wild house dust mites: environmental Der p 1 and Der p 2 sequence polymorphisms. *J Allergy Clin Immunol* 2001;107:985–992.

117. Piboonpocanun S, Malainual N, Jirapongsananuruk O, Vichyanond P, Thomas WR: Genetic polymorphisms of major house dust mite allergens. *Clin Exp Allergy* 2006;36:510–516.
118. Sigurs N, Hattveig G, Kjellman B, Kjellman NI, Nilsson L, Björkstén B: Appearance of atopic disease in relation to serum IgE antibodies in children followed up from birth for 4 to 15 years. *J Allergy Clin Immunol* 1994;94:757–763.
119. Upham JW, Holt PG, Taylor A, Thornton CA, Prescott SL: HLA-DR expression on neonatal monocytes is associated with allergen-specific immune responses. *J Allergy Clin Immunol* 2004;114:1202–1208.
120. Miles EA, Warner JA, Jones AC, Colwell BM, Bryant TN, Warner JO: Peripheral blood mononuclear cell proliferative responses in the first year of life in babies born to allergic parents. *Clin Exp Allergy* 1996;26:780–788.
121. Prescott SL, Macaubas C, Holt BJ, et al: Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J Immunol* 1998;160:4730–4737.
122. Smillie FI, Elderfield AJ, Patel F, et al: Lymphoproliferative responses in cord blood and at one year: no evidence for the effect of in utero exposure to dust mite allergens. *Clin Exp Allergy* 2001;31:1194–1204.
123. Chan-Yeung M, Ferguson A, Chan H, et al: Umbilical cord blood mononuclear cell proliferative response to house dust mite does not predict the development of allergic rhinitis and asthma. *J Allergy Clin Immunol* 1999;104:317–321.
124. van der Velden VH, Laan MP, Baert MR, de Waal Malefyt R, Neijens HJ, Savelkoul HF: Selective development of a strong Th2 cytokine profile in high-risk children who develop atopy: risk factors and regulatory role of IFN-gamma, IL-4 and IL-10. *Clin Exp Allergy* 2001;31:997–1006.
125. Marks GB, Zhou J, Yang HS, Joshi PA, Bishop GA, Britton WJ: Cord blood mononuclear cell cytokine responses in relation to maternal house dust mite allergen exposure. *Clin Exp Allergy* 2002;32:355–360.
126. Thornton CA, Upham JW, Wikstrom ME, et al: Functional maturation of CD4+CD25+CTLA4+CD45RA+ T regulatory cells in human neonatal T cell responses to environmental antigens/allergens. *J Immunol* 2004;173:3084–3092.
127. Devereux G, Seaton A, Barker RN: In utero priming of allergen-specific helper T cells. *Clin Exp Allergy* 2001;31:1686–1695.
128. Piccinni MP, Mecacci F, Sampognaro S, et al: Aeroallergen sensitization can occur during fetal life. *Int Arch Allergy Immunol* 1993;102:301–303.
129. Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG: Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999;353:196–200.
130. Yabuhara A, Macaubas C, Prescott SL, et al: TH2-polarized immunological memory to inhaled allergens in atopics is established during infancy and early childhood. *Clin Exp Allergy* 1997;27:1261–1269.
131. Tedeschi A, Barcella M, Bo GA, Miadonna A: Onset of allergy and asthma symptoms in extra-European immigrants to Milan, Italy: possible role of environmental factors. *Clin Exp Allergy* 2003;33:449–454.
132. 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;124:630–635.
133. Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TA: Antigenic and structural analysis of group II allergens (Der f II and Der p II) from house dust mites (*Dermatophagoides* spp). *J Allergy Clin Immunol* 1989;83:1055–1067.
134. Ramos JD, Teo AS, Ou KL, et al: Comparative allergenicity studies of native and recombinant *Blomia tropicalis* Paramyosin (Blo t 11). *Allergy* 2003;58:412–419.
135. Li CS, Hsu CW, Chua KY, Hsieh KH, Lin RH: Environmental distribution of house dust mite allergen (Der p 5). *J Allergy Clin Immunol* 1996;97:857–859.
136. Shen HD, Lin WL, Tsai LC, et al: Characterization of the allergen Der f 7 from house dust mite extracts by species-specific and crossreactive monoclonal antibodies. *Clin Exp Allergy* 1997;27:824–832.
137. Stewart GA, Kollinger MR, King CM, Thompson PJ: A comparative study of three serine proteases from *Dermatophagoides pteronyssinus* and *D. farinae*. *Allergy* 1994;49:553–560.
138. Weghofer M, Thomas WR, Pittner G, Horak F, Valenta R, Vrtala S: Comparison of purified *Dermatophagoides pteronyssinus* allergens and extract by two-dimensional immunoblotting and quantitative immunoglobulin E inhibitions. *Clin Exp Allergy* 2005;35:1384–1391.
139. Batard T, Hrabina A, Bi XZ, et al: Production and proteomic characterization of pharmaceutical-grade *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* extracts for allergy vaccines. *Int Arch Allergy Immunol* 2006;140:295–305.
140. Holt PG, Clough JB, Holt BJ, et al: Genetic “risk” for atopy is associated with delayed postnatal maturation of T-cell competence. *Clin Exp Allergy* 1992;22:1093–1099.
141. Epton MJ, Smith W, Hales BJ, Hazell L, Thompson PJ, Thomas WR: Non-allergenic antigen in allergic sensitization: responses to the mite ferritin heavy chain antigen by allergic and non-allergic subjects. *Clin Exp Allergy* 2002;32:1341–1347.