House dust mite allergy

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Abstract

The importance of the allergens in sensitisation and disease can be readily demonstrated in regions with and without mite infestation. Dose-response relationships in regions with high infestation are less easy to detect pointing to the importance of variations of other factors. The relationship between allergic disease and IgE antibody shows that only people with very high responses become persistent asthmatics and many people with high titres are healthy. A major burden of disease is however found in children with lower IgE titres and intermittent asthma, and this cannot be predicted with the current tests. Considerable advances have been made on recognising the important allergens. There is a hierarchical response, for both high and low responders, with the group 1 and 2 allergens accounting for 50-60% of the IgE binding and the mid-range group 4, 5 and 7 allergens accounting for about 20-30%. The IgE responses to most other allergens are low and they do...
not induce non-allergenic responses, as judged by IgG antibody. Possible important allergens that have not been quantitatively investigated are the group 11, 14 and 15, that are respectively paramyosin, large lipid transfer protein and chitinase. The importance of the Th2 cytokine responses of T cells is well established but the role of Th1 responses in either exacerbating disease or modifying disease is uncertain, as is the role of regulatory T cell responses. A major limitation of many studies is that allergen extracts have been used instead of allergens so the doses have not been optimised and the responses to all the significant allergens have not been measured. The effect of non-allergenic antigens and pro-inflammatory molecules in extracts are further problems. The end results of mite allergen immunotherapy show beneficial effects for disease similar to pollen immunotherapy. New types of immunotherapy or vaccination could therefore have a large impact on disease. Mite avoidance treatments for homes on the other hand have been shown to be largely ineffective probably because the mite reductions that can be achieved are modest. Healthy people exposed to house dust mites make non-harmful responses to mite allergens. They do not result in significant antibody responses of any isotype, but are large as shown by T-cell precursor analysis.

I. Exposure sensitisation and allergic disease

Verhorst and colleagues first proposed that the pyroglyphid mite *Dermatophagoides pteronyssinus* was the major source of allergen in house dust (1). The potent allergenicity of proteins produced by the house dust mite (HDM) and importance of the allergy in asthma and other allergic disease remain unquestioned. In regions with high mite infestation, 20-40% of people become sensitised as shown by skin tests or IgE measurement, and about half these develop symptoms of asthma (2-4). Of the asthmatics, about half take daily medication and one quarter can be defined as moderate asthmatics who, despite medication, have frequent symptoms, or need of hospitalisation or emergency treatment (4, 5).

The expected requirement for mites to be present in the environment for the development of allergic sensitisation is shown by the lack of IgE and skin test reactivity found in the inhabitants of regions with few mites, such as those found in deserts (4), cold climates (6) and at altitude (7). The people in these areas develop sensitisation to other prevailing allergens such as animals and moulds, so reasons other than the exposure to allergen are unlikely to be involved. Analyses of less contrasting climates have also shown the relationship between allergen in the environment and sensitisation (8). Here the prevalence of mite and cockroach allergy in different cities of the United States of America was found to be associated with the abundance of each allergen source. In contrast to comparisons in different geographical regions, some studies of exposure in different homes within the same environment have not shown a relationship (9, 10) or have only shown a weak association (11, 12). A strong association was found in Germany (13), which might, from the low levels of mites reported, be attributed to dose-response relationships existing only at low doses. This finding is backed by Cullinan et al. (3) who found a sharp dose response at low allergen levels and an attenuation resulting in drop at higher doses. The species-specificity of mite allergy also shows the relationship between exposure and sensitisation. Shen et al. showed that in Taiwan, where *D. pteronyssinus* predominates over *D. farinae*, that the IgE antibody responses to Der p 7 was far higher than the antibody titre to Der f 7 (14). Likewise Hales et al. showed that in another area,
Western Australia, where *D. pteronyssinus* predominates that peripheral blood mononuclear cell (PBMC) responses of the residents responded better to the peptides with Der p 1 compared to Der f 1 sequences (15).

HDM sensitivity is a strong independent risk factor for asthma even when considered independently from other allergen sensitisations (16, 17). Curiously, despite the associations between exposure and sensitisation and between sensitisation and disease, a direct relationship between exposure and disease has not been obvious. Studies in regions with a high infestation have shown no relationship between exposure and the prevalence or severity of disease (17, 18), as have studies in regions with moderate infestations (8, 10). Associations of the presence of asthma (12) and asthma exacerbations (19) can however be found provided the studies are sufficiently powered to stratify for exposure of people who have, or develop, sensitivity. A recent longitudinal study from Germany (20) found a clear association with the persistence of asthma from early childhood to 13 years of age. It was however critical to restrict the analysis to the progressive disease of children with an established association of atopy and asthma. One reason for not finding a relationship is the poor reliability of the measurement of the mites (21, 22). Even the studies reported to have good reproducibility (23, 24) only had correlation for repeated measurements of about 0.4 - 0.7, which is level of error that can seriously undermine multivariate analyses of complex outcomes. It has now been reported that climatic variations in different years can also markedly affect the level of mite infestation (25), which could seriously affect prospective studies. The appropriateness of sampling dust in carpets or mattresses has also been questioned. A recent study compared dust sampling with sampling of allergen in inhaled air with intranasal filters. The allergen levels in inhaled air did not correlate with the amount of allergen in the reservoir dust, and only the reservoir samples had associations with asthma and sensitisation (12).

Deliberate interventions to reduce allergen levels could provide controlled methods of studying the effect of allergen exposure. Indeed the earliest observations pointing to importance of mite allergy and disease were the relief from symptoms and bronchial hyperreactivity found when asthmatics took a sojourn in a mite-free environment, such as a hospital (26, 27) or high-altitude retreat (28). Reductions in inflammation shown by eosinophilia (29) and eosinophil mediator release (29, 30) have also been detected. The conclusions drawn from these observations have been questioned because of possible confounding effects such as the reduction of pollution. They cannot be strictly eliminated but it should be noted that pollution only has a small effect on asthma (31) and that the symptoms suffered by asthmatics are worse at altitude (32). A feature of these studies is that a very substantial relief was obtained in a period of only a few months. The consistent effects found in mite-free environments contrast with the indifferent effects of mite avoidance measures taken in homes (33). The results from a large number of trials suggest there may be a small degree of relief for children but none for adults. A major problem remains in obtaining sufficient mite reduction (34-36). Even in studies where the experimenters implemented seemingly stringent measures, only 50-60% reductions were achieved, still leaving behind high-level exposure (34, 36).

Mite avoidance in the primary prevention has produced similar results. Children reared in homes treated to reduce mites do not show a decrease in sensitisation or symptoms (35, 36). An improvement in some measures of airway function has been
reported (34) but the same study found that the prevalence of mite sensitisation increased in the avoidance group. Since the mites were still present in substantial quantities after the avoidance, it is possible that the increase was due to the removal of the high-dose attenuation found by Cullinan et al. (3). A recent study, which has combined food allergen and mite allergen avoidance, has shown a remarkable reduction of sensitisation and disease, lasting for at least 8 years (37). Further studies of this procedure will be of immense interest.

II. Correlation of IgE and allergic disease

Despite the strong association of sensitisation and allergic disease not all people with high IgE antibody develop disease. Shibisaki et al., for example, showed that while asthma in school-aged children, defined as recurrent wheezing with shortness of breath, was almost exclusively found in those with the highest titres, only about 20% with high titres had asthma (2). Moreover the same prevalence was found across a 30-fold range of titres beginning at about 50 IU/ml of IgE (extrapolated from the assumption that the limit of RAST detection by Shibasaki was about 0.35 IU/ml). The children below this level had asthmatic symptoms described as wheezing. These observation match analyses with CAP scores and receiver operating characteristic (ROC) calculations (38). A value above 44 IU/ml of antibody had a 70-80% sensitivity and specificity of identifying people with more serious persistent asthma and values below this had a similar ability to identify intermittent asthma. These studies make the important observation that people with high anti-HDM titres have more disease but several critical points need to be considered: 1) That levels of 50 IU/ml are very high representing the top 20-30% of people with mite sensitisation. 2) People defined as having persistent asthma are very ill having three or more days of asthma symptoms per week. 3) 75% of children and 75% of hospital admissions for asthma are for exacerbations of intermittent asthma (39). 4) People with intermittent asthma not only have serious exacerbations but also have tissue remodelling (40). 5) Many people with the high levels of IgE are asymptomatic. 6) Specificity and sensitivity values of 70% are not very accurate.

Analyses of IgE with the clinical significance of intermittent asthma have been made. Soderstrom et al. compared the IgE antibody levels of prospective mite-allergic patients with blinded clinical assessments (41). The results from three centres showed that the probability that a doctor would implicate mite allergy as a cause of disease was 50% when anti mite titres were about 1 IU/ml, and almost 100% with titres of 3.5 IU/ml. Similarly analyses conducted to measure actual outcomes in a longitudinal study showed a 50% probability of current wheeze at 30 IU/ml (42). The probability of wheeze and reduction in lung function were however continuous variables with changes at 1 IU/ml and as expected from previous results even children with high titres only had a 60% probability of wheeze. A study comparing IgE titres in asymptomatic allergic people and asthmatics found a cut-off of 8 IU/ml for disease but this only had 60% specificity and sensitivity (43). The study also defined a skin size of 31 mm2 for defining clinically important allergy but this had even less specificity and sensitivity than the IgE titre.

III. Immunotherapy

The significance of the ability to treat allergic disease with immunotherapy is frequently overlooked. The treatment leaves much to desired in terms of the time required
for an effect and the protracted injection regimens, but its ability to produce symptom relief and reduce the need for medication has been well documented in placebo controlled trials (44, 45), including for patients with moderate and severe asthma (46). Symptom relief can be achieved within a year although, by analogy with grass pollen immunotherapy, it probably needs to be continued for several years for lasting effects. 10-year post-treatment follow-ups of children receiving immunotherapy for 5 years showed the persistence of lasting benefit (47). Changes in the inflammatory responses induced by allergen can be seen in decreased skin test reactivity (44, 48-50), eosinophil activation (45), and bronchial challenge (50). Most studies however have found that serum IgE antibodies were not decreased (45, 49, 50), even when decreased skin test reactions were found (49, 50). Mastrandrea et al. however found decreased IgE antibody to the major Der p 1 and Der p 2 allergens but only after several years of immunotherapy (51). Increased levels of IgG4 antibodies, which could have blocking effects, have been detected (48, 49) along with decreases in antigen-specific Th1 and Th2 cytokine responses of CD4 T cells (52) and decreased Th1 responses of CD8 T cells (53). The ability of the desensitisation to alter the symptoms provides a causal connection between the allergy and disease. The elucidation of the change responsible for this could thus provide important information on the events that produce disease.

IV. House dust mite species and distribution

Species from the family Pyroglyphidae are the most important source of HDM allergens, including in tropical and subtropical regions where they co-exist with the glycyphagid mite Blomia tropicalis (54). The most important species are D. pteronyssinus and D. farinae and to a much lesser extent Euroglyphus maynei. D. pteronyssinus is the most prevalent species found in abundance in Europe, North and South America, Africa, Asia and Australasia. It is almost the only species in found in England (55), Australia (56, 57) and New Zealand (58). D. pteronyssinus and D. farinae have inaccurately been labelled as the European and American mites and D. farinae has been incorrectly portrayed as the dominant mite species in the United States and Japan. D. pteronyssinus is in fact the most prevalent mite in most regions of these countries (59, 60) but depending on both the geographical region and individual homes, infestation with D. farinae can be important, and can even exceed the infestation with D. pteronyssinus. The infestations in the northeastern regions of North America have a bias to D. farinae (61) and the west coast a bias to D. pteronyssinus (59). In Europe, Sweden (62), Poland (63) and some regions of Italy (64) are biased to D. farinae while the species distribution in most other countries is mixed (64). Most of the areas documented in Asia also show mixed populations often with substantial representation of B. tropicalis (65-68). South America has predominantly D. pteronyssinus and B. tropicalis (69).

The IgE antibody to the allergens from different Dermatophagoides species cross-react but typically with 10-50% of the IgE binding to the sensitising allergen (14, 70). This can however vary enormously for different people showing from zero to over a 1000 fold difference (Horn 1987). The allergens of each species have about a 20% sequence disparity, a level that causes major antigenic changes in other antigens while maintaining the protein folds (71-73). The glycyphagid mites that include several species of storage mites and B. tropicalis typically have 65% sequence disparity with the pyroglyphid mites.
The group 1 allergens (74) and group 5 allergens (75, 76) show no cross reactivity between *B. tropicalis* and pyroglyphid mites. Other allergens like the group 2 allergens show a low level of cross reactivity (77) while other highly conserved allergens such as tropomyosin with 95% sequence identity, cross react extensively (78). Knowledge of the mites found in different regions is required to interpret studies on the allergenicity and epitopes. The low IgE binding to Der f 7 compared to the binding to Der p 7 found in Taiwan (14) is probably due to the fact that the study population had not been significantly exposed to *D. farinae*. There are in fact few other studies of cross reactivities with defined mite allergens and those conducted have examined sera from people exposed to mixed species so further investigation is required. The sequence disparity also causes differences in T-cell epitope recognition, as shown for the group 1 house dust mite allergens (15). This was however only apparent when peptide mapping was used. Responses to the whole allergen measured by in vitro allergen stimulation assays showed no effect (79, 80).

While from the current perspective *D. pteronyssinus*, *D. farinae* and *B. tropicalis* appear to be the most important mites there are numerous studies showing that other species can be important on a more regional basis. These must be considered both with respect to diagnosing, studying and treating the allergy in those regions, and as confounding factors in the interpretation of studies that do not take mite species into account. The possible importance of the glycyphagid storage mites *Lepidoglyphus destructor* and *Tyrophagus putrescentiae* is well known (81). Another interesting example is the recent discovery that the mite *Chortoglyphus arcuatus* is a frequent house dust mite found in mattresses in northern Spain (82).

V. House dust mite allergens
At least 21 different mite proteins have been shown to bind IgE in the sera of at least some people with HDM allergy. The group 1 and 2 allergens are however dominant specificities that typically bind IgE with titres of about 50 ng/ml (83, 84) and constitute about 50% of IgE binding to all the HDM allergens regardless of whether the total titres of the sera are high or low (84).

The group 1 allergens are cysteine proteases (85) found in the gut and dung balls (86). The recently determined X-ray crystal structures for the proenzyme and mature form of protein have shown the expected structure of papain-related enzymes (87, 88). 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 (89, 90). The mechanism might be related to its ability to cleave a number of immunologically related (91), or as suggested from *in vitro* studies, increasing the permeability of intercellular junctions (92), activating antigen-presenting cells (93) and inducing the release of chemokines from respiratory epithelium (94). 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 (95-97). The recent finding that Der p 1 probably exists as a dimer (88) may be more important in promoting allergenicity by aiding cross linking of receptors. The group 1 allergen was shown to be the 31st most abundant protein produced by *D. pteronyssinus* (98).
The group 2 allergens are proteins of the ML-domain family, related to the toll like receptor 4 (TLR-4) co-receptor MD2. (99-101) and to Niemann Pick Type 2 proteins (102). They consist of a single domain with an immunoglobulin like-fold creating a hydrophobic cavity. The Niemann Pick proteins show cholesterol-like ligand binding in the cavity so the allergens probably have a similar function. The group 2 allergen was the 41st most abundant protein shown to be produced by mites (98). They are found in the dung balls of male and female mites (103, 104).

The group 3 allergens are trypsins. They are major constituents of mite faeces but they are often present in low concentration in body extracts (105). Recent quantitative studies have confirmed the early results, reviewed by Thomas et al. (106) showing they only induce low IgE antibody titres (84). They do not appear to be important allergens and this casts doubt on an adjuvant role for proteolytic activity.

The group 4 allergens are one of the most important allergens besides Der p 1 and 2 (84). They are typical alpha-amylases with a sequence that models well with the structure of the family 13 glycosyl hydrolases (107). They are 50% identical to insect and mollusc α-amylases but this is due to amylase-specific sequences conserved throughout the animal kingdom, such that humans show the same identity.

As shown for Der p 5, the group 5 allergens have a similar degree of IgE binding to the group 4 and are thus important mid-range allergens (84). The C-terminal region has a sequence strongly predicted to be coiled coil and this agrees with the predictions for a recently described similar allergen, Der p 21. Here short angle X-ray scattering analysis confirmed the prediction (108). These allergens are also important because Blo t 5 from B. tropicalis may be the most important allergen of this species (76, 109).

The group 6 allergens are chymotrypsins that do not cross-reactive with the trypsin allergens and bind even less IgE (110, 111).

The group 7 allergens are one of the three known significant mid-potency allergens. Little is known about their structure. One important aspect is the high and variable glycosylation. The recombinant polypeptide has a molecular mass of 22kDa but the allergen is found in mite extracts as molecules of 26, 30 and 31 kDa, (112, 113) with the abundance of each species varying from extract to extract (112). N-glycosidase treatment reduced the molecules to 26 kDa. There are no unambiguous homologues that might indicate their biochemical identity. They are the 44th most abundant protein made by mites (98) but are very minor proteins in extracts in keeping with the demonstrated degradation in these concoctions (14, 112).

The group 8 glutathione-S-transferase allergens frequently bind IgE but at very low titre (84, 114). Huang also showed evidence for low but frequent, cross reactivity with glutathione-S-transferase from the American cockroach.

The group 9 are serine proteases with collagenolytic activity (111). Their IgE binding activity is less than the low IgE binding Der p 3. Recent sequences for Der p 9 (AAN02511, AAP57077) show a protein with the known N-terminal of the natural allergen and a 38% amino acid sequence identity to Der p 3 trypsin and 40% identity to Der p 6 chymotrypsin, with expected differences in the predicted substrate binding pocket.

The group 10 allergens are tropomyosins. Their sequences are evolutionary conserved being 96% identical to the tropomyosins of glycyphagid mites, 80% to crustaceans, 75% to insect tropomyosins and 50% to human tropomyosins. The cross
reactivity of mite tropomyosin with cockroach and shrimp is well known (115-117). The original description of Der f 10 from Japan showed a very high frequency and high degree of IgE binding (118). Subsequent studies in Europe (119), Australia (84), and Singapore (78) found a low frequency and amount of reactivity to Der p 10 and to glycyphagid Blo t 10 tropomyosin. IgE binding assays in Africa however showed frequent responses (119) suggesting the presence of a cross reacting sensitisation.

Studies from Taiwan and Singapore have reported a high frequency of IgE binding by the group 11 paramyosin allergens from *D. farinae*, *D. pteronyssinus* and *B. tropicalis* (120-123) but the assays used were not quantitative. It was also shown that the natural allergen was degraded and present in mite extracts at less than 1 μg/ml (123). The reactivity of paramyosin with IgE from sera from other populations has not been examined but Western blotting studies have reported prominent IgE binding bands that could be due to this allergen. Paramyosins from a number of parasites are prominent antigens and, while this could conceivably affect regional responses by cross reactivity, they are not evolutionary conserved like tropomyosin.

The group 12 allergen has only been described for *B. tropicalis* (124) although sequence database entry has been made for a homologous protein from *L. destructor*. Blo t 12 has regions of sequence similarity with chitin binding domains of larger chitinases. It bound IgE in half the allergic sera with strong immunostaining in a phage plaque assays suggesting it could be important.

The group 13 fatty acid binding proteins were the 15th most abundant proteins shown to be produced by *D. pteronyssinus*, ahead of all the other denominated allergens (98). Despite their denomination as allergens and abundance the lack of allergenicity is their most prominent feature. Only occasional people produce IgE antibody to this protein in both the glycyphagid mites (125) and for Der f 13 (126) and Der p 13 (unpublished). The amino acid sequence is quite disparate from mammalian homologues (40%) so the reason for its lack of allergenicity may be interesting.

The group 14 allergens are members of the large lipid transfer (LLTP) protein family that includes the egg storage vitellogenins and the lipid transporting apolipoporins, as well as other molecules of the haematopoietic and defence system. The N-terminal 250 amino acids form a highly conserved beta barrel structure called the lipid transfer module (127, 128). The allergens were originally described as apolipoparin-like proteins because of their similarity to insect apolipoporins but the sequences are just as similar to crustacean vitellogenins (129). Antibodies against Der f 14 (130) and Der p 14 (131) stain the haemocoels and react by immunoblot with male and female mites (unpublished). The lack of a sex difference indicates a function other than a vitellogenin. Complete cDNA sequences are available for the allergens from *D. pteronyssinus* and *E. maynei* while, presumably due to the technical difficulties in making large full length cDNA, only C-terminal regions determination have been made for other mites. For *D. farinae* the protein was defined as M-177 by immunoblotting with antibodies prepared against C-terminal recombinant peptide fragments. These fragments with laboratory designations of Mag-1 and Mag-3 are products of incomplete cDNA transcripts with no known or suspected significance as natural entities. The studies of Fujikawa showed high reactivity of the native M-177 allergen (130). Unpublished studies by the authors using a combination of the recombinant peptides 1-260 and 1322-1662 showed a possible significant activity with IgE binding one third of the allergic subjects at levels of 5-10
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ng/ml. The allergens are highly susceptible to proteolytic degradation in mite extracts (132), but this process that may not occur in nature where, even after death, compartmentalisation from the digestive enzymes could be retained.

The group 15 chitinase allergens are the major allergens of HDM-allergic dogs, binding IgE in almost all dogs compared to less than 50% binding shown by the group 1 and 2 allergens (95, 96). Their sequences show they are family 18 chitinases and have the characteristic PEST region of O-glycosylation (95, 133). Their cDNAs encode polypeptides of 61 kDa but the natural allergens have molecular masses of 98 and 106 kDa almost certainly due to glycosylation of the proline, serine rich PEST region (95). A semi quantitative assay of the IgE binding of sera from HDM-allergic people to a recombinant Der p 15 polypeptide showed that it bound very frequently at 70% (133). Another interesting property is that they are found in the gut but not the faecal pellets, showing they are distributed differently from the group 1 and 2 allergens (95).

The IgE binding to Der f 16 and 17 has been detected from screening cDNA libraries. The binding was found in about 50% of subjects and was low compared to other allergens (134).

The group 18 chitinase-like allergens have sequences that resemble chitin binding domains but the second catalytic domain is truncated and does not contain the critical Glu 160 (97, 133). They also do not have a PEST region. Natural Der f 18 was shown to bind IgE in 54% of humans (97), with a weaker binding activity, compared to the signal reported by the same laboratory for Der f 15 (95). The reactivity with dog IgE was at a high frequency of 77% but was also less than Der f 15. The study of O’Neil in humans showed that the frequency IgE binding to a recombinant polypeptide produced in E. coli was, at 63%, less than Der p 15. Note that the group 15 and 18 only have 25% sequence identity and as expected from this they have no serological cross reactivity (133). Like Der f 18 the Der f 15 was found in the upper digestive system but not the faeces.

The group 19 designation was given to an as yet unpublished and poorly allergenic homologue of an anti microbial peptide from B. tropicalis.

The group 20 is the mite arginine kinase, which was investigated because its sequence is highly conserved amongst invertebrates showing 80% identity to crustaceans and 75% to insects compared with 45% for mammalian enzymes. It was shown to bind IgE in 40% of sera from mite allergic people, but with low titres (84). Isoforms of arginine kinases are the 29th and 30th most abundant proteins in mite extracts (98).

The group 21 allergen entered into the sequence (ABC73706) and allergen databases is a protein shown to bind IgE antibodies in 30% of sera from mite-allergic people Austria (108). It is of structural interest because its sequence has similarity to the group 5 allergens, as discussed above.

VI. Antibody responses in allergy

IgE antibodies are traditionally considered to mediate allergic response by the classical induction of mediator release by the IgE antibody and allergen-induced cross-linking mast cell FcER1 receptors. Therapy with anti-IgE monoclonal antibodies (135) clearly show the importance of IgE in disease but the mechanism is uncertain. The activation of mast cells by cross-linking FcER1 induces a wide array of cytokine-mediated effects besides the release of histamine and leukotriene mediators (136). IgE antibodies can also facilitate the presentation of allergen to T cells by antigen presenting
cells, at least in in vitro cultures (137). IgE immunoglobulin binding to the FcER1 receptors also activates signalling pathways that regulate the activation state of mast cells (136), and since allergen-induced IgE antibody constitutes a major proportion of the total IgE immunoglobulin, including for house dust mite allergy (138), this can also be important for disease. The role of IgG antibody has mostly been considered to be a regulatory one. Recent data in pollen immunotherapy indicate a role in blocking IgE facilitated allergen presentation (137), although studies in mice show blocking can act by a number of mechanisms, including simple allergen sequestration (139).

The IgE binding activities of HDM allergens show a hierarchical pattern with the individual allergens inducing the same hierarchy of response in different people. For *D. pteronyssinus* the IgE binding of Der p 1 and 2 accounts for 50-60% of the total anti-HDM response while three mid-range allergens, Der p 4, 5 and 7 each have about 10% of the anti-HDM titre. The relative proportions of binding to these specificities were remarkable constant across a wide range of anti HDM titres. Their average IgE binding of 10-15 ng/ml is higher than the responses of many people to major allergens, and is level that can, as outlined earlier, have clear significance for disease. 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 (84), and since the group 6 and 9 allergens bind even less IgE than Der p 3 (110, 111, 140) they are low. The IgE binding for the group 16, 17 (134) are also low in comparison to other allergens. Only occasional people have IgE to the group 13 fatty-acid-binding-protein allergens (126) and IgE binding to the group 10 tropomyosin allergens have been shown to be low in Australia (84), Europe (119), USA (117) and Singapore (78). High reactivity has been found in Japan (118) and Africa (119). The geographical inconsistency suggests a cross reactivity, which is plausible because tropomyosin has a very highly conserved sequence.

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 (141). Increased IgG1 and IgG4 in sera from allergic subjects has been described for Der p 1 (84, 142) and Der p 2 (84, 143) as well as pan IgG anti-Der p 1 (144). Some studies however have reported that increased titres are confined to the IgG4 subclass (145, 146). A study of school children which found no difference in the pan-IgG, IgG1 and IgG4 antibody titres to Der p 2 is difficult to reconcile (147). On balance the most consistent finding is that only allergic people make high IgG responses to HDM allergens and they make both IgG1 and IgG4. The latter shows that they induce both Th1 and Th2 responses (84, 142).

The association of IgG responses with allergenicity can also be seen by comprising responses to different allergens (84). Der p 1 and 2 frequently bound IgG1 and IgG4 antibodies while the mid potency Der p 4, 5 and 7 also bound, but to a lesser degree and the weak allergens Der p 3, 8, 10 and 20 had essentially no IgG binding activity. From these studies there appears to be no non-allergic responses that manifest with IgG without IgE.

### VII. T Cell responses in allergy

T cells are not only required for the antibody responses but they appear to have a more direct role in the allergic reactions (148), especially for the late phase reactions that are accompanied by a mixed cellular infiltrate including eosinophils and T cells. They are
produced by people with higher T-cell reactivity as shown by in vitro responses of PBMC to HDM extracts (149, 150). They can however, at least for skin reactions, be induced by the injection of anti-IgE antibodies indicating that T cells are not absolutely required (151). Anti-IgE therapy also removes late phase reactions (135) but this could act via IgE antibody facilitated antigen presentation to T cells. The studies of Haselden et al. provide direct evidence for T cell involvement by showing that cat allergic patients injected with peptides representing the T-cell epitopes of Fel d 1 develop late phase bronchoconstriction (152). The reactions were however not associated with an increase in inflammation or T-cell infiltration into the airways.

Lung biopsies of asthmatics show T cells producing Th2 cytokines in the airway walls. The Th1-type IFN-γ-producing cells are low for both normal and asthmatic people (148). Bronchial challenge with HDM extract induces a rapid decrease in number of allergen-reactive T cells in the peripheral blood indicating an infiltration of the airways (153) which can be seen by the appearance of T cells producing Th2 cytokines and the Th2 master transcription factor GATA-3 in the airways (148, 154). T-cell responses following bronchial challenge with major allergens have been demonstrated for Der p 1 and Der p 2 (155). They induced early asthmatic reactions, increased serum IL-5 and late phase reactions. The IL-5 and late responses were less than those produced with a dose of HDM extract that induced the same immediate reactions. This indicates the importance of other allergens or perhaps different dose-response characteristics of early and late reactions.

Anti-HDM reactive T cell clones provided the first evidence that Th1 and Th2 cytokine polarisation could be induced in cultures of human T cells (156). It is however now well established that HDM allergens mostly induce similar quantities of IFN-γ from the PBMC of both allergic and non-allergic subjects (53, 80, 142, 157-161). The results from the cloning experiments probably showed the strong in vitro polarising activity of IL-4 produced by cells from the allergic subjects. It has been reported that Th1 cytokine release can be induced by higher doses of allergen (162) but other studies have found that increased doses increase both Th1 and Th2 cytokines (80, 159-161). The induction of IFN-γ has been demonstrated with purified Der p 1 and Der p 2 allergens as well as extracts (53, 80, 142, 157, 160, 161). Similar findings for IFN-γ release have also been found for pollen allergens (160, 163) but it is possible that the IFN-γ is reduced in severe long-term allergy (157, 164, 165).

There is little definitive data on the T-cell responses elicited by purified allergens other than the major group 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 (166) 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 (80) but this is now thought to result from effects of impurities. Proliferative responses of PBMC to the group 14 allergens have been shown to be similar to those induced by major allergens by two research groups (167, 168). Studies with extracts have also shown that T cell responses to antigens other than group 1and 2 allergens can be high (169, 170).

T-cell cloning has definitively shown that non-allergic subjects make T-cell responses to HDM allergens (156, 171). Some reports have shown that PBMC from allergic and non-allergic subjects proliferate equally well to HDM extracts or major
allergens (15, 80, 172-175) while others have shown that PBMC from allergic subjects produce higher responses (158, 176-178, 167, 179). The precursor frequencies of T cells responding to defined allergens have not been examined, but HDM extracts have been studied. Limiting dilution assays have reported frequencies in the order of 0.01 to 0.1%. Some studies have found about a 5-fold increase in the frequency for cells from allergic subjects (149, 180, 181) although similar frequencies for PBMC from allergic and non-allergic people have also been reported (172). Precursor T cell frequencies found after vaccinations of microbial antigens are about 0.02% (182, 183) so it appears that anti-HDM precursor frequencies are high even in the non-allergic people.

Given the high precursor frequencies of T cell responses in non-allergic people, immunoregulation rather than a lack of stimulation is probably important. Allergen-stimulated PBMC from allergic subjects have however been shown to make more of the regulatory IL-10 than cells from non-allergic subjects, (142, 161, 184, 185). Some indication of regulation was indicated by the finding of a negative correlation between the IL-10 responses and the size of skin prick test of the donors (142, 161) but more IL-10 production was also reported for the responses of severe asthmatics (184). Evidence for a role of IL-10 in down regulating responses has been obtained by different types of analyses. The addition of anti-IL-10 to cultures of PBMC from non-allergic subjects enhanced proliferative responses to Der p 1 (186) and a follow up showed that cultures from healthy people had more IL-10 producing T-cells when measured 12 hours after stimulation with allergen (160). 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 day culture periods used by others. Indeed cytokines produced by the PBMC of allergic subjects could 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 allergens (187). 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 (158). 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 (188). It is possible these effects are linked to increased IL-2 production by the higher responses of allergic subjects.

The HDM extract-responsive T cells of allergic subjects are mainly in the memory CD45RO+ population (175, 180) in both allergic and non-allergic people. Th2 cytokines were released from the CD45RO+ cells of atopic subjects as is the induction of the Th2 transcription factor GATA-3 (189).

The division of allergen-responsive cells into populations with different chemokine receptors is a measure that reflects T-cell polarisation 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 (190). Bronchial challenge of birch or HDM allergic patients with allergen extracts induces an infiltration of CCR4+ cells with few CXCR3-bearing Th1 cells (191, 192). Airway challenge also induces the production of MDC and TARC (192-194) that can attract Th2 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 and could be detected on 40% of T cells from allergic people responding to pollen and not on the allergen-responsive cells of healthy people (195).

The Th2 cytokine and GATA-3 responses of PBMC (176, 189) and the immunohistochemistry of biopsies (196) show that CD4+ cells predominate in allergic reactions. Allergen responsive CD8+ cells are however present in the peripheral blood as demonstrated with Der p 1 (53). CD8+ cells from allergic subjects released more IFN-γ than cells from non-allergic subjects and the release was frequently higher for CD8 cells compared to the release from unfractionated PBMC. 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 (197).

As reviewed (198), several studies have indicated an involvement of HDM allergy and genes in the HLA class II region, but associations with particular alleles have varied. Responses of T-cell clones have shown a varied HLA restriction, including restriction by HLA-DRB1, DRB3, DQ and DP alleles. As expected from this diversity, people can respond to many T-cell epitopes but three studies have shown biased responses for Der p 1. All showed that T-cells preferentially responding to peptides from a domain-joining loop in the center of the molecule (15, 199-201). Kirchner et al. showed a further bias of the responding T cells in the use of Vβ18.1+ Vα2,3+ T-cell receptors. This TCR usage pattern had been previously noted (202). The immunodominance is not due to a restricted MHC presentation because the region contains multiple T-cell epitopes (15, 200) and the responses are presented by multiple MHC class II molecules (200). This differs from responses that have MHC-based epitope immunodominance as found for Art v 1 from mugwort (203). Responses to Der p 2 show no immunodominant region (204, 205, 206).

Major mite allergens have highly polymorphic amino acid sequences (207, 208) 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.

### VIII. Development of house dust mite allergy

Children do not show HDM allergy until 3-5 years of age. This contrasts with food allergy where IgE antibodies appear early and then often disappears. T-cell responses can however be detected earlier and there is even debate about responses of the foetus. Allergen has been reported to be detected in the amniotic fluid (209) and there are plausible transport mechanisms via IgG antibody. A less controversial observation is that cord blood of subjects who develop allergies contains more activated T cells (210), but is not known if this is due to allergen stimulation. Many studies have demonstrated in vitro proliferation of allergen-stimulated cord blood mononuclear cells (CBMC) and that this is higher in children who become allergic (211) or high-risk children (212, 213). Contrary results have been reported but both studies that drew these conclusions only diagnosed allergy in early infancy so their validity is questionable (214, 215). The increased responsiveness may however be an intrinsic characteristic of children who become allergic and not be due to allergen exposure. No association has been demonstrated with HDM allergen exposure in pregnancy (213, 216). Thornton et al. showed that allergen-
responsive cord cells had a regulatory phenotype and apoptose in the absence of anti-apoptotic cytokines like IL-7 (217). It was concluded from this that \textit{in utero} stimulation would not lead to T-cell memory but this ignores the fact that the necessary cytokines abound in the placenta. Devereux 2001 \textit{et al.} did find that 50\% of the cells responding to allergen in the cord were of the memory CD45RO+ phenotype, indicating antigenic stimulation (218). 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 or other proteins are responsible for their observations. It has however been reported that CBMC proliferate in response to Der p 1 (219) and Der p 1 and 2 (210).

The cytokine responses of allergen-stimulated CBMC show a Th2 bias for both allergic and non-allergic subjects with very little IFN-\gamma production (212, 220). The responses at 2 years show that Th2 cytokine responses of children that become allergic are in fact lower than the non-allergic and that the Th1 responses are still very low for all children (220). The Th2 responses of the children who become allergic then increases during infancy while the Th1 responses of both allergic and non-allergic subjects develop similarly (220, 221). By 6 years the responses of PBMC of children are similar to adults with Th1 and Th2 cytokines produced by the T cells of allergic children, and only Th1 cytokine by the non-allergic (142). These studies have not been conducted with purified allergens so the responding specificities are unknown. It should also be noted that adult immigrants newly exposed to mite and other allergens develop allergies with a similar propensity and time course to infants (222) so the neonatal immaturity is not essential for sensitisation.

People with severe allergy in adulthood had severe allergy in childhood so the early events can be persistent (223). Asthma can however resolve or ameliorate in adolescence and adulthood with estimates given from 30-80\%. A study of adults showed people with persistent asthma had higher IgE anti-HDM titres than people with resolved asthma but this was not associated with a reduction in the ability of HDM extracts to induce Th2 cytokines. The resolved asthmatics also had similar T-cell proliferative responses. Reduced production of IFN-\gamma production, below the level found in non-asthmatic people and people without HDM allergy, was however found (224). It is not clear if this is a result of feedback from long-term Th2 responses or if this is important for the resolution of disease. A similar pattern was noted in adolescence although also with reduced HDM-extract-induced Th2 cytokine production and T-cell proliferation (225). Changes in HDM extract stimulated IL-10 production have been reported, although dose response relationships were complex and difficult to interpret (226). While these studies are of interest, the greater persistence of asthma children with strong sensitivity (223) shows that longitudinal studies are required.

Conclusions

The discovery that house dust mites are the major source of indoor allergen was a major advance. It was also equally significant for subsequent studies to show that other sources such as cockroaches were important, so that observations on allergy and allergic disease need to be made in the knowledge of the prevailing allergens. The discovery of the importance of the glycyphagid mite \textit{B. tropicalis} in regions such as tropical Asia and South America is similarly highly significant. Epidemiological studies have shown that exposure to mite allergen is important for the development of sensitisation and disease
and it is possible to demonstrate an association of the mite exposure and allergic disease for sensitised people. The modest strength of the relationships however points to the importance of other factors. The relationship between sensitisation measured by IgE antibody and disease is also complex. Only people with very high responses become persistent asthmatics and many people with high titres are healthy. A major burden of disease is however found in children with lower IgE titres and intermittent asthma, and this cannot be predicted with the current tests. Studies of the allergens have now identified the dominant group 1 and 2 allergens and the potential importance of the group 4, 5 and 7 allergens. The IgE responses to most other allergens are known to be low and they do not induce non-allergic responses, at least as judged by IgG antibody production. Non-allergic people also rarely show IgG antibodies to the allergens. The hierarchical nature of the response and the restriction of significant IgE binding to a limited number of allergens bode well for the development of formulations of purified allergens for more accurate diagnosis and treatment. The importance of the Th2 cytokine responses of T cells is well established but the role of Th1 responses in either exacerbating disease or modifying disease is uncertain, as is the role of regulatory T cell responses. A major limitation of many studies is that allergen extracts have been used instead of allergens so the doses have not been optimised and the effect of other antigen specificities and proinflammatory molecules in the extracts cannot be considered. There is a high degree of irreproducibility in many investigations and this could be anticipated from the use of undefined and non-standardised reagents. The species of mite in the study areas are also poorly recognised and inappropriate allergens used. While the use of the current types of immunotherapy has several logistical drawbacks, the end results have been shown to be very beneficial for disease. This shows the central role of the allergy in disease, and that new, especially faster acting, types of immunotherapy or immunological prophylaxis could have a large impact. Healthy people exposed to HDM make non-harmful responses to HDM allergens and although the nature of the responses is poorly characterised, they are large as shown by T-cell precursor analysis.

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