

# House Dust Mite Allergens: New Discoveries and Relevance to the Allergic Patient

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## Abstract

**Purpose of Review** Recent findings on house dust allergens and their contribution to knowledge that will significantly impact on current and future allergy treatments are appraised.

**Recent Findings** Quantitation of IgE binding to a spectrum of allergen components in several independent studies in varying locations has largely affirmed the main components as the groups 1 and 2 and possibly 23 allergens with mid-tier contributions from the groups 4, 5, 7, and 21. Prevalent binding to Der p 23 has been recapitulated sometimes with low titers. The IgE of non-asthmatic atopic subjects binds at lower titer and to fewer components than that of asthmatics, and their IgG binding relative to IgE is higher especially for children hospitalized for exacerbation. The higher IgG ratios were associated with increased IL-10 a cytokine more readily induced from T cells of allergic subjects. Peptides representing the groups 1 and 2 allergens can be used to stimulate *ex vivo* T cells showing responses correlating with IgE binding and providing a valuable tool for ascertaining the contribution of IgE and T cells to disease. Also, the induction of Th2 and follicular helper T cells are shown to make different contributions in mice. Cross-reactivity of IgE binding assays with high-titer cross-reactive antibodies induced by scabies is a problem in the many areas of the world where scabies is highly prevalent and endemic and from recent increases in immigration.

**Summary** In the last few years, allergen research has produced results that warrant rapid translation into diagnostic tools and the formulation of allergen components for immunotherapy.

**Keywords** *Dermatophagoides* · House dust mite · Allergen · IgE · IgG · T cell

## Introducing Quantitation

The relationship between IgE antibody titers to house dust mite (HDM) allergens and asthma shows that disease is uncommon in subjects with concentrations below about 3.5 IU/ml [1], which is tenfold above the 0.35 IU/ml concentration typically used as the cutoff to define atopy and similarly well above the titers required for skin prick test positivity [2]. The likelihood of developing asthma increases with increasing titers [1, 3], along with the likelihood of diagnosis [4], persistence [5], severity [5, 6], and lung inflammation [7]. Similar correlations have been documented for skin prick test responses, for example, with 28 % of subjects with wheals of 3 mm having asthma rising to 50 % for wheals of 9 mm [8]. IgE antibody by its ability to arm mast cells for degranulation and cytokine release and to bind receptors of antigen presenting cells can be causal for both early and late phases of asthma, and since T cell help is required for IgE production, it is then a measure of T cell function. Recent recognition of the role of follicular helper T cells (T<sub>fh</sub>) in T cell help [9•, 10•, 11•], a lineage different to Th2 cells, however, shows a need for measuring the responses of these lineages. Variations of the responses of patients to anti-IgE monoclonal antibody therapy indicate independent contributions to disease by T cells and IgE [12].

IgE is part of the normal antibody responses being readily observable in the responses of non-atopic subjects to bacterial

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antigens such as those of *Haemophilus influenzae* [13, 14], shown to be Th1 biased [15]. This and collateral priming where Th2 adaptive immunity stimulates Th2 responses to bystander antigens [16] show the need to identify the components that drive allergic responses. Assessments also must appreciate that allergy diagnosis by history and physical examination is biased to false positives [17], with one assessment finding as few as 62 % of a sample of US patients being treated for allergic rhinitis having IgE antibody to the prevailing allergens [18].

### Comparative Responses to Allergen Components

The first quantitated comparison of IgE binding by HDM allergens [19•] used *Dermatophagoides pteronyssinus* components that could absorb, on average, 76 % of RAST binding to a HDM extract and most of the frequent high staining spots of 2D immunoblotting [20]. The summated IgE binding to the components correlated with and on average exceeded the ImmunoCAP score. Inclusion of the now-recognized groups 21 and 23 allergens would make this higher. The few discordances were mainly binding to Der p 4 that, see below, might be attributed to scabies. The combined binding to Der p 1 and 2 correlated highly with the total binding constituting about ca. 50 % of the total IgE binding, recapitulating the findings of Trombone et al. [21]. Binding to the mid-tier allergens, Der p 4, 5, and 7, although only found in 40–70 % of subjects, correlated with the binding to Der p 1 and 2 and collectively accounted for most of the remaining titer. Binding to Der p 3, 8, 10, and 20 was low and sporadic. A more comprehensive study in Singapore [22] and analyses across Europe that included Der p 21 were largely in agreement [23]. The Der p 23 peritrophin-like constituent of the dung ball [24] was later shown to bind IgE at high prevalence with titers similar to Der p 1 and 2. This led to a proposal that Der p 1, 2, and 23 were immunodominant with Der p 4, 5, 7, and 21 being mid-tier with most other allergens denominated in the IUIS allergen database being minor [25•] (Table 1).

Three recent studies have now compared IgE binding to allergen components including Der p 23 in North America, Asia, and Europe [26•, 27•, 28•]. The high IgE binding to Der p 23 was recapitulated with sera from Germany [26•] and the USA [27•] but, although prevalent, low titers were found in other samples from Europe, Canada, and Japan [27•] and in another study for allergic rhinitis patients from the USA [28•]. A report from Thailand described a 50 % prevalence with titers lower than Der p 2 [29] but with high potency in basophil degranulation assays. The dominance of the groups 1 and 2 and the mid-tier binding to Der p 4, 5, 7 and 21 were found [26•, 27•] with low responses to Der p 10, 13 and 15 [27•]. As shown in their supplementary data, Resch et al. [25•] found that the mid-tier IgE-binding was, with one exception, in proportion to the binding to Der p 1 and 2 [26•].

Of 11 % of subjects without IgE binding to Der p 1 or 2, nearly half had IgE binding to Der p 23 and were atopic but not asthmatic [26•] and most of the others lacked IgE binding even to extracts. The rhinitis patients of Mueller et al. [28•] showed that IgE binding to glutathione-S-transferase Der p 8 constituted a significant proportion of the binding of subjects with very low titers, who, since cat-allergic subjects were included, might not have had HDM allergy. Comparison of atopic asthmatic and atopic non-asthmatic sera showed that the latter had lower overall titers and IgE binding to fewer components [26•] making an interesting comparison with Hales et al. [19•, 30] who found that children susceptible to asthma exacerbation had higher IgE binding but without binding more components.

Batard et al. [27•] found that IgE from 13 % of adults but no children did not bind either Der p 1 or 2, while Resch et al. found this for 2/102 in asthmatics [26•]. A Japanese sample [31] showed that all patients that responded to bronchial provocation with HDM extract had IgE to Der p 1 plus 2 as did all, except for one, subjects with ImmunoCAPs over 3.5 IU/ml. This differs from other studies that show about 20 % disparity between binding to Der p 1 and 2 [19•, 21, 26•, 27•]. In older studies, Hales et al. [19•] found that all sera from allergic subjects reacted with either Der p 1 or 2, and mostly both, as did Trombone et al. for subjects with greater than 2 IU/ml of IgE binding [21]. The South American sample of Trombone et al. however did find sera with low-titer IgE binding to an extract without binding to Der p 1 or 2, as did a sample from Singapore [22]. Both these regions have complex acarofauna that includes *Blomia tropicalis* [32] and a high prevalence of scabies [33•] so cross-reactions should be expected.

### The Response to *Dermatophagoides farinae*

IgE binding to *D. farinae* allergens has not been studied to the same degree as *D. pteronyssinus* allergens but a similarity would be expected from their 80–90 % sequence identity. Early US studies [34] showed prevalent IgE binding to Der f 1 and 2 and strong skin test reactivity with low responses to Der f 3, but since both species were in the study region, the cause of sensitization is unknown. In Korea, where *D. farinae* is essentially the only species across most of the country, 79.1 % of allergic subjects bound Der f 1, 79.1 % Der f 2, 9.3 % Der f 6 6.2 % Der f 8, 6.2 %, Der f 10, and 6.6 % Der f 20 [35]. Low IgE binding signals were evident for Der f 8 and 10 in a country where the potentially cross-reactive cockroach allergy is prevalent. IgE binding prevalences of 65 % for Der f 5 and 21 [36] in Korea indicate similar mid-tier responses to their *D. pteronyssinus* counterparts. HDM-sensitized patients in Korea also show the expected 50–100 IU/ml titers of IgE binding to the groups 1 and 2 allergens [37]. In one of the many regions of China shown to be biased for *D. farinae* [38], the pattern of *D. farinae* IgE binding examined by crossed

**Table 1** Hierarchy of IgE reactivity of denominated *Dermatophagoides* house dust mite allergens (by group number)

|                                                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                    |
|------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Immunodominant                                                                                                                           | 1 Cysteine protease<br>2 ML domain<br>23 Peritrophin-like <sup>a</sup>                                                                                                                                                                                                                                                                                             |
| Mid-tier                                                                                                                                 | 4 Amylase<br>5 Unknown with coiled coil bundle structure<br>7 LPS-binding/ bactericidal/permeability-increasing protein-like<br>21 Group 5 homologue (paralogue)                                                                                                                                                                                                   |
| Minor                                                                                                                                    | 3 (trypsin), 6 (chymotrypsin), 8 (glutathione-S-transferase) 9 (collagenolytic serine protease), 10 (tropomyosin), 11 (paramyosin), 12 (not found in pyroglyphid mites), 13 (fatty acid binding protein), 15 (chitinase-like), 16 (gelsolin), 17 (EF-hand protein), 18 (inactive chitinase-like), 19 (not found in <i>Dermatophagoides</i> ), 20 (arginine kinase) |
| Not determined (no gravimetric estimates of IgE binding to whole allergen or comparative titrations with known immunodominant allergens) | 14 (Large lipid transfer), 22 (ML-domain-like), 24 (ubiquinol-cytochrome c reductase), 25 (triosephosphate isomerase), 26 (myosin alkali light chain), 27 (serpin), 28 (heat shock protein 70), 29 (peptidyl-prolyl <i>cis-trans</i> isomerase/cyclophilin), 30 (ferritin heavy chain), 31 (cofilin), 32 (inorganic secreted pyrophosphatase), 33 (alpha-tubulin)  |

<sup>a</sup> Group 23 allergens repeatedly shown to have high prevalence of IgE binding but the quantity of binding has been found to be both high and low (see text)

radio-immunoelectrophoresis of HDM extracts resembled that to *D. pteronyssinus* [39] with a high prevalence of IgE binding to the groups 1 and 2 shown in patients from four cities [40]. Measurement of IgE binding to Der p 1, 2, and 10 in Guangzhou [41], a region biased to *D. farinae* exposure, found that 91.5 % of patients were sensitized to Der p 1 and/or Der p 2 with a high correlation to binding determined with extract.

New allergenic proteins designated by Der f 24–33 have been described posing the possibility of an unrecognized complexity for *D. farinae*, and since the reports have been from China, the possibility of unrecognized environmental influences. The data summarized in Table 2 show that the reports have not provided quantitative titrations or comparisons with known immunodominant allergens, so it is not possible to estimate their allergenicity. There are also discrepancies in the allergenicity data found in different reports for Der f 26, 28, 29, 30, 31, and 32 that might be expected for weak allergens or cross-reactivities. Since the studies have only examined small numbers of allergic subjects (under 20) and fewer control subjects in single regions of China, more extensive studies on their significance and possible cross reactivities are required for further consideration. The IgE binding protein aconitate hydratase detected by a proteomic study in Thailand [48] should be similarly analyzed.

### Group 23 Peritrophin-Like Allergen Structure

The structure of Der f 23 has been solved by X-ray crystallography, which from modeling should be similar for Der p 23 [28••]. It contains two anti-parallel  $\beta$ -sheets held together by a disulphide bond and aromatic stacking of opposing phenylalanine and tryptophans. Apart from a chitin binding domain, it has little similarity to other proteins and does not bind chitin or

related oligosaccharides [24, 28••, 29]. It apparently binds something else on the dung ball, which is not novel for this diverse family of proteins. Soh et al. demonstrated that the O-glycosylation did not influence IgE binding [29] but interestingly, a reported Der f 23 mRNA sequence (KU166910) encoding a 175 amino acids instead of the previously described 93 for KM009999 includes a massively expanded threonine proline rich region typical of O-glycosylation sites. Similar but smaller insertions have been reported for Der p 23 (KP895831, KP895832) compared to the clone original (EU414751) [24]. There appears from BLAST to be only one gene in the *D. farinae* genome [42••], which is intronless and identical to the long form. Perhaps the changes are allelic with their distribution differing in their geographical distribution.

### IgG Antibody Binding

HDM allergens components induce IgG1 and IgG4 antibodies in allergic subjects with the propensity for each component being commensurate with its ability to induce IgE [19••]. As measured by solid phase microtiter plate assays Der p 1 and 2 each induce about 5 and 0.25  $\mu\text{g/ml}$  [19••, 49••] with using a published calibrator [50], similar titers measured by microarray [26••]. These titers are of similar magnitude to responses induced by viral and bacterial infections [14, 51, 52] measured by the same methods and are of the same high affinity of antibodies produced by immunizing animals with adjuvant [53] and in subjects undergoing immunotherapy [54]. The affinity of IgE antibodies is higher but overlapping [53, 54]. IgG and IgE to cross-reacting allergen carbohydrate determinants have similar high affinity [55]. Inhibition titration curves show the same affinity for IgG and IgE antibodies to HDM extract [56], and the human IgE and

**Table 2** Newly denominated *D. farinae* allergens

| Denomination | Biochemical                                              | Allergenicity                                                                                                                                                                                                                                                  |
|--------------|----------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Der f 24     | Ubiquinol-cytochrome c reductase                         | 100 % IgE binding to recombinant by ELISA [42••]<br>50 % skin test positivity [42••]                                                                                                                                                                           |
| Der f 25     | Triosephosphate isomerase                                | 76 % by immunoblotting of electrophoresed natural allergen [43]<br>100 % IgE binding to partially purified natural by ELISA [43]<br>60 % skin test positivity [43]                                                                                             |
| Der f 26     | Myosin alkali light chain                                | 100 % IgE binding by five subjects by immunoblot (IUIS database entry)<br>29 % skin test positive (IUIS database entry)<br>No IgE binding to recombinant by ELISA [42••]                                                                                       |
| Der f 27     | Serpin                                                   | 2D electrophoresis immunoblotting of extract [43]<br>IgE binding of three subjects to recombinant by ELISA [44]<br>42 % skin test reactivity to recombinant [44]                                                                                               |
| Der f 28     | Heat shock protein 70                                    | 68 % by immunoblotting of electrophoresed natural allergen [43]<br>100 % IgE binding to partially purified natural by ELISA [43]<br>70 % skin test positivity [43]<br>10 % IgE binding to recombinant by immunoblotting [45]                                   |
| Der f 29     | Peptidyl-prolyl <i>cis-trans</i> isomerase (cyclophilin) | 86 % by immunoblotting of electrophoresed natural allergen [43]<br>100 % IgE binding to partially purified natural by ELISA [43]<br>70 % skin test positivity [43]<br>IgE binding by ELISA to recombinant by 3 subjects [46]<br>24 % skin test reactivity [46] |
| Der f 30     | Ferritin heavy chain                                     | 63 % by immunoblotting of electrophoresed natural allergen [43]<br>100 % IgE binding to partially purified natural by ELISA [43]<br>60 % skin test positivity [43]<br>No IgE binding to recombinant by ELISA [42••]                                            |
| Der f 31     | Cofilin                                                  | IgE binding to recombinant by five subjects by ELISA and immunoblot (IUIS database)<br>31 % skin test reactivity to recombinant (IUIS database)<br>Weak IgE binding to recombinant by ELISA [42••]                                                             |
| Der f 32     | Inorganic secreted pyrophosphatase                       | 15 % skin test positivity to recombinant (IUIS database)<br>IgE binding by five subjects by immunoblot and ELISA to recombinant (IUIS database)<br>Weak IgE binding to recombinant by ELISA [42••]                                                             |
| Der f 33     | Alpha-tubulin                                            | 24 % skin positivity to recombinant [47]<br>IgE binding by four subjects to recombinant by ELISA [47]                                                                                                                                                          |

IgG binding curves to Der p 2 show a similar affinity to themselves and a reference monoclonal antibody [21, 57].

IgG1 and IgG4 titers were found to be strikingly reduced in children hospitalized for asthma exacerbations [19••, 31] being found in under 20 % of subjects compared to 70 % of stable asthmatics with fivefold to tenfold lower titers. Follow-up found children with persistent asthma or frequent exacerbations had lower IgG titers than those with intermittent exacerbations and that it did not increase after convalescence [31]. Microarray analysis has now also found lower IgG binding to HDM components in asthmatics compared to atopic non-asthmatics [26••] showing heterogeneity in the asthmatics possibly due the nature of the disease. A high prevalence of low-titer IgG antibody in non-atopic subjects was also reported but at levels close to the

myeloma controls used in assays that reported a low prevalence [19••]. The protective association has been further corroborated for IgG antibody responses to Der p 1 in unselected birth cohorts in Australia and England and a further cohort selected from atopic parents [49••]. The IgG/IgE ratio was about twofold lower for asthmatics compared to non-asthmatics in the birth cohorts, sevenfold for the high-risk children, and over tenfold less hospitalized versus stable asthmatics. The IgG4/IgE ratio was not different, although IgG4 titers were significantly reduced. Since IgG4 production requires Th2-cell help, there might be a more complex relationship than for IgG1 that is helped by Th17 cytokines and can be amplified by IL-10. A similar analysis for cat allergy also implicated higher IgG/IgE ratios for reduced disease [58], and Th2-dependent IgG4 has been associated with

protection in subjects chronically exposed to cats [59]. With respect to a blocking model for IgG as demonstrated in vitro for IgG induced by natural HDM exposure [49•], the finding that immunization with different variants of Der p 2 induces IgG with different binding to other variants [60] shows that strategies to induce IgG for therapy need to consider the variants.

The high IgG/IgE ratios were associated with the production of IL-10 by CD4+ T cells stimulated with HDM extract. Remarkably, analysis of transcripts found that IL-10 was the only significant positive association thus not including IL-21 pathway genes that would be expected for help. IL-10 can be induced by the engagement of the antigen receptors of CD4+ cells that have differentiated down one of the Th1, Th2, and Th17 pathways, with the production being dependent on the transcription factors important for each pathway, or independently by extraneously produced cytokines as demonstrated for IL-27, IL-6, IL-12, and TGF- $\beta$  [61]. HDM extracts could thus induce IL-10 via allergens or cytokines induced by other constituents. Previously IL-10 induced in peripheral blood mononuclear cells (PBMC) by Der p 1, but not HDM extract, was found to be inversely correlated with wheal size [62], so the mechanisms would be of considerable interest.

### Allergen Cross-Reactivities Important for Diagnosis

Component-resolved diagnosis can distinguish the cause of allergies from cross-reactivities. IgE binding to Blo t 5 to distinguish sensitization to *B. tropicalis* from sensitization by *D. pteronyssinus* has provided such an assay important for allergies in several very populous countries [32]. The paralogous Blo t 21 however has shown significant cross-reactivity with Der p 5 [63]. Further recent examination of the groups 5 and 21 allergens from *D. farinae*, *B. tropicalis*, and *Tyrophagus putrescentiae* in Korea found that IgE binding to Der f 5 and 21 by different subjects was highly correlated but not cross-reactive despite their ca. 50 % sequence identity [36]. Binding to Tyr p 5 and 21 also correlated but was nearly all cross-reactivity to Der f 21 and to a lesser extent Der f 5, as was the IgE binding to Blo t 5 with rBlo t 21, noting that *B. tropicalis* is not found in Korea. The investigation thus demonstrates the allergens of storage mites could be confounding cross-reactivities that should be examined. In Brazil where *B. tropicalis* is abundant and IgE antibodies induced by *Ascaris lumbricoides* bind to *B. tropicalis* extracts, IgE binding to Blo t 5 and 21 detected 96.4 % of sera that showed IgE binding to *B. tropicalis* extract without cross-reactions with *A. lumbricoides* [64]. The known geographical distribution of *B. tropicalis* allergy has now been extended to Sub Saharan Africa since skin test reactivity to *B. tropicalis* without reactions to *D. pteronyssinus* is prevalent in the KwaZulu-Natal Province of South Africa [65].

While cross-reactivity of anti-scabies antibodies and HDM allergens has been well documented [66], recent measurements

of high IgE binding to defined HDM allergens by indigenous and non-indigenous subjects currently infected by or exposed to scabies in Northern Australia has made the problem strikingly evident [67•]. Subjects with crusted and ordinary scabies had high titers of IgE antibodies to natural Der p 4 and recombinant Der p 20 (amylase and arginine kinase). The titers were of similar magnitude to titers to Der p 1 and 2 in asthmatics, but there was no essentially no IgE binding to these or the mid-tier HDM allergens. Subjects who had been exposed to scabies but were uninfected had high anti-Der p 4 titers suggesting persistence of IgE from prior infection. The high IgE binding focused on Der p 4 is the pattern previously reported for indigenous Australians selected by skin reactions to HDM extracts [68]. Tropomyosin was not cross-reactive, but a comprehensive study of the allergen components appears warranted. Scabies might also be implicated in a study showing high-titer IgE binding to Blo t 4 in the inland Chengdu province of China where there are few HDM of any species [38] and no antibody to the immunodominant specificity Blo t 5 [69].

Reported prevalences of scabies include 0.27 % for the UK [70] and 0.07 % in the USA [71], so if scabies antibodies persist for years as indicated for indigenous Australians, a prevalence of anti scabies IgE in 1 % of these populations would seem a conservative estimate. The problem is however far more serious for regions where scabies infestation is endemic and for countries that accommodate the migration of people from these regions. It has allegedly become widespread due to the accommodation of displaced persons in crowded transitional camps. Current scabies infestations are found in an astonishing 40 % of some indigenous populations of Northern Australia and the inhabitants of Pacific and Indian Ocean Islands and in over 10 % of people in South American countries and 5 % in Supra and Sub Saharan Africa [72]. The prevalence in children is higher than adults in both endemic and non-endemic regions [71, 72]. The diagnosis and study of HDM allergy with HDM extracts would seem impossible in these regions given that Der p 4 and 20 are highly abundant in extracts [27•]. Increases in scabies infestation due to immigration have been reported across Europe. As an example, medical records in Italy [73] found a 1.4 % incidence for native Italians and a 14 % incidence for immigrants being 33 % for people from Southeast Asia. Immigrants from the Eastern Mediterranean with an incidence of 23 % however constituted the largest caseload. Given that patients in France have been found to first seek medical help up to 52 weeks after infestation [74] and subjects in the Cameroons up to 2 years [75], undiagnosed disease must be prevalent. The genomic sequence of dog scabies that has been solved and annotated [76•] as well as this and the currently less assembled data for human scabies [77] will provide a valuable resource for identifying IgE binding proteins to discriminate between responses of HDM allergy and scabies infection especially, combined with the *D. farinae* genome [42•].

Studies summarized elsewhere have described the ability of the group 10 allergens to cross-react with tropomyosin from other sources, but it is not prevalent or high titered even for subjects allergic to sources with known cross-reactive tropomyosins [25•].

### Allergen-Induced T cell Responses

In vitro culture with HDM extracts exposes cells to unknown inflammatory stimuli of unknown significance, which for peripheral blood mononuclear cells (PBMC), would not be encountered either because they are not inhaled at all or in active concentrations or are not known to breach the mucosal barrier.

Read outs that induce responses via the engagement of the T cell receptors without such stimuli in contrast reveal the differentiation and activation states of cells produced by encounter with allergen in vivo in the presence of physiologically relevant innate immune signals. The induction of IL-10 release from human memory B-cells by non-specific stimuli in HDM extracts [78], for example, can greatly modify responses. Investigations with purified natural allergens need to assess their purity and those with recombinant allergens problems with folding and aggregation and different post-translational modifications [79]. Additionally, allergens have biological activities capable of influencing immune responses [73, 80•]. Contrarily, peptides representing T cell epitopes can signal via T cell receptors with minimal extraneous stimuli providing a measure of prior in vivo events similar for all allergens but to date have only induced low responses from human PBMC without an amplification step of in vitro stimulation, often with allergen extract [81]. From the patterns of epitope recognition for the groups 1 and 2 allergens of *D. pteronyssinus* and *D. farinae* by human PBMC, Hinz et al. [82••] have now described a pool of HDM peptides that directly stimulate *ex vivo* responses. Unlike responses to extracts, the responses correlated with HDM specific IgE titers and induced a Th2 response from PBMC of allergic donors being high for IL-5 and IL-10 and low for IFN- $\gamma$  and IL-17. In keeping with many reports summarized previously [83], however, the IFN- $\gamma$  and IL-10 production was higher for allergic subjects, especially IL-10. Peptides representing Der p 23 only induced low responses that would concur with the low titer IgE found by some investigators.

A key area of investigation flagged by recent studies in mice is to ascertain the relative participation of IL-4 producing Tfh and Th2 cells in humans. In mice, the T cell help required for sustained IgE production to antigens including HDM extract [9••, 10••, 11••], and Der p 1 [11••] is provided by Tfh and not by Th2 cells that have different pathways of differentiation and trafficking. Th2 cells only provide transient help for IgE but mediate large Th2 inflammatory responses [9••, 11••] importantly producing IL-13 that is not produced by Tfh cells. The Tfh

differentiation [84] is controlled by the transcription factor Bcl6, not GATA-3, and for humans, different to mice, is driven by a constellation of IL-1 $\beta$ , TGF- $\beta$ , IL-12, and IL-23 with negative regulation by IL-2 and IL-10. Tfh function has not been reported for human allergy but it has been reported that asthmatics have increased circulating Tfh cells with the defining CXCR5+ receptor [85]. Different types of antigens induced different balances of Tfh and Th2 in mice [9••] perhaps due to differences in their induction of innate cytokines that might differ between HDM allergen components. The different role of IL-1 $\beta$  and IL-33 in inducing Tfh and Th2 cells, respectively, and the differential expression of the IL-33 receptor on Th2 cells [9••, 86] would promote different contributions from different antigen presenting and group 2 innate lymphoid cells. The peptide assays could be the basis of assays for correlations of T cell function with responses of subjects to anti-cytokine and anti-IgE therapy.

CD4+ T cells of HDM-allergic children with low IgG/IgE ratios, and thus a more severe asthma phenotype, have been found to express transcripts of Toll-like receptor (TLR) -2, -4, and -9 [49••] (TLR2 in supplementary data of reference 49). This needs to be distinguished from the familiar expression of TLR by antigen presenting cells known to be important in the initiation of immune responses. Most naive human CD4+ T cells show intracellular production of TLRs and when activated via the T cell receptor, this is increased with TLR2 and TLR4 being expressed at the cell surface [87]. Exposure to LPS did not reveal a function for TLR4, but lipoproteins stimulated TLR2 to increase cytokines indicating, along with its constitutive expression on memory T cells a physiologically relevant function. Studies in mice have revealed a number of negative and positive regulatory functions for the engagement of TLR on T cells [88–91] suggesting that this might warrant closer investigation in allergy.

### Conclusions

Quantitative comparisons of IgE binding to the different HDM allergens, sometimes called components, have established that HDM allergy is mostly constituted by a hierarchical pattern of responses to a relatively small number of allergens with the groups 1 and 2 and possibly 23 being dominant and the groups 4, 5, 7, and 21 being mid-tier. The group 23 induces prevalent responses but inter-investigational discrepancies in the size need resolution. When measured, IgE binding to minor allergens has been found to constitute a small fraction of total responses. Responses that do not include the dominant allergens should be investigated for cross-reactivities. The prevalence of skin test positivity and detectable IgE binding (0.35 IU/ml) remain as poor measures. Cross-

reactivity with IgE induced by scabies infection is a large problem for diagnosis and investigation in the many countries with high infestation and is rising elsewhere due to immigration of displaced persons. The increased risk of disease in allergic subjects with low IgG antibody responses has now been repeatedly demonstrated with an extremely high risk for frequent and persistent asthma exacerbation. The associations of high IgG/IgE ratios with increased IL-10 responses to HDM extracts and reduced T cell expression of TLR provide avenues for further investigation. The elucidation of a peptide pool representing the groups 1 and 2 allergens that stimulates *ex vivo* T cell responses proportional to donor IgE titers provides a means for such studies and for investigating the roles of Th2 and Tfh cells.

### Compliance with Ethical Standards

**Conflict of Interest** Dr. Thomas declares numerous patents licensed by the Telethon Kids Institute for royalties

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

### References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Simpson A, Soderstrom L, Ahlstedt S, Murray CS, Woodcock A, Custovic A. IgE antibody quantification and the probability of wheeze in preschool children. *J Allergy Clin Immunol*. 2005;116:744–9.
2. Haahtela T, Jaakonmäki I. Relationship of allergen-specific IgE antibodies, skin prick tests and allergic disorders in unselected adolescents. *Allergy*. 1981;36:251–6.
3. Holt PG, Rowe J, Kusel M, Parsons F, Hollams EM, Bosco A, et al. Toward improved prediction of risk for atopy and asthma among preschoolers: a prospective cohort study. *J Allergy Clin Immunol*. 2010;125(3):653–9.
4. Soderstrom L, Kober A, Ahlstedt S, de Groot H, Lange CE, Paganelli R, et al. A further evaluation of the clinical use of specific IgE antibody testing in allergic diseases. *Allergy*. 2003;58:921–8.
5. Matricardi PM, Bockelbrink A, Keil T, Gruber C, Niggemann B, Hamelmann E, et al. Dynamic evolution of serum immunoglobulin E to airborne allergens throughout childhood: results from the Multi-Centre Allergy Study birth cohort. *Clin Exp Allergy*. 2009;39:1551–7.
6. Wang J, Visness CM, Calatroni A, Gergen PJ, Mitchell HE, Sampson HA. Effect of environmental allergen sensitization on asthma morbidity in inner-city asthmatic children. *Clin Exp Allergy*. 2009;39:1381–9.
7. Crane J, Lampshire P, Wickens K, Epton M, Siebers R, Ingham T, et al. Asthma, atopy and exhaled nitric oxide in a cohort of 6-yr-old New Zealand children. *Pediatr Allergy Immunol*. 2012;23:59–64.
8. Haahtela T, Burbach GJ, Bachert C, Bindslev-Jensen C, Bonini S, Bousquet J, et al. Clinical relevance is associated with allergen-specific wheal size in skin prick testing. *Clin Exp Allergy*. 2014;44:407–16.
9. Kobayashi T, Iijima K, Dent AL, Kita H. Follicular helper T cells mediate IgE antibody response to airborne allergens. *J Allergy Clin Immunol*. 2016; doi: [10.1016/j.jaci.2016.04.021](https://doi.org/10.1016/j.jaci.2016.04.021). [Epub ahead of print]. **One of several papers showing that in mice, help for sustained IgE antibody is provided by follicular helper T cells, while Th2 cells help transient IgE and produce inflammation. Allergen differs in the induction of the lineages.**
10. Ballesteros-Tato A, Randall TD, Lund FE, Spolski R, Leonard WJ, León B. T Follicular helper cell plasticity shapes pathogenic T helper 2 cell-mediated immunity to inhaled house dust mite. *Immunity*. 2016;44:259–73. **Shows that in mice help for sustained IgE antibody is provided by follicular helper T cells while Th2 cells help transient IgE and produce inflammation.**
11. Noble A, Zhao J. Follicular helper T cells are responsible for IgE responses to Der p 1 following house dust mite sensitization in mice. *Clin Exp Allergy*. 2016; doi: [10.1111/cea.12750](https://doi.org/10.1111/cea.12750). [Epub ahead of print] **This study shows that in mice, help for sustained IgE antibody is provided by follicular helper T cells while Th2 cells help transient IgE and produce inflammation.**
12. Wahn U, Martin C, Freeman P, Blogg M, Jimenez P. Relationship between pretreatment specific IgE and the response to omalizumab therapy. *Allergy*. 2009;64:1780–7.
13. Hollams EM, Hales BJ, Bachert C, Huvenne W, Parsons F, de Klerk NH, et al. Th2-associated immunity to bacteria in asthma in teenagers and susceptibility to asthma. *Eur Resp J*. 2010;36:509–16.
14. Hales BJ, Chai LY, Elliot CE, Pearce LJ, Zhang G, Heinrich TK, et al. Antibacterial antibody responses associated with the development of asthma in house dust mite-sensitized and non-sensitized children. *Thorax*. 2012;67:321–7.
15. Epton MJ, Hales BJ, Thompson PJ, Thomas WR. T-cell cytokine responses to outer membrane proteins of *Haemophilus influenzae* and the house dust mite allergens of Der p 1 in allergic and non-allergic subjects. *Clin Exp Allergy*. 2002;32:1589–95.
16. Eisenbarth SC, Zhadkevich A, Ranney P, Herrick CA, Bottomly K. IL-4-dependent Th2 collateral priming to inhaled antigens independent of Toll-like receptor 4 and myeloid differentiation factor 88. *J Immunol*. 2004;172:4527–34.
17. Williams PB, Ahlstedt S, Barnes JH, Soderstrom L, Portnoy J. Are our impressions of allergy test performances correct? *Ann Allergy Asthma Immunol*. 2003;91:26–33.
18. Szeinbach SL, Williams B, Muntendam P, O'Connor RD. Identification of allergic disease among users of antihistamines. *J Manag Care Pharm*. 2004;10:234–8.
19. Hales BJ, Martin AC, Pearce LJ, Laing IA, Hayden CM, Goldblatt J, et al. IgE and IgG anti-house dust mite specificities in allergic disease. *J Allergy Clin Immunol*. 2006;117:275–82. **Original quantitative comparison of IgE and IgG binding to allergen components showing that IgE binding to mid-tier allergens is proportional to the binding to the groups 1 and 2 and that, from a large sample, children hospitalized for asthma exacerbation have vanishing low titers of IgG antibody to allergen components.**
20. 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;35:1384–91.
21. Trombone AP, Tobias KR, Ferriani VP, Schuurman J, Aalberse RC, Smith AM, 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;32:1323–8.
22. Kidon MI, Chiang WC, Liew WK, Ong TC, Tiong YS, Wong KN, et al. Mite component-specific IgE repertoire and phenotypes of allergic disease in childhood: the tropical perspective. *Pediatr Allergy Immunol*. 2011;22:202–10.
  23. Weghofer M, Thomas WR, Kronqvist M, Mari A, Purohit A, Pauli G, et al. Variability of IgE reactivity profiles among European mite allergic patients. *Eur J Allergy Clin Immunol*. 2008;38:959–65.
  24. Weghofer M, Grote M, Resch Y, Casset A, Kneidinger M, Kopec J, et al. Identification of Der p 23, a peritrophin-like protein, as a new major *Dermatophagoides pteronyssinus* allergen associated with the peritrophic matrix of mite fecal pellets. *J Immunol*. 2013;190:3059–67.
  25. Thomas WR. Hierarchy and molecular properties of house dust mite allergens. *Allergology Int*. 2015;64:304–11. **Recent review of dominant, mid-tier and minor house allergens with cross reactivities and critique of claims for the importance of biological activity for allergens.**
  26. Resch Y, Michel S, Kabesch M, Lupinek C, Valenta R, Vrtala S. Different IgE recognition of mite allergen components in asthmatic and nonasthmatic children. *J Allergy Clin Immunol*. 2015;136:1083–91. **Array analysis of allergen components showing IgE and IgG binding and responses of asthmatic and non-asthmatic atopic subjects. Der p 23 binding. Supplementary data is important.**
  27. Batard T, Baron-Bodo V, Martelet A, Le Mignon M, Lemoine P, Jain K, et al. Patterns of IgE sensitization in house dust mite-allergic patients: implications for allergen immunotherapy. *Allergy*. 2016;71:220–9. **Array analysis of allergen components in countries in three continents showing IgE binding including Der p 23 and a focus on the groups 1 and 2 from *Dermatophagoides* species and allergens detectable in extracts.**
  28. Mueller GA, Randall TA, Glesner J, Pedersen LC, Perera L, Edwards LL, et al. Serological, genomic and structural analyses of the major mite allergen Der p 23. *Clin Exp Allergy*. 2016;46:365–76. **Array analysis of allergen components binding IgE from US rhinitis patients including Der p 23 and provides structure of Der f 23.**
  29. Soh WT, Le Mignon M, Suratannon N, Satitsuksanoa P, Chatchatee P, Wongpiyaboron J, et al. The house dust mite major allergen Der p 23 displays o-glycan-independent IgE reactivities but no chitin-binding activity. *Int Arch Allergy Immunol*. 2015;168:150–60.
  30. Hales BJ, Martin AC, Pearce LJ, Rueter K, Zhang G, Khoo SK, et al. Anti-bacterial IgE in the antibody responses of house dust mite allergic children convalescent from asthma exacerbation. *Clin Exp Allergy*. 2009;39:1170–8.
  31. Minami T, Fukutomi Y, Lidholm J, Yasueda H, Saito A, Sekiya K, et al. IgE Abs to Der p 1 and Der p 2 as diagnostic markers of house dust mite allergy as defined by a bronchoprovocation test. *Allergol Int*. 2015;64:90–5.
  32. Thomas WR. Geography of house dust mite allergens. *Asian Pac J Allergy Immunol*. 2010;28:211–24.
  33. Romani L, Steer AC, Whitfield MJ, Kaldor JM. Prevalence of scabies and impetigo worldwide: a systematic review. *Lancet Infect Dis*. 2015;15:960–7. **Widespread global prevalence of scabies infestation with 10–40 % incidences.**
  34. 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;83:1055–67.
  35. Jeong KY, Lee JY, Son M, Yi MH, Yong TS, Shin JU, et al. Profiles of IgE Sensitization to Der f 1, Der f 2, Der f 6, Der f 8, Der f 10, and Der f 20 in Korean house dust mite allergy patients. *Allergy Asthma Immunol Res*. 2015;7:483–8.
  36. Kim CR, Jeong KY, Yi MH, Kim HP, Shin HJ, Yong TS. Cross-reactivity between group-5 and -21 mite allergens from *Dermatophagoides farinae*, *Tyrophagus putrescentiae* and *Blomia tropicalis*. *Mol Med Rep*. 2015;12:5467–74.
  37. Kim HS, Kang SH, Won S, Lee EK, Chun YH, Yoon JS, et al. Immunoglobulin E to allergen components of house dust mite in Korean children with allergic disease. *Asia Pac Allergy*. 2015;5:156–62.
  38. Zheng YW, Lai XX, de Zhao Y, Zhang CQ, Chen JJ, Zhang L, et al. Indoor allergen levels and household distributions in nine cities across China. *Biomed Environ Sci*. 2015;28:709–17.
  39. Zhang C, Gjesing B, Spangfort MD, Xu J, Zhong N. The allergen-specific IgE reactivity pattern of Chinese house dust mite allergic patients. *Allergy*. 2008;63:1640–1.
  40. Zheng YW, Li J, Lai XX, Zhao DY, Liu XF, Lin XP, et al. Allergen micro-array detection of specific IgE-reactivity in Chinese allergy patients. *Chin Med J*. 2011;124:4350–4.
  41. Zeng G, Luo W, Zheng P, Wei N, Huang H, Sun B, et al. Component-resolved diagnostic study of *Dermatophagoides pteronyssinus* major allergen molecules in a Southern Chinese cohort. *J Investig Allergol Clin Immunol*. 2015;25:343–51.
  42. Chan TF, Ji KM, Yim AK, Liu XY, Zhou JW, Li RQ, et al. The draft genome, transcriptome, and microbiome of *Dermatophagoides farinae* reveal a broad spectrum of dust mite allergens. *J Allergy Clin Immunol*. 2015;135:539–48. **Genome sequence of *Dermatophagoides farinae*.**
  43. An S, Chen L, Long C, Liu X, Xu X, Lu X, et al. *Dermatophagoides farinae* allergens diversity identification by proteomics. *Mol Cell Proteomics*. 2013;12:1818–28.
  44. Lin J, Li M, Liu Y, Jiang C, Wu Y, Wang Y, et al. Expression, purification and characterization of Der f 27, a new allergen from *Dermatophagoides farinae*. *Am J Transl Res*. 2015;7:1260–70.
  45. Aki T, Fujikawa A, Wada T, Jyo T, Shigeta S, Murooka Y, et al. Cloning and expression of cDNA coding for a new allergen from the house dust mite, *Dermatophagoides farinae*: homology with human heat shock cognate proteins in the heat shock protein 70 family. *J Biochem*. 1994;115:435–40.
  46. Lin J, Wang H, Li M, Liang Z, Jiang C, Wu Y, et al. Characterization and analysis of a cDNA coding for the group 29b (Der f 29b) allergen of *Dermatophagoides farinae*. *Am J Transl Res*. 2016;8:568–77.
  47. Wang H, Lin J, Liu X, Liang Z, Yang P, Ran P, et al. Identification of alpha-tubulin, Der f 33, as a novel allergen from *Dermatophagoides farinae*. *Immunobiology*. 2016;221:911–7.
  48. Choopong J, Reamtong O, Sookkrung N, Seesuy W, Indrawattana N, Sakolvaree Y, et al. Proteome, allergenome, and novel allergens of house dust mite, *Dermatophagoides farinae*. *J Proteome Res*. 2016;15:422–30.
  49. Holt PG, Strickland D, Bosco A, Belgrave D, Hales B, Simpson A, et al. Distinguishing benign from pathologic TH2 immunity in atopic children. *J Allergy Clin Immunol*. 2016;137:379–87. **Multiple cohorts show that children prone to asthma have low IgG/IgE ratios of antibodies to Der p 1 especially in a reanalysis of hospitalized children. IL-10 induced by mite extracts was the only cytokine associated with the low ratios.**
  50. Gallerano D, Wollmann E, Lupinek C, Schleiderer T, Ebner D, Harwanegg C, et al. HIV microarray for the mapping and characterization of HIV-specific antibody responses. *Lab Chip*. 2015;15:1574–89.
  51. Iwasaki J, Smith WA, Khoo SK, Bizzintino J, Zhang G, Cox DW, et al. Comparison of rhinovirus antibody titers in children with asthma exacerbations and species-specific rhinovirus infection. *J Allergy Clin Immunol*. 2014;134:25–32.
  52. Iwasaki J, Chai LY, Khoo SK, Bizzintino J, Laing IA, Le Souëf PN, et al. Lower anti-echovirus antibody responses in children

- presenting to hospital with asthma exacerbations. *Clin Exp Allergy*. 2015;45:1523–30.
53. Hantusch B, Schöll I, Harwanegg C, Krieger S, Becker WM, Spitzauer S, et al. Affinity determinations of purified IgE and IgG antibodies against the major pollen allergens Phl p 5a and Bet v 1a: discrepancy between IgE and IgG binding strength. *Immunol Lett*. 2005;97:81–9.
  54. Svenson M, Jacobi HH, Bødtger U, Poulsen LK, Rieneck K, Bendtzen K. Vaccination for birch pollen allergy. Induction of affinity-matured or blocking IgG antibodies does not account for the reduced binding of IgE to Bet v 1. *Mol Immunol*. 2003;39: 603–12.
  55. Jin C, Hantusch B, Hemmer W, Stadlmann J, Altmann F. Affinity of IgE and IgG against cross-reactive carbohydrate determinants on plant and insect glycoproteins. *J Allergy Clin Immunol*. 2008;12: 185–90.
  56. Boluda L, Gallego MT, Gonzalez Romano ML, Cambra O, Berrens L. Evaluation of the potency of allergenic extracts by inhibition of IgG-antibody binding. *Biologicals*. 1994;22:171–7.
  57. Schuurman J, Perdok GJ, Mueller GA, Benjamin DC, Yong Tan K, Chapman MD, et al. Mouse/human chimeric IgG1 and IgG4 antibodies directed to the house dust mite allergen Der p 2: use in quantification of allergen specific IgG. *Clin Exp Allergy*. 1997;27:1095–102.
  58. Custovic A, Soderstrom L, Ahlstedt S, Sly PD, Simpson A, Holt PG. Allergen-specific IgG antibody levels modify the relationship between allergen-specific IgE and wheezing in childhood. *J Allergy Clin Immunol*. 2011;127:1480–5.
  59. Renand A, Archila LD, McGinty J, Wambre E, Robinson D, Hales BJ, et al. Chronic cat-allergen exposure induces a TH2 cell-dependent IgG4 response related to low-sensitization. *J Allergy Clin Immunol*. 2015;136:1627–35.
  60. Tanyaratrisakul S, Jirapongsananuruk O, Kulwanich B, Hales BJ, Thomas WR, Piboonpocanun S. Effect of amino acid polymorphisms of house dust mite Der p 2 variants on allergic sensitization. *Allergy Asthma Immunol Res*. 2016;20168:55–62.
  61. Gabrysova L, Howes A, Saraiva M, O'Garra A. The regulation of IL-10 expression. *Curr Top Microbiol Immunol*. 2014;380:157–90.
  62. Heaton T, Rowe J, Turner S, Aalberse RC, de Klerk N, Suriyaarachchi D, 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: 142–9.
  63. Tan KW, Ong TC, Gao YF, Tiong Y, Wong KN, Chew FT, et al. NMR structure and IgE epitopes of Blo t 21, a major dust mite allergen from *Blomia tropicalis*. *J Biol Chem*. 2012;287:34776–85.
  64. Carvalho Kdos A, de Melo-Neto OP, Magalhaes FB, Ponte JC, Felipe FA, dos Santos MC, et al. *Blomia tropicalis* Blo t 5 and Blo t 21 recombinant allergens might confer higher specificity to serodiagnostic assays than whole mite extract. *BMC Immunol*. 2013;14:11. doi:10.1186/1471-2172-14-11.
  65. Jeevarathnum AC, van Niekerk A, Green RJ, Becker P, Masekela R. Prevalence of *Blomia tropicalis* allergy in two regions of South Africa. *S Afr Med J*. 2015;105:567–9.
  66. Arlian LG, Feldmeier H, Morgan MS. The potential for a blood test for scabies. *PLoS Negl Trop Dis*. 2015;9:. doi:10.1371/journal.pntd.0004188. eCollection 2015.
  67. Walton SF, Slender A, Pizutto S, Mounsey KE, Oprescu F, Thomas WR, et al. Analysis of IgE binding patterns to house dust mite allergens in scabies-endemic communities: insights for both diseases. *Clin Exp Allergy*. 2015;45:1868–72. **IgE antibodies of scabies infected and exposed subjects bind Der p 4 and Der p 20 at high titer apparently persisting for Der p 4.**
  68. Hales BJ, Laing IA, Pearce LJ, Hazell LA, Mills KL, Chua KY, et al. Distinctive IgE and IgG4 anti-house dust allergen binding specificities in a tropical Australian Aboriginal Community. *Clin Exp Allergy*. 2007;37:1357–63.
  69. Cheong N, Ramos JD, Tang CY, Chng HH, Yao R, Liang Z, et al. Mite amylase from *Blomia tropicalis* (Blo t 4): differential allergenicity linked to geographical regions. *Int Arch Allergy Immunol*. 2009;149:25–32.
  70. Fuller LC. Epidemiology of scabies. *Curr Opin Infect Dis*. 2013;26: 123–6.
  71. Owusu-Edusei K, Chesson HW, Gift TL. The economic burden of pediculosis pubis and scabies infections treated on an outpatient basis in the United States: evidence from Private Insurance Claims Data, 2001–2005. *Sex Transm Dis*. 2009;36:297–9.
  72. Romani L, Steer AC, Whitfield MJ, Kaldor JM. Prevalence of scabies and impetigo worldwide: a systematic review. *Lancet Infect Dis*. 2015;15:960–7.
  73. Limina RM, Baitelli G, Marcantoni C, Covolo L, Festa A, Spezziani F, et al. Frequency of infectious diseases in immigrants in a Western European country: a population-based study. *J Immigr Minor Health*. 2015;17:66–75.
  74. De Sainte MB, Mallet S, Gaudy-Marqueste C, Baumstarck K, Bentaleb N, Loundou A, et al. Therapeutic failure in scabies: an observational study. [French]. *Ann Dermatol Venerol*. 2016;143: 9–15.
  75. Kouotou EA, Nansseu JR, Sieleunou I, Defo D, Bissek AC, Ndam EC. Features of human scabies in resource-limited settings: the Cameroon case. *BMC Derm*. 2015;15:12.
  76. Rider SG, Morgan MS, Arlian LG. Draft genome of the scabies mite. *Parasit Vectors*. 2015;8:585. **Sequence of scabies genome.**
  77. Mofiz E, Holt DC, Seemann T, Currie BJ, Fischer K, Papenfuss AT. Genomic resources and draft assemblies of the human and porcine varieties of scabies mites, *Sarcoptes scabiei* var. *hominis* and var. *suis*. *GigaScience*. 2016;5:23.
  78. Milovanovic M, Heine G, Zuberbier T, Worm M. Allergen extract-induced interleukin-10 in human memory B cells inhibits immunoglobulin E production. *Clin Exp Allergy*. 2009;39:671–8.
  79. Vrtala S, Huber H, Thomas WR. Recombinant house dust mite allergens. *Methods*. 2014;66:67–74.
  80. Thomas WR. Innate affairs of allergens. *Clin Exp Allergy*. 2013;43: 152–63. **Detailed assessment of evidence that the biological properties of aeroallergens and interactions with innate immune receptors affect allergenicity.**
  81. Wambre E, Bonvalet M, Bodo VB, Maillere B, Leclert G, Moussu H, et al. Distinct characteristics of seasonal (Bet v 1) vs. perennial (Der p 1/Der p 2) allergen-specific CD4(+) T cell responses. *Clin Exp Allergy*. 2011;41:192–203.
  82. Hinz D, Oseroff C, Pham J, Sidney J, Peters B, Sette A. Definition of a pool of epitopes that recapitulates the T cell reactivity against major house dust mite allergens. *Clin Exp Allergy*. 2015;45:1601–12. **Pool of peptides from T cell epitopes of groups 1 and 2 allergens provides direct ex vivo stimulation of T cells from human peripheral blood and hence determinations of their differentiation and activation induced by natural exposure.**
  83. Thomas WR. House dust allergy and immunotherapy. *Hum Vaccin Immunother*. 2012;8:1469–78.
  84. Vinuesa CG, Linterman MA, Yu D, MacLennan CM. Follicular helper T cells. *Ann Rev Immunol*. 2016;34:335–68.
  85. Gong F, Su Q, Jiang D, Chen J, Pan Y, Huang X. High frequency of circulating follicular helper T cells in patients with bronchial asthma. *Clin Lab*. 2014;60:963–8.
  86. Chen X, Yang X, Li Y, Zhu J, Zhou S, Xu Z, et al. Follicular helper T cells promote liver pathology in mice during *Schistosoma japonicum* infection. *PLoS Pathog*. 2014;10:e1004097. doi:10.1371/journal.ppat.1004097.
  87. Komai-Koma M, Jones L, Ogg GS, Xu D, Liew FY. TLR2 is expressed on activated T cells as a costimulatory receptor. *Proc Natl Acad Sci U S A*. 2004;101:3029–34.

88. Reynolds JM, Martinez GJ, Chung Y, Dong C. Toll-like receptor 4 signaling in T cells promotes autoimmune inflammation. *Proc Natl Acad Sci U S A*. 2012;109:13064–9.
89. Zanin-Zhorov A, Tal-Lapidot G, Cahalon L, Cohen-Sfady M, Pevsner-Fischer M, Lider O, et al. Cutting edge: T cells respond to lipopolysaccharide innately via TLR4 signaling. *J Immunol*. 2007;179:41–4.
90. Caramalho I, Lopes-Carvalho T, Ostler D, Zelenay S, Haury M, Demengeot J. Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide. *J Exp Med*. 2003;197: 403–11.
91. Gonzalez-Navajas JM, Fine S, Law J, Datta SK, Nguyen KP, Yu M, et al. TLR4 signaling in effector CD4<sup>+</sup> T cells regulates TCR activation and experimental colitis in mice. *J Clin Invest*. 2010;120: 570–81.