

REVIEW

Innate affairs of allergens

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Clinical & Experimental Allergy

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Cite this as: W. R. Thomas, *Clinical & Experimental Allergy*, 2013 (43)

152–163

Summary

Activation of receptors of the innate immune system is a critical step in the initiation of immune responses. It has been shown that dominant allergens have properties that could allow them to interact with toll-like and C-type lectin receptors to favour Th2-biased responses and many bind lipids and glycans that could associate with ligands to mimic pathogen-associated microbial patterns. In accord with the proposed allergen-specific innate interactions it has been shown that the immune responses to different allergens and antigens from the same source are not necessarily coordinately regulated.

Introduction

The concepts of the innate immunity provide a framework for understanding how allergens initiate immune responses and from this the natural events underpinning allergic sensitization and how allergen delivery in allergen immunotherapy and vaccination might be better designed. The cat-and-mouse-game played out by infectious agents and the innate immune system has left us with valuable insights into how innate immunity can be subverted, for example with inactive decoy receptors, and how innate immunity in turn has countered with the evolution of diverse families of receptors and multiple integrated signalling pathways [1–4]. Given the molecular complexity of inhaled particles, dominant allergens must have attitude to become important specificities, even if a helping hand is provided by adjuvant material in the environment or by the constitution of the host. The interaction of allergens with the innate immune system and their abundance are properties that would direct the size and nature of the responses to individual allergens. Co-exposure with natural immunological adjuvants such as the Th2-inducing eicosanoids and nucleosides of pollen [5–8] might be catalysts for allergic sensitization, but they are more likely to assist sensitization to all antigens. Similarly, it has been shown that animals sensitized to one allergen have an enhanced propensity to become sensitized to co-presented antigens [9–11], but this

would be expected to proportionally stimulate all responses. Recent demonstrations that allergens can interact with the innate immune system in ways that would be favourable for allergenicity provide a new platform for investigating allergic sensitization.

The innate immune system evolved to respond to pathogens by recognizing evolutionarily conserved microbial structures termed pathogen-associated molecular patterns (PAMPs). Detection of PAMP by antigen presenting cells occurs by families of pattern recognition receptors that are very important for dendritic cells [12]. The most important receptors for exogenous antigens such as allergens are the transmembrane-protein family of Toll-like receptors (TLRs) and the transmembrane-protein family of C-type lectin receptors. There are two families of cytoplasmic pattern recognition receptors, the NOD-like receptors and RIG-I-like receptors, and these are very important for viruses and self antigens. There are nine TLR members that variously signal via the adaptor proteins MyD88 to activate nuclear factor κ B (NF- κ B), and TRIF to activate interferon regulatory factor (IRF)-responsive genes and induce type 1 interferons. TLR1, 2, 4, 5, and 6 are primarily found on the plasma membrane, where they sense surface and secreted molecules of microbes while TLR3, 7, 8, and 9 are found in the endolysosomal compartments, where they recognize RNA and DNA but TLR4 in particular traffics to the endosomes where its signalling changes to favour type 1 interferon activation via the

TRIF adaptor [13]. The TLRs here can also be modified and modulated by the endosomal cysteine proteases [14]. TLR-4, which binds lipopolysaccharide (LPS) and TLR-2, which binds lipopeptides, are the most important for allergen adjuvanticity. The C-type lectin membrane receptors consist of a diverse group many of which are preferentially expressed on dendritic cells and macrophages where they bind microbial and other glycans as well as endogenous ligands. The C-type lectins initiate the endocytosis of antigens as well as activating signalling pathways that modify the responses to TLR.

Dominant allergens

Sensitization to many sources of allergen is preferentially directed to a small number of proteins, the dominant allergens that logically must have preferentially stimulated the immune system. Over 80% of IgE antibody induced by birch and olive pollen is, respectively, directed to Bet v 1 [15] and Ole e 1 [16] and collectively the group 1 and group 5-like grass pollen allergens bind 80% of the IgE antibody directed to pollen proteins [17]. A family of proteins that constitute the Amb a 1 and Amb a 2 proteins bind an average of 50% of anti-ragweed antibodies with values for individuals varying from 25 to 85% [18]. Recent studies with allergen arrays indicate binding closer to 85% [19]. The group 1 and 2 allergens of *Dermatophagoides* spp. typically account for 50–80% of the IgE binding attributed to house dust mite [HDM] extracts [20, 21] and Blo t 5, 7 and 21 from *Blomia tropicalis*, an important mite in tropical and subtropical regions account for 60% of its IgE-binding activity [22]. Although to a lesser degree, the groups 5, 7 and 21 are also important for *Dermatophagoides* spp. [21].

Allergen dominance might not be found for some sources of allergen or might only be found in certain circumstances. In the one quantitative comparison reported for IgE binding to cockroach, Bla g 2 and Bla g 5 on average bound 50% of the antibodies directed to the German cockroach allergens, but there was considerable heterogeneity with some large responses to Bla g 4 [23]. Depletion of Fel d 1 from cat extracts has been shown to reduce the IgE binding by 50% for most of a small number of individuals examined [24, 25]. From this and a study of a serum pool [26] Fel d 1 has been regarded as dominant but this needs to be reinvestigated taking into account recent information on the heterogeneity of anti-Fel d 1 antibodies responses and the antibody titres to newly defined allergens [27–30]. In particular adults with cat-induced rhinoconjunctivitis have low IgE anti-Fel d 1 titres and a high probability that more IgE is directed to other allergens. Low titres and a broad spread of IgE binding specificities are features of IgE responses to dogs [31] and, although the specificities responsible

for the discrepancy are not known, anti-Mus m1 antibodies account for very little of the IgE antibodies of half the mouse-allergic subjects [32].

Food allergens can have major allergens such as the related Ara h 2 and 6 allergens that sensitize most peanut-allergic subjects. They are, however, first processed by the gut acid and hydrolytic enzymes so molecular properties of resilience to processing are important and the altered structures and quantities of allergen presented to the immune system are not known [33].

Abundance of allergens

The dominant pollen allergens are the most abundant proteins in their respective pollen grains. Bet v 1 constitutes 12–30% of protein of birch pollen [34, 35], with Phl p 5 being 10–15% for grass [36], Amb a 1 9% for ragweed [37] and Ole 1 5–20% for olive [38]. Measurements of the allergen content of air in the pollen season show exposure to 1–3 ng/m³ of allergen for Bet v 1 [39, 40] 10–15 ng/m³ for Phl p 5 [36], 6 ng/m³ for Ole 1 [41], and from the data of Gunawan *et al.* [42] and Turkeltaub *et al.* [43] a representative pollen count of 400 grains of ragweed pollen/m³ would contain 6 ng/m³ of Amb a 1. Sensitization and allergic rhinitis can, however, be prevalent in regions where pollen counts only reach levels of 10% of these levels [44] and taking Ole 1 as an example symptoms develop when the airborne allergen reaches 0.9 ng/m³ [41]. The estimation of inhaled pollen allergen, however, is not that simple. Pollen counts and airborne allergen roughly correlate at the peak of the pollination, but high levels of airborne allergen associate with small particles, [45] which are found before and after this period. Indeed the incidence of ragweed asthma peaks in autumn, well after pollination and only exposure to subpollen particles can induce bronchial constriction [46]. This is not surprising given that pollens with diameters of 20–50 µm would deposit in the proximal regions of the nares. Subpollen particles can not only be inhaled, but also have enzymes that produce reactive oxygen species [47] and constituents that enhance mucosal persistence by binding surfactant [48]. The allergen content of the subpollen particle thus might be more informative. In addition, the allergen content of pollen grains is transiently high at the peak of pollination, but outside this period there is 10-fold less allergen [40] and a greater proportion of other proteins.

The group 1 allergens constitute 1–3% of the protein of *Dermatophagoides* spp. extracts [49, 50] and the group 2 allergens 0.2–1.2%. The extracts are, however, made from well-fed, pampered mites that produce large quantities of excrement accounting for 50% of the major allergen content [51]. This does not accumulate and declines rapidly when HDM cultures overgrow [51,

52] with other proteins becoming more abundant [52]. Also mites living in atmospheres with less than 75% relative humidity, conditions frequently encountered in homes in temperate regions, eat and defecate sparingly [53] so the allergen profile in the environment may be quite different than that determined for the allergists' extracts.

The proteome of *D. pteronyssinus* cultures, determined without aqueous extraction [54] showed that Der p 1 and Der p 2 were the 31st and 41st most abundant proteins with the mid-potency Der p 7 being present in similar quantity to Der p 2 and the minor allergen Der p 20 in similar quantities to Der p 1. The non-allergenic ferritin and occasional allergen Der p 13 [22] were present in higher quantity than both along with the minor tropomyosin allergen. Taking 2 mg protein per gram as the protein content for house dust [55], estimates show that Der p 1 would constitute 0.05% of dust protein [56]. For HDM proteins besides the group 1 and 2 allergens Der p 5 has been reported to be about 0.1 µg per gram of dust [57] and although not published the author has found the levels of Der p 13 in dust to be about the same as those for Der p 1. The major HDM allergens only become airborne when repositories such as bedclothes are disturbed and are predominantly found on non-respirable particles greater than 10 µm of unknown protein content [56, 58]. The aerodynamics of other HDM proteins is unknown so a greater and more consistent exposure cannot be excluded.

Comparisons of the dominant allergens of *Dermatophagoides* spp. and *B. tropicalis* provide a useful comparison with allergenicity. In Singapore, where both species are abundant, Blo t 5, the major allergen of *B. tropicalis*, is found at 1 µg per gram of dust [59], which is 10-fold higher than Der p 5 [57], a less important allergen of *D. pteronyssinus*. Measurement from a pool of dust showed that Blo t 1 was present at 46 ng/mg of dust protein [60], around 100 ng per gram of dust, much lower than the microgram quantities found for Der p 1. Extracts of *B. tropicalis* had 500 ng of Blo t 5 per mg of protein [60], also 10–50-fold more than Der p 5. The abundance of the poorly allergenic Blo t 2 would be of interest, but is unreported.

Cat dander and hair extracts contain 300–500 µg/mL of protein [61] 15–30 µg of which is the major allergen Fel d 1 and 30–60 µg of which is the less important albumin, Fel d 2 [62]. Fel d 1 and albumin are found in similar ratios in saliva [24] with Fel d 1 at 10 µg/mL constituting about 0.06% of salivary protein [63]. Eight times more Fel d 1 than albumin can, however, be washed from the fur [64] indicating that saliva is not the source of the allergen in the hair. Fel d 1 is famously abundant in respirable particles in ambient air [56], typically found in homes with cats at 20 ng/m³ [65]. This exceeds occupational threshold levels reported for pure

allergens: fungal alpha-amylase allergen (8 ng/m³) [66] and Mus m 1 for mouse 0.14 ng/mL [67]. Data for other cat allergens are sparse. The von Ebner gland lipocalin Fel d 7, constitutes 0.21% of the protein of saliva, a higher quantity than Fel d 1, and 0.023% of the protein of hair extract [30]. An intriguing concept of allergen dispersal, however, is indicated by another lipocalin Fel d 4. It is a kairomone, which is specifically produced to be inhaled by other animals to convey chemical messages, in this case, fear of the cat [68].

Mimicry

There is evidence that the group 2 HDM allergens have enhanced immunogenicity because they mimic their human homologue, myeloid differentiation factor-2 (MD-2). Both are β-cups where a layer of β-sheet is folded over like a taco-shell creating a hydrophobic cavity [69, 70]. The discovery that MD-2 loads LPS onto TLR-4 to activate inflammatory responses suggested the possibility of mimicry [71], subsequently demonstrated by the ability of Der p 2 expressed in MD-2 deficient human embryonic kidney cells to restore their ability to produce LPS-induced IL-8 [72] and to enhance the response in cells replete with MD-2. It was further demonstrated that intranasal administration of recombinant Der p 2 and LPS sensitized mice for a pulmonary eosinophilic hypersensitivity in a TLR-4-dependent but MD-2 independent manner [72]. Although this demonstrates the possibility of Der p 2-mediated TLR4 loading, experiments of others have shown that LPS administered with ovalbumin, a serpin with no lipid-binding function, enhanced sensitization [73]. The circumstances that would benefit from MD-2 mimicry therefore need to be better defined and additionally the sensitization needs to be distinguished from transient sensitization known to precede tolerance [74].

Human MD2 has four well-defined ligand interactions [75]: 1) the binding of TLR4 by an interface of basic residues; 2) the tethering of LPS; 3) secondary binding to LPS to perturbate the structure of MD2 to create a second TLR-4-binding region; and 4) the dimerization of the complex. Der f 2 binds LPS with nanomolar affinity with one to one stoichiometry [76] and as revealed by nuclear magnetic resonance (NMR) perturbates the structure around the mouth of the hydrophobic cavity, consistent with the creation of the second TLR-4 binding region. The functional residues of Der f 2 as reckoned and illustrated by Ichikawa *et al.* are shown in Fig. 1. The three basic residues that mediate the binding of free MD-2 to TLR-4 are conserved in Der f 2 with K48, K77 and K82 being equivalent to K82, R106 and K109 of MD-2. The LPS tethering region of MD-2 F119, S120 and F121 correspond to Y90, T91 and W92 of Der f 2. They not only have conserved

Df2.0101	48	KTAKIEIKASLDGLEIDVPGIDTNACH-FVKCPVKGQYDIKYTW-NVPKIAP	99
Ld2.0101	47	AKVTIKVLAKVAGTTIQVPGLETGCK-FIKCPVKKGEALDFIYSG-TIPAITP	98
Ld2.0102	47	AKVTIKVLTKVAGTTIQVPGLETGCK-FIKCPVKKGEALDFIYSG-TIPAITP	98
Ld2.0201	47	NKVTIKVLAKVAGTTIQVPGLETGCK-VLKCPVKKGEALDFNYGM-TIPAITP	98
Gd2.0101	47	ASVGLVLTAIIDGDIADIPGLETNACKLMKCPVIRKGEHQLIYNIIGEIPDATP	100
Gd2.0201	47	TKATIKVLAKVAGTPIQVPGLETGCK-FVKCPVKKGDPIDFKYTT-TVPAILP	98
Bt2.0101	47	EKLEIKISAIMNNIEVPVPGVDKDGCK-HTTCPLKKGQKYELDYSL-IIPTILP	98
Bt2.0102	47	EKLEIKISAIMNNIEVPVPGVDKDGCK-HTTCPLKKGQKYELDYSL-IIPTILP	98
Bt2.0103	47	EKLEIKISAIMNNIEVPVPGVDKDGCK-HTTCPLKKGQKYELDYSL-IIPTILP	98
Tp2.0101	47	CKIEVKVTGQLNGLEVPIPGIETDGCK-VLKCPVKKGKYTMNYSV-NVPSVVP	98
Sm2.0101	47	PTAVIKISAKVNGVELQVPGIETNGCH-HMKCPVKGQSYQFKYDL-VIPQILP	98
As2.0101	48	AHVKIQLTASMGGEIIPVPGVGTGCK-YIKCPVKGKKTFPPTY-LVPKILP	99
Ao2.0101	47	EKVEIKIIAKVNGLEIPVPGVESNGCN-HMKCPVTKGQKYTFNYGI-SIPKLLP	98

KKK	Basic amino acids that attach to TLR4
LLIP...IAP	Amino acids perturbed by LPS binding to create second TLR4 binding site
F	No TLR4 binding when this aromatic amino acid is mutated to alanine
YTW	Amino acids that tether LPS

Fig. 1. Comparison MD-2-like amino acids of group 1 allergens. Alignment is shown with reference to the LPS and TLR4 binding amino acids of Der f 2 proposed from the analysis of Ichikawa *et al.* [76]. Sequence from the group 2 allergens (with variant numbers) are shown for *Dermatophagoides farinae* (Df), *Lepidoglyphus destructor* (Ld), *Blomia tropicalis* (Bt), *Glycyphagus domesticus* (Gd), *Tyrophagus putrescentiae* (Tp), *Acarus siro* (As), *Aleuroglyphus ovatus* (brown legged grain mite) (Ao) and *Suidasia medanensis* (Sm).

structural coordinates but are perturbed by LPS-binding. The hydrophobic residues that are then perturbed by the LPS-binding are partially conserved with L58, L61, I63, P66, I97, A98 and P99 being equivalent to V82, M85, L87, R90, I124, K125 and F126 of MD2. Der f 2 lack the basic residues of MD-2 in this region, but the preservation is still striking considering that the proteins only have 21% sequence identity.

The group 2 allergens of *Dermatophagoides* spp. and the glycyphagoide (storage-type) mites only have 30% amino acid sequence identity and the glycyphagoides only show 50% identity between species. It would be of interest to know if the functional residues are conserved and how this compares to allergenicity. The MD-2-like residues of the glycyphagoide mites mostly align very well with those of Der f 2 (Fig. 1). One of hydrophobic residues that are perturbed by LPS-binding (residue 63) is not hydrophobic for *L. destructor* and *G. domesticus*, but is for the others species and in any case this interface is not that well conserved between MD-2 and the allergens. The standout observation, however, is that all the variants of Blo t 2 lack the key basic TLR-4-binding residue K77. In addition, an aromatic residue at position 75, shown to be important for TLR-4 binding (Tyr102 of MD-2) [77] was histidine for all Blo t 2 sequences and valine for Tyr p 2. It is therefore speculated that low TLR4 binding by Blo t 2 accounts for its low allergenicity [22, 78] and for the lack of non-cross-reactive anti-Tyr p 2 antibodies [79, 80]. The ability of Blo t 2 and Tyr p 2 to interact with TLR-4 therefore might be very informative along with determining their abundance in the environment. The group 2 allergens of *L. destructor* [81] and *G. domesticus* are known to induce antibodies so inter-species comparisons can be made.

Infiltrating cysteine protease regulation

Contrary to popular representation protease activity is not a common property of allergens. Only

Dermatophagoides spp. have a dominant cysteine protease allergen and serine proteases even from this source induce little IgE antibody [21, 22]. Fungal and insect extracts are rich in proteases [82], but reports of allergenicity are equivocal. A vacuolar serine protease reported to bind IgE from over 50% of *Cladosporium* spp.-sensitized subjects in Taiwan [83] only bound IgE in 15% of sensitized subjects in Europe [84] and similarly although a cockroach serine protease has been reported [85] as an important allergen in India, the allergens described in other regions can account for the known IgE-binding [23, 86].

Recent experiments conducted with papain, a homologue of Der p 1 and a potent occupational allergen, have nevertheless demonstrated the Th2 adjuvant activity of a cysteine protease [87–91] and its ability to stimulate Th2 responses to bystander antigens, independently from the immune response to itself [91]. Experiments in which Der p 1 and Der f 1 were injected with the Th2-inducing alum adjuvant similarly found lower IgE responses if the proteolytic activity was irreversibly inactivated [92, 93] although contrarily respiratory challenge of sensitized mice with activated Der p 1 augmented the Th1-type macrophage over the Th2-type lung eosinophilia [94]. Many possible adjuvant mechanisms have been proposed based on cysteine proteases' ability to breach epithelial barriers and to cleave important immunological extracellular receptors [95]. Recent experiments where activated papain was injected into the tissue have pointed to a not necessarily unrelated role for basophils [89, 90]. Although these mechanisms might operate in some circumstances, cysteine proteases from HDM extracts are inactive [96–98] as would be expected from their sensitivity to oxidation and the extracellular fluid into which they are deposited following inhalation is patently oxidizing [99]. Chromatographic fractions containing Der p 1 are demonstrably devoid of proteolytic activity without first adding a reducing agent [96], as is Der p 1 purified from extracts

[97], and there is no activity in unfractionated extracts as shown the inability to cleave Boc-FSR-MCA or the Boc-LLVY-MCA substrates [98], both of which are excellent substrates for activated Der p 1 and Der f 1 [100].

Endosomes and lysosomes would be the logical site for the action of cysteine proteases. Cystine is taken up by cells, reduced in the cytoplasm by glutathione and the resulting cysteine is translocated to lysosomes via a transport mechanism unique to these organelles [99]. Here it activates up to eight resident cysteine proteases that have critical functions for immune responses [14, 101] that might be mimicked or corrupted by endocytosed cysteine protease antigens. The functions include the degradation of the invariant chain of the MHC class II molecules to initiate peptide loading and the modification of lysosomal TLR as shown for cathepsin K, which interestingly cleaves TLR9 to increase MyD88 signalling. The lysosomal cysteine proteases can also enter the cytosol and help drive the cysteine-protease caspases of the inflammasomes [102]. Indeed it has recently been demonstrated that Der p 1 in mite extracts activated caspase 1 in NLP3-type inflammasomes of keratinocytes resulting in the release of IL-1 β and IL-18 [103]. The experiments did not activate the Der p 1 by reduction so it would be informative to determine if it acted extracellularly or was internalized by the endocytic activity of the keratinocytes [104]. The IL-1 β has been shown to preferentially stimulate Th2 and Th17 responses compared to Th1 [105] and IL-18 is a potent inducer of IL-13 production from both Th1 and Th2 cells [106].

Cysteine proteases produced by *Leishmania* spp. and helminthes deviate the host immune responses from Th1 to non-protective Th2 responses. For example *Leishmania mexicana* genetically engineered for impaired cysteine protease production induce Th1 instead of Th2 responses and less virulent infections [107]. In what might be informative for allergy, the cysteine protease from the helminth *Fasciola hepatica* acts within the macrophage endosome to cleave TLR-3 leaving TLR-4 intact [108]. This stopped the production of proinflammatory mediators, especially IL-12, induced through the TRIF-signalling pathway not only by removing TLR3 but also by an unknown mechanism, via TLR4. It is probable that the diminution of type 1 interferons induced by TRIF-signalling is of critical importance since their signalling functions block Th2 responses via GATA-3 [109] and mediate synergistic signalling from TLR4 and TLR3 to induce IL-12 [110]. In contrast to the endosomal action the induction of TSLP from epithelial cells by activated by papain is TRIF-dependent indicating a different mechanism for this experimental model [90]. Experiments with reversible oxidatively inactive cysteine protease are indicated.

C-type lectin receptor binding

Microbial carbohydrates, especially from parasites, can trigger Th2 instead of Th1-type immunity by targeting C-type lectin receptors of antigen presenting cells such the mannose receptor, DC-sign and dectin [111, 112]. A recent study with a HDM extract showed that the engagement of dectin-2 with unknown ligands induced Th2-inducing cysteinyl leukotrienes [113], however, studies of allergen-specific effects have focussed on DC-sign and the mannose receptors. When these receptors are engaged with glycans, TLR-induced signals are modified to induce Th2-and reduce Th1 cytokines [111, 112]. This is followed by positive reinforcement where IL-4 and IL-13 direct the alternate activation pathway of macrophages, which further increases the expression of these receptors [114–116]. As well as being induced by parasite and some other infections, alternatively activated macrophages are induced during wound healing [117], by interactions with stem cells [118] and by IL-33 [119], which is released by tissue damage. Both macrophages and dendritic cells can undergo this differentiation [120]. Many aeroallergens are glycosylated so engagement of lectin receptors should be expected to be part of their interaction with the innate immune system. Very efficient uptake of Der p 1 by the mannose receptor [121] has been described and, more recently, down-regulation of the mannose receptor by gene silencing technology was shown to inhibit the uptake of Der p 1, Der p 2, Ara h 1, Bla g 2 and Can f 1 by human cells [122, 123]. Moreover, mannose-receptor-depleted dendritic cells produced more of the immune regulator indoleamine 2,3-dioxygenase (IDO) after incubation with LPS and Der p 1, and when used to stimulate T cells they induced less IL-4 and interferon- γ by an IDO-dependent mechanism [122]. These experiments, plus the demonstration that mannose-receptor knockout mice injected with Fel d 1 in alum could not make IgE antibody [123] are intriguing. Previous investigations, however, have convincingly shown that blockade of the mannose receptor did not affect T-cell cytokine responses [124] to the glycosylated allergens in Japanese cedar pollen extract [125] and pure non-glycosylated proteins Bet v 1 [35] and β -lactoglobulin [126] readily induce IgE in mice [127, 128].

Mannose receptors have a broad specificity for carbohydrate ligands [129] so they would have the ability to affect responses to the important glycosylated aeroallergens Der f 1 [130] (and by analogy Der p 1), Fel d 1 [131], Phl p 1 [132], Ole 1 [133] and Bla g 2 [134]. In addition, Bla g 4 is probably glycosylated [85]. Important aerollergens that are not glycosylated are Der p 2 [135], Bet v 1 [35], group 5 grass allergens [17, 136] and Amb a 1 [137]. Natural Der p 2 by mass spectros-

copy has precisely the molecular weight expected from its polypeptide chain and this agrees with the lack of an N-glycosylation and O-glycosylation motifs [135]. The mannose receptor results of Royer *et al.* for Der p 2 [122] therefore should be questioned, but as discussed below could be due to associated carbohydrate.

PAMP ligands

Dominant allergens are frequently lipid-binding molecules. The first structures of allergens revealed that Bet v 1 [138] and Der p 2 and Der f 2 [69, 70] had hydrophobic cavities. Fel d 1 proved to be a typical uteroglobin that would be expected to bind lipid mediators [139] and most of the other important mammalian allergens are lipid-binding lipocalins [140]. Bla g 4 from the cockroach binds the lipid juvenile growth hormone [141]. The group 5 HDM allergens [142] and by inference the homologous group 21 form a multimeric cage with a hydrophobic cavity indicating a lipid-ligand function and the next most important HDM allergens the group 7 are structurally members of the LPS [143] and odorant binding protein families [144] that can bind the lipid polymyxin [143]. Thus, these allergens would be expected to be complexed with lipid and glycolipid ligand and be able to engage TLR2 and TLR4 and through the binding of glycolipids the C-type lectins. There are also structures for direct carbohydrate binding. The group 1 grass allergen maize Zea m 1, consistent with its expansin function, has a family-III carbohydrate-binding module with the archetypal aromatic carbohydrate-binding residues on a concave surface of a beta sandwich [145]. Such a module been proposed for Der p 2 [146], however, it does not have surface exposed aromatic residues and has an internal hydrophobic pocket [69, 70]. The major ragweed, cedar and cypress allergens are from their structures pectate lyases that bind to complex carbohydrates [147] and interestingly the cedar pectate lyase [148] has been shown only to have minimal enzymatic activity indicating a noncatalytic binding function.

Interactions with pattern receptor signalling

As described, interactions with C-type lectin receptors and the modification of TLRs by cysteine proteases could both initiate immune responses and bias them to the Th2 pathway. The TLR4 activation induced by MD-2 mimicry would not necessarily lead to a Th2 bias, however, the activation of TLR4 on non haemopoietic cells of the lung does lead to Th2 responses [149] and chronic stimulation of TLR4 can down-regulate Th1 in favour of Th2 responses [150]. The diverse ligand bind-

ing activity indicated by the structures of allergens indicates that a plethora of mimics of pattern associated molecular patterns might exist showing a need to ascertain the ligands associating with natural allergens. Allergens can stimulate by more than one pattern recognition pathway so the interactions need to be understood. The modification of the TLR signalling by C-type lectin signalling is well known, as introduced above, and evidence has been presented for the engagement of both pathways by Der p 2. As shown by the modification of TLR4 signalling that followed the cleavage of TLR3 by parasite cysteine protease [108] there are uncharacterized interactions of signalling from different TLR. Further still, at least for TLR4, there can be interaction between the different signalling from the same receptor in different compartments, namely the endosomal and cell membranes [13]. The C-type lectin signalling pathways also vary and although they often have anti-inflammatory properties, their functions, as exemplified by DC-sign, are dependent on signalling from other pattern recognition receptors and on the stimulating glycan ligand [151], even to the point of enhancing Th1-type inflammatory responses [151]. The abundant mannose receptor has a major function in antigen transport and presentation to T-cell receptors [152], which can greatly influence responses by this mechanism, for example, by efficient release and feedback from cytokines from T-cell interacting with antigen presenting cells. Recent experiments with human cell culture have shown that in contrast to the effect of mannose receptor, knockdown of DC-sign expression enhanced Th2 responses to the Der p 1 and Fel d 1 allergens indicating opposite functions [153] at least under the culture conditions used.

Relevance

The extent to which the proposed innate interactions (Table 1) occur in the allergic sensitization remains to be determined, but there is convincing evidence that the regulation of immune responses to an allergen is determined by the allergen itself. Information on non-allergic inhaled proteins that are not antigens of infecting microbes is alarmingly sparse but immune responses to the non-allergenic HDM ferritin have been investigated and the results are highly informative. HDM ferritin induced large T-cell proliferative responses that were strongly correlated with the size of the response to Der p 2 [154]. Both Th1 and Th2 cytokines were released, but with a more balanced mix than that found for responses from allergic subjects to Der p 2 and the responses were similar for HDM allergic and non-allergic subjects. Importantly, the Th2 cytokine release induced by Der p 2 was higher than that induced by ferritin for allergic subjects, but lower than

Table 1. Proposed innate interactions

Innate interaction	Allergens with known activity or structural characteristics	Mechanism
MD-2 mimicry	Der p 2 and Der f 2	Allergens bind TLR4 and LPS to activate MyD88 and TRIF inflammatory pathways
Infiltration of endosomal cysteine protease regulation	Der p 1 and Der f 1, Activity shown by parasite (<i>Fasciola hepatica</i>) cysteine protease	Cleavage of innate receptors e.g. TLR3 in reducing (permissive) environment
Binding C-type Lectin Receptors	Fel d 1, Der p 1, Der p 2*, Bla g 2, Can f 1(*Der p 2 must bind glycan)	Signalling induced by mannose binding receptor and dectin initiated Th2 responses and promote alternatively activated antigen presenting cell
Allergen ligands mimicking pathogen-associated molecular patterns	Group 5, 7, 21 HDM allergens, Bet v 1, Fel d 1 and lipocalins Fel d 4, 7 and 8, Can f 1, Mus m 1, Group 1 grass.	Many allergens show structures that bind lipid and glycans that could mimic PAMPS

that induced by ferritin in non-allergic subjects. Their responses were therefore not coordinated and the type of response induced by HDM ferritin, an abundant protein, was regulated differently to the responses to Der p 2. Similarly, when comparing different allergens, the chitinase and chitinase-binding Der p 15 and 18 induce IgE with titres that do not correlate with those to the major allergens [155, 156] or to the titres to the second tier allergens Der p 5 and 7 [156], all of which show excellent correlations with each other. The titres to Der p 15 and 18, allergens that are not cross-reactive and only have 29% sequence identity, however, show excellent correlation with each other. They are therefore, despite only being found in subjects with IgE antibodies to the major HDM allergens, regulated by a separate common mechanism, perhaps to due an association with chitin. The observation made with ferritin and the chitinase and chitinase-binding proteins therefore show qualitative differences that are unlikely to be determined by source-specific adjuvants such as accompanying microbial products, chitin, inflammatory lipids [157] or even help from proteases. The paucity of allergic responses to the abundant group 13 allergens also shows that the milieu during antigen presentation is of

limited importance. Similar results were found from a comparison of the types of immune responses induced by different grass pollen allergens [158]. Here the group 1 and 11 allergens induced pure Th2 responses while the responses to other allergens, which included the groups 4 and 5, induced a broad mix of Th1, Th2 and Th17 cytokines. The design of this investigation, which was a blanket analysis of responses to allergen peptides, precluded the influence of the properties of the allergens having an effect on *in vitro* cultures and the presence of biological impurities.

Investigating why *B. tropicalis*-exposed people have low responses to Blo t 2 would be a productive line of investigation since its ability to mimic MD-2 could be examined along with its abundance in the environment. The general lack of responses reported to storage mite cysteine proteases would also be a very useful area of investigation especially if their substrate specificities vary. In addition, if T-cell sensitization to allergens is elicited following interactions with different innate immune receptors then different allergens would be expected to have different gene expression profiles. Such experiments would seem straight forward, for example using the HDM and pollen allergens described above. Animal experiments with defined antigens or exposure to allergen sources that resemble the natural process might be useful, but the delivery may be difficult given the inherent difference in the airways and breathing patterns of rodents [159]. The size of respirable particles and particles that lodge in the nose are, however, not that different for rats and humans so some experiments could be done. The aerodynamics of the airborne Fel d 1-containing particles of the cat is for example well defined and they are readily available from their natural source. Models for HDM and pollen allergy should not unreasonably be expected to show that they recapitulate the allergen hierarchy and the qualitative differences in regulation of responses to different allergens and antigens demonstrated for humans.

Understanding the interaction of allergens with innate immunity is fundamental to understanding the sensitizing process and also the process by which non-allergic subjects develop resistance. It would be important to determine if the innate immune system interactions are control points for the development of allergy, if environmental and genetic factors impinge on the interactions and how they differ for different allergens. For example processes that generate alternatively activated macrophages such as wound healing would increase Th2 responses to allergens that engage with C-type lectins and probably not to others. Thus, the interactions between allergens and innate immunity, shown in principle to operate, could be game changers in how people respond to allergens, a contention

already supported by differences in immune responses to different allergens and accompanying antigens. The question of whether or not allergens interact with innate immunity should more productively be changed to how they interact.

Conflict of interests:

Wayne Thomas is an inventor on patents on house dust mite allergens obtained and assigned by the Telethon Institute for Child Health Research.

References

- Bowie AG, Unterholzner L. Viral evasion and subversion of pattern-recognition receptor signalling. *Nat Rev Immunol* 2008; 8:911–22.
- Alcami A. New insights into the subversion of the chemokine system by poxviruses. *Eur J Immunol* 2007; 37:880–3.
- Potempa J, Pike RN. Corruption of innate immunity by bacterial proteases. *J Innate Immunol* 2009; 1:70–87.
- Hooper LV. Do symbiotic bacteria subvert host immunity? *Nat Rev Micro* 2009; 7:367–74.
- Plotz SG, Traidl-Hoffmann C, Feussner I *et al.* Chemotaxis and activation of human peripheral blood eosinophils induced by pollen-associated lipid mediators. *J Allergy Clin Immunol* 2004; 113:1152–60.
- Behrendt H, Kasche A, Ebner von Eschenbach C, Risse U, Huss-Marp J, Ring J. Secretion of proinflammatory eicosanoid-like substances precedes allergen release from pollen grains in the initiation of allergic sensitization. *Int Arch Allergy Immunol* 2001; 124:121–5.
- Gilles S, Fekete A, Zhang X *et al.* Pollen metabolome analysis reveals adenosine as a major regulator of dendritic cell-primed T(H) cell responses. *J Allergy Clin Immunol* 2011; 127:454–61.
- Gilles S, Jacoby D, Blume C *et al.* Pollen-derived low-molecular weight factors inhibit 6-sulfo LacNAc⁺ dendritic cells' capacity to induce T-helper type 1 responses. *Clin Exp Allergy* 2010; 40:269–78.
- Dittrich AM, Chen HC, Xu L *et al.* A new mechanism for inhalational priming: IL-4 bypasses innate immune signals. *J Immunol* 2008; 181:7307–15.
- Blumchen K, Gerhold K, Schwede M *et al.* Effects of established allergen sensitization on immune and airway responses after secondary allergen sensitization. *J Allergy Clin Immunol* 2006; 118:615–21.
- Wang LF, Chen JS, Hsu CJ *et al.* Antigen-driven bystander effect accelerates epicutaneous sensitization with a new protein allergen. *J Biomed Sci* 2009; 16:28.
- Levitz SM, Golenbock DT. Beyond empiricism: informing vaccine development through innate immunity research. *Cell* 2012; 148:1284–92.
- Kagan JC, Su T, Horng T, Chow A, Akira S, Medzhitov R. TRAM couples endocytosis of Toll-like receptor 4 to the induction of interferon-beta. *Nat Immunol* 2008; 9:361–8.
- Watts C. The endosome-lysosome pathway and information generation in the immune system. *Biochim Biophys Acta* 2012; 1824:14–21.
- Moverare R, Westritschnig K, Svensson M *et al.* Different IgE reactivity profiles in birch pollen-sensitive patients from six European populations revealed by recombinant allergens: an imprint of local sensitization. *Int Arch Allergy Immunol* 2002; 128:325–35.
- Lombardero M, Quirce S, Duffort O *et al.* Monoclonal antibodies against Olea europaea major allergen: allergenic activity of affinity-purified allergen and depleted extract and development of a radioimmunoassay for the quantitation of the allergen. *J Allergy Clin Immunol* 1992; 89:884–94.
- Andersson K, Lidholm J. Characteristics and immunobiology of grass pollen allergens. *Int Arch Allergy Immunol* 2003; 130:87–107.
- Zeiss CR, Levitz D, Suszko IM. Quantitation of IgE antibody specific for ragweed and grass allergens: binding of radiolabeled allergens by solid-phase bound IgE. *J Allergy Clin Immunol* 1978; 62:83–90.
- Gadermaier G, Wopfner N, Wallner M *et al.* Array-based profiling of ragweed and mugwort pollen allergens. *Allergy* 2008; 63:1543–9.
- Trombone AP, Tobias KR, Ferriani VP *et al.* Use of a chimeric ELISA to investigate immunoglobulin E antibody responses to Der p 1 and Der p 2 in mite-allergic patients with asthma, wheezing and/or rhinitis. *Clin Exp Allergy* 2002; 32:1323–8.
- Hales BJ, Martin AC, Pearce LJ *et al.* IgE and IgG anti-house dust mite specificities in allergic disease. *J Allergy Clin Immunol* 2006; 118:361–7.
- Kidon MI, Chin CW, Kang LW *et al.* Mite component-specific IgE repertoire and phenotypes of allergic disease in childhood: the tropical perspective. *Ped Allergy Immunol* 2011; 22:202–10.
- Satinover SM, Reefer AJ, Pomes A, Chapman MD, Platts-Mills TA, Woodfolk JA. Specific IgE and IgG antibody-binding patterns to recombinant cockroach allergens. *J Allergy Clin Immunol* 2005; 115:803–9.
- Lowenstein H, Lind P, Weeke B. Identification and clinical significance of allergenic molecules of cat origin. Part of the DAS 76 Study. *Allergy* 1985; 40:430–41.
- de Groot H, van Swieten P, van Leeuwen J, Lind P, Aalberse RC. Monoclonal antibodies to the major feline allergen Fel d 1. I. Serologic and biologic activity of affinity-purified Fel d 1 and of Fel d 1-depleted extract. *J Allergy Clin Immunol* 1988; 82:778–86.
- van Ree R, van Leeuwen WA, Bulder I, Bond J, Aalberse RC. Purified natural and recombinant Fel d 1 and cat albumin in *in vitro* diagnostics for cat allergy. *J Allergy Clin Immunol* 1999; 104:1223–30.
- Gronlund H, Adedoyin J, Reininger R *et al.* Higher immunoglobulin E antibody levels to recombinant Fel d 1 in cat-allergic children with asthma compared with rhinoconjunctivitis. *Clin Exp Allergy* 2008; 38:1275–81.
- Erwin EA, Custis NJ, Satinover SM *et al.* Quantitative measurement of IgE antibodies to purified allergens using streptavidin linked to a high-capacity solid phase. *J Allergy Clin Immunol* 2005; 115:1029–35.

- 29 Smith W, Butler AJ, Hazell LA *et al.* Fel d 4, a cat lipocalin allergen. *Clin Exp Allergy* 2004; 34:1732–8.
- 30 Smith W, O'Neil SE, Hales BJ *et al.* Two newly identified cat allergens: the von Ebner gland protein Fel d 7 and the latherin-like protein Fel d 8. *Int Arch Allergy Immunol* 2011; 156:159–70.
- 31 Mattsson L, Lundgren T, Olsson P, Sundberg M, Lidholm J. Molecular and immunological characterization of Can f 4: a dog dander allergen cross-reactive with a 23 kDa odorant-binding protein in cow dander. *Clin Exp Allergy* 2010; 40:1276–87.
- 32 Krop EJM, Matsui EC, Sharrow SD *et al.* Recombinant major urinary proteins of the mouse in specific IgE and IgG testing. *Int Arch Allergy Immunol* 2007; 144:296–304.
- 33 Koppelman SJ, Hefle SL, Taylor SL, de Jong GA. Digestion of peanut allergens Ara h 1, Ara h 2, Ara h 3, and Ara h 6: a comparative *in vitro* study and partial characterization of digestion-resistant peptides. *Mol Nut Food Res* 2010; 54:1711–21.
- 34 Erler A, Hawranek T, Kruckemeier L *et al.* Proteomic profiling of birch (*Betula verrucosa*) pollen extracts from different origins. *Proteomics* 2011; 11:1486–98.
- 35 Swoboda I, Jilek A, Ferreira F *et al.* Isoforms of Bet v 1, the major birch pollen allergen, analyzed by liquid chromatography, mass spectrometry, and cDNA cloning. *J Biol Chem* 1995; 270:2607–13.
- 36 Schappi GF, Taylor PE, Pain MC *et al.* Concentrations of major grass group 5 allergens in pollen grains and atmospheric particles: implications for hay fever and allergic asthma sufferers sensitized to grass pollen allergens. *Clin Exp Allergy* 1999; 29:633–41.
- 37 King TP. Chemical and biological properties of some atopic allergens. *Adv Immunol* 1976; 23:77–105.
- 38 Fernandez-Caldas E, Carnes J, Iraola V, Casanovas M. Comparison of the allergenicity and Ole e 1 content of 6 varieties of *Olea europaea* pollen collected during 5 consecutive years. *Ann Allergy Asthma Immunol* 2007; 98:464–70.
- 39 Schappi GF, Suphioglu C, Taylor PE, Knox RB. Concentrations of the major birch tree allergen Bet v 1 in pollen and respirable fine particles in the atmosphere. *J Allergy Clin Immunol* 1997; 100:656–61.
- 40 Buters JT, Weichenmeier I, Ochs S *et al.* The allergen Bet v 1 in fractions of ambient air deviates from birch pollen counts. *Allergy* 2010; 65:850–8.
- 41 Brito FF, Gimeno PM, Carnes J *et al.* *Olea europaea* pollen counts and aeroallergen levels predict clinical symptoms in patients allergic to olive pollen. *Ann Allergy Asthma Immunol* 2011; 106:146–52.
- 42 Gunawan H, Takai T, Kamijo S *et al.* Characterization of proteases, proteins, and eicosanoid-like substances in soluble extracts from allergenic pollen grains. *Int Arch Allergy Immunol* 2008; 147:276–88.
- 43 Turkeltaub PC, Campbell G, Mosimann JE. Comparative safety and efficacy of short ragweed extracts differing in potency and composition in the treatment of fall hay fever. Use of allergenically bioequivalent doses by parallel line bioassay to evaluate comparative safety and efficacy. *Allergy* 1990; 45:528–46.
- 44 Nja F, Roksund OD, Svidal B, Nystad W, Carlsen KH. Asthma and allergy among schoolchildren in a mountainous, dry, non-polluted area in Norway. *Ped Allergy Immunol* 2000; 11:40–8.
- 45 Feo Brito F, Mur Gimeno P, Carnes J *et al.* Grass pollen, aeroallergens, and clinical symptoms in Ciudad Real, Spain. *J Invest Allergol Clin Immunol* 2010; 20:295–302.
- 46 Rosenberg GL, Rosenthal RR, Norman PS. Inhalation challenge with ragweed pollen in ragweed-sensitive asthmatics. *J Allergy Clin Immunol* 1983; 71:302–10.
- 47 Bacsı A, Choudhury BK, Dharajiya N, Sur S, Boldogh I. Subpollen particles: carriers of allergenic proteins and oxidases. *J Allergy Clin Immunol* 2006; 118:844–50.
- 48 Schleh C, Erpenbeck VJ, Winkler C *et al.* Allergen particle binding by human primary bronchial epithelial cells is modulated by surfactant protein D. *Resp Res* 2010; 11(83):2010.
- 49 Ford AW, Rawle FC, Lind P, Spiekma FT, Lowenstein H, Platts-Mills TA. Standardization of *Dermatophagoides pteronyssinus*: assessment of potency and allergen content in ten coded extracts. *Int Arch Allergy Appl Immunol* 1985; 76:58–67.
- 50 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.
- 51 Yella L, Morgan MS, Arlian LG. Population growth and allergen accumulation of *Dermatophagoides pteronyssinus* cultured at 20 and 25 degreesC. *Exp Appl Acarol* 2011; 53:103–19.
- 52 Eraso E, Guisantes JA, Martinez J *et al.* Kinetics of allergen expression in cultures of house dust mites, *Dermatophagoides pteronyssinus* and *D. farinae* (Acari: Pyroglyphidae). *J Medical Entomol* 1997; 34:684–9.
- 53 Arlian LG. Water balance and humidity requirements of house dust mites. *Exp Appl Acarol* 1992; 16:15–35.
- 54 Batard T, Hrabina A, Bi XZ *et al.* Production and proteomic characterization of pharmaceutical-grade *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* extracts for allergy vaccines. *Int Arch Allergy Immunol* 2006; 140:295–305.
- 55 Haselbarth-López MM, Neria JP, Espinosa RA. Caracterización preliminar de la composición proteica del polvo de casas localizadas en diferentes puntos de la Zona Metropolitana de la Ciudad de México. *Rev Inst Nal Enf Resp Mex* 2002; 15:206–13.
- 56 Tovey ER, Chapman MD, Wells CW, Platts-Mills TA. The distribution of dust mite allergen in the houses of patients with asthma. *Am Rev Respir Dis* 1981; 124:630–5.
- 57 Li CS, Hsu CW, Chua KY, Hsieh KH, Lin RH. Environmental distribution of house dust mite allergen (Der p 5). *J Allergy Clin Immunol* 1996; 97:857–9.
- 58 de Blay F, Heymann PW, Chapman MD, Platts-Mills TA. Airborne dust mite allergens: comparison of group II allergens with group I mite allergen and cat-allergen Fel d I. *J Allergy Clin Immunol* 1991; 88:919–26.
- 59 Yi FC, Lee BW, Cheong N, Chua KY. Quantification of Blo t 5 in mite and dust extracts by two-site ELISA. *Allergy* 2005; 60:108–12.
- 60 Ramos JD, Cheong N, Kuo Teo AS, IC, Lee BW, Chua KY. Production of monoclonal antibodies for immunoaffinity purification and quantitation of

- Blo t 1 allergen in mite and dust extracts. *Clin Exp Allergy* 2004; 34:604–10.
- 61 May JC, Sih JT, Miller JR, Seligmann EB. Optimization of parameters in protein nitrogen unit precipitation procedure for allergenic extracts. *J Allergy Clin Immunol* 1979; 63:87–97.
 - 62 Esch RE. Allergen source materials and quality control of allergenic extracts. *Methods* 1997; 13:2–13.
 - 63 Anderson MC, Baer H. Allergenicity active components of cat allergen extracts. *J Immunol* 1981; 127:972–5.
 - 64 Ohman JL, Baer H, Anderson MC, Leitemann K, Brown P. Surface washes of living cats: an improved method of obtaining clinically relevant allergen. *J Allergy Clin Immunol* 1983; 72:288–93.
 - 65 Luczynska CM, Li Y, Chapman MD, Platts-Mills TA. Airborne concentrations and particle size distribution of allergen derived from domestic cats (*Felis domesticus*). Measurements using cascade impactor, liquid impinger, and a two-site monoclonal antibody assay for Fel d I. *Am Rev Resp Dis* 1990; 14:361–7.
 - 66 Houba R, Heederik DJ, Doekes G, van Run PE. Exposure-sensitization relationship for alpha-amylase allergens in the baking industry. *Am J Respir Crit Care Med* 1996; 154:130–6.
 - 67 Schweitzer IB, Smith E, Harrison DJ *et al.* Reducing exposure to laboratory animal allergens. *Comp Med* 2003; 53:487–92.
 - 68 Papes F, Logan DW, Stowers L. The vomeronasal organ mediates interspecies defensive behaviors through detection of protein pheromone homologs. *Cell* 2010; 141:692–703.
 - 69 Derewenda U, Li J, Derewenda Z *et al.* The crystal structure of a major dust mite allergen Der p 2, and its biological implications. *J Mol Biol* 2002; 318:189–97.
 - 70 Johannessen BR, Skov LK, Kastrup JS *et al.* Structure of the house dust mite allergen Der f 2: implications for function and molecular basis of IgE cross-reactivity. *FEBS Lett* 2005; 579:1208–12.
 - 71 Thomas WR, Hales BJ, Smith WA. Structural biology of allergens. *Curr Allergy Asthma Rep* 2005; 5:388–93.
 - 72 Trompette A, Divanovic S, Visintin A *et al.* Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. *Nature* 2009; 457:585–8.
 - 73 Piggott DA, Eienbarth SC, Xu L *et al.* MyD88-dependent induction of allergic Th2 responses to intranasal antigen. *J Clin Invest* 2005; 115:459–67.
 - 74 Holt PG, Batty JE, Turner KJ. Inhibition of specific IgE responses in mice by pre-exposure to inhaled antigen. *Immunol* 1981; 42:409–17.
 - 75 Park BS, Song DH, Kim HM, Choi BS, Lee H, Lee JO. The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. *Nature* 2009; 458:1191–5.
 - 76 Ichikawa S, Takai T, Yashiki T *et al.* Lipopolysaccharide binding of the mite allergen Der f 2. *Genes Cells* 2009; 14:1055–65.
 - 77 Re F, Strominger JL. Separate functional domains of human MD-2 mediate Toll-like receptor 4-binding and lipopolysaccharide responsiveness. *J Immunol* 2003; 171:5272–6.
 - 78 Tsai JJ, Yi FC, Chua KY, Liu YH, Lee BW, Cheong N. Identification of the major allergenic components in *Bloimia tropicalis* and the relevance of the specific IgE in asthmatic patients. *Ann Allergy Asthma Immunol* 2003; 91:485–9.
 - 79 Liao EC, Ho CM, Lin MY, Tsai JJ. *Dermatophagoides pteronyssinus* and *Tyrophagus putrescentiae* allergy in allergic rhinitis caused by cross-reactivity not dual-sensitization. *J Clin Immunol* 2010; 30:830–9.
 - 80 Park JW, Ko SH, Yong TS, Ree HI, Jeoung BJ, Hong CS. Cross-reactivity of *Tyrophagus putrescentiae* with *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* in urban areas. *Ann Allergy Asthma Immunol* 1999; 83:533–9.
 - 81 van Hage-Hamsten M, Johansson SG, Johansson E, Wiren A. Lack of allergenic cross-reactivity between storage mites and *Dermatophagoides pteronyssinus*. *Clin Allergy* 1987; 17:23–31.
 - 82 Esch RE. Allergen immunotherapy: what can and cannot be mixed? *J Allergy Clin Immunol* 2008; 122:659–60.
 - 83 Chou H, Tam MF, Lee LH *et al.* Vacuolar serine protease is a major allergen of *Cladosporium cladosporioides*. *Int Arch Allergy Immunol* 2008; 146:277–86.
 - 84 Poll V, Denk U, Shen HD *et al.* The vacuolar serine protease, a cross-reactive allergen from *Cladosporium herbarum*. *Mol Immunol* 2009; 46:1360–73.
 - 85 Sudha VT, Arora N, Gaur SN, Pasha S, Singh BP. Identification of a serine protease as a major allergen (Per a 10) of *Periplaneta americana*. *Allergy* 2008; 63:768–76.
 - 86 Chuang JG, Su SN, Chiang BL, Lee HJ, Chow LP. Proteome mining for novel IgE-binding proteins from the German cockroach (*Blattella germanica*) and allergen profiling of patients. *Proteomics* 2010; 10:3854–67.
 - 87 McGlade JP, Gorman S, Lenzo JC *et al.* Effect of both ultraviolet B irradiation and histamine receptor function on allergic responses to an inhaled antigen. *J Immunol* 2007; 178:2794–802.
 - 88 Sokol CL, Barton GM, Farr AG, Medzhitov R. A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nat Immunol* 2008; 9:310–8.
 - 89 Sokol CL, Chu NQ, Yu S, Nish SA, Laufer TM, Medzhitov R. Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nat Immunol* 2009; 10:713–20.
 - 90 Tang H, Cao W, Kasturi SP *et al.* The T helper type 2 response to cysteine proteases requires dendritic cell-basophil cooperation via ROS-mediated signaling. *Nat Immunol* 2010; 11:608–17.
 - 91 Cunningham PT, Elliot CE, Lenzo JC *et al.* Sensitizing and Th2 adjuvant activity of cysteine protease allergens. *Int Arch Allergy Immunol* 2012; 158:347–58.
 - 92 Gough L, Schulz O, Sewell HF, Shakib F. The cysteine protease activity of the major dust mite allergen Der p 1 selectively enhances the immunoglobulin E antibody response. *J Exp Med* 1999; 190:1897–902.
 - 93 Kikuchi Y, Takai T, Kuhara T *et al.* Crucial commitment of proteolytic activity of a purified recombinant major house dust mite allergen Der p1 to sensitization toward IgE and IgG responses. *J Immunol* 2006; 177:1609–17.
 - 94 Gough L, Campbell E, Bayley D, Van Heeke G, Shakib F. Proteolytic activ-

- ity of the house dust mite allergen Der p 1 enhances allergenicity in a mouse inhalation model. *Clin Exp Allergy* 2003; 33:1159–63.
- 95 Shakib F, Ghaemmaghami AM, Sewell HF. The molecular basis of allergenicity. *Trends Immunol* 2008; 29:633–42.
 - 96 Stewart GA, Kollinger MR, King CM, Thompson PJ. A comparative study of three serine proteases from *Dermatophagoides pteronyssinus* and *D. farinae*. *Allergy* 1994; 49:553–60.
 - 97 Zhang J, Saint-Remy JM, Garrod DR, Robinson C. Comparative enzymology of native and recombinant house dust mite allergen Der p 1. *Allergy* 2009; 64:469–77.
 - 98 Ino Y, Ando T, Haida M *et al.* Characterization of the proteases in the crude mite extract. *Int Arch Allergy Appl Immunol* 1989; 89:321–6.
 - 99 Yan Z, Banerjee R. Redox remodeling as an immunoregulatory strategy. *Biochem* 2010; 49:1059–66.
 - 100 Takai T, Kato T, Sakata Y *et al.* Recombinant Der p 1 and Der f 1 exhibit cysteine protease activity but no serine protease activity. *Biochem Biophys Res Comm* 2005; 328:944–52.
 - 101 Colbert JD, Matthews SP, Miller G, Watts C. Diverse regulatory roles for lysosomal proteases in the immune response. *Eur J Immunol* 2009; 39:2955–65.
 - 102 Sharp FA, Ruane D, Claass B *et al.* Uptake of particulate vaccine adjuvants by dendritic cells activates the NALP3 inflammasome. *Proc Nat Acad of Sci* 2009; 106:870–5.
 - 103 Dai X, Sayama K, Tohyama M *et al.* Mite allergen is a danger signal for the skin via activation of inflammasome in keratinocytes. *J Allergy Clin Immunol* 2011; 127:806–14.
 - 104 Schwarz G, Boehncke WH, Braun M *et al.* Cathepsin S activity is detectable in human keratinocytes and is selectively upregulated upon stimulation with interferon-gamma. *J Invest Dermatol* 2002; 2002(119):44–9.
 - 105 Ben-Sasson SZ, Hu-Li J, Quiel J *et al.* IL-1 acts directly on CD4 T cells to enhance their antigen-driven expansion and differentiation. *Proc Nat Acad Sci* 2009; 106:7119–24.
 - 106 Nakanishi K, Tsutsui H, Yoshimoto T. Importance of IL-18-induced super Th1 cells for the development of allergic inflammation. *Allergol Int* 2010; 59:137–41.
 - 107 Bryson K, Besteiro S, McGachy HA, Coombs GH, Mottram JC, Alexander J. Overexpression of the natural inhibitor of cysteine peptidases in *Leishmania mexicana* leads to reduced virulence and a Th1 response. *Infect Immun* 2009; 77:2971–8.
 - 108 Donnelly S, O'Neill SM, Stack CM *et al.* Helminth cysteine proteases inhibit TRIF-dependent activation of macrophages via degradation of TLR3. *J Biol Chem* 2010; 285:3383–92.
 - 109 Huber JP, Ramos HJ, Gill MA, Farrar JD. Cutting edge: Type I IFN reverses human Th2 commitment and stability by suppressing GATA3. *J Immunol* 2010; 185:813–7.
 - 110 Krummen M, Balkow S, Shen L *et al.* Release of IL-12 by dendritic cells activated by TLR ligation is dependent on MyD88 signaling, whereas TRIF signaling is indispensable for TLR synergy. *J Leuk Biol* 2010; 88:189–99.
 - 111 Harn DA, McDonald J, Atochina O, Da'dara AA. Modulation of host immune responses by helminth glycans. *Immunol Rev* 2009; 230:247–57.
 - 112 Wills-Karp M. Current Allergen-specific pattern recognition receptor pathways. *Curr Opin Immunol* 2010; 22:777–82.
 - 113 Barrett NA, Rahman OM, Fernandez JM *et al.* Dectin-2 mediates Th2 immunity through the generation of cysteinyl leukotrienes. *J Exp Med* 2011; 208:593–604.
 - 114 Muller U, Stenzel W, Kohler G *et al.* IL-13 induces disease-promoting type 2 cytokines, alternatively activated macrophages and allergic inflammation during pulmonary infection of mice with *Cryptococcus neoformans*. *J Immunol* 2007; 179:5367–77.
 - 115 Gomez-Garcia L, Rivera-Montoya I, Rodriguez-Sosa M, Terrazas LI. Carbohydrate components of *Taenia crassiceps* metacystodes display Th2-adjuvant and anti-inflammatory properties when co-injected with bystander antigen. *Parasitol Res* 2006; 99:440–8.
 - 116 Fairweather D, Cihakova D. Alternatively activated macrophages in infection and autoimmunity. *J Autoimmun* 2009; 33:222–30.
 - 117 Daley JM, Brancato SK, Thomay AA, Reichner JS, Albina JE. The phenotype of murine wound macrophages. *J Leuk Biol* 2010; 87:59–67.
 - 118 Kim J, Hematti P. Mesenchymal stem cell-educated macrophages: a novel type of alternatively activated macrophages. *Exp Hematol* 2009; 37:1445–53.
 - 119 Kurowska-Stolarska M, Stolarski B, Kewin P *et al.* IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. *J Immunol* 2009; 183:6469–77.
 - 120 Chieppa M, Bianchi G, Doni A *et al.* Cross-linking of the mannose receptor on monocyte-derived dendritic cells activates an anti-inflammatory immunosuppressive program. *J Immunol* 2003; 171:4552–60.
 - 121 Deslée G, Charbonnier AS, Hammad H *et al.* Involvement of the mannose receptor in the uptake of Der p 1, a major mite allergen, by human dendritic cells. *J Allergy Clin Immunol* 2002; 110:763–70.
 - 122 Royer PJ, Emara M, Yang C *et al.* The mannose receptor mediates the uptake of diverse native allergens by dendritic cells and determines allergen-induced T cell polarization through modulation of IDO activity. *J Immunol* 2010; 185:1522–31.
 - 123 Emara M, Royer PJ, Abbas Z *et al.* Recognition of the major cat allergen Fel d 1 through the cysteine-rich domain of the mannose receptor determines its allergenicity. *J Biol Chem* 2011; 286:13033–40.
 - 124 Okano M, Kino K, Takishita T *et al.* Roles of carbohydrates on Cry j 1, the major allergen of Japanese cedar pollen, in specific T-cell responses. *J Allergy Clin Immunol* 2001; 108:101–8.
 - 125 Maeda M, Kamamoto M, Hino K *et al.* Glycoform analysis of Japanese cedar pollen allergen, Cry j 1. *Biosci Biotech Biochem* 2005; 69:1700–5.
 - 126 Koistinen H, Koistinen R, Seppala M, Burova TV, Choiset Y, Haertle T. Glycodelin and beta-lactoglobulin, lipocalins with a high structural similarity, differ in ligand binding properties. *FEBS Lett* 1999; 450:158–62.
 - 127 Vrtala S, Mayer P, Ferreira F *et al.* Induction of IgE antibodies in mice and rhesus monkeys with recombinant birch pollen allergens: different allergenicity of Bet v 1 and Bet v 2. *J Allergy Clin Immunol* 1996; 98:913–21.

- 128 Adel-Patient K, Nahori MA, Proust B *et al.* Elicitation of the allergic reaction in beta-lactoglobulin-sensitized Balb/c mice: biochemical and clinical manifestations differ according to the structure of the allergen used for challenge. *Clin Exp Allergy* 2003; 33:376–85.
- 129 Martinez-Pomares L, Linehan SA, Taylor PR, Gordon S. Binding properties of the mannose receptor. *Immunobiol* 2001; 204:527–35.
- 130 Chruszcz M, Chapman MD, Vailes LD *et al.* Crystal structures of mite allergens Der f 1 and Der p 1 reveal differences in surface-exposed residues that may influence antibody binding. *J Mol Biol* 2009; 386:520–30.
- 131 Seppala U, Hagglund P, Wurtzen PA *et al.* Molecular characterization of major cat allergen Fel d 1: expression of heterodimer by use of a baculovirus expression system. *J Biol Chem* 2005; 280:3208–16.
- 132 Wicklein D, Lindner B, Moll H *et al.* Carbohydrate moieties can induce mediator release: a detailed characterization of two major timothy grass pollen allergens. *Biol Chem* 2004; 385:397–407.
- 133 Napoli A, Aiello D, Di Donna L, Moschidis P, Sindona G. Vegetable proteomics: the detection of Ole e 1 isoallergens by peptide matching of MALDI MS/MS spectra of underivatized and dansylated glycopeptides. *J Proteome Res* 2008; 7:2723–32.
- 134 Li M, Gustchina A, Glesner J *et al.* Carbohydrates contribute to the interactions between cockroach allergen Bla g 2 and a monoclonal antibody. *J Immunol* 2011; 186:333–40.
- 135 Lienard D, Tran Dinh O, van Oort E *et al.* Suspension-cultured BY-2 tobacco cells produce and mature immunologically active house dust mite allergens. *Plant Biotech J* 2007; 5:93–108.
- 136 van Oort E, Lerouge P, de Heer PG *et al.* Substitution of Pichia pastoris-derived recombinant proteins with mannose containing O- and N-linked glycans decreases specificity of diagnostic tests. *Int Arch Allergy Immunol* 2004; 135:187–95.
- 137 Wopfner N, Gadermaier G, Egger M *et al.* The spectrum of allergens in ragweed and mugwort pollen. *Int Arch Allergy Immunol* 2005; 138:337–46.
- 138 Gajhede M, Osmark P, Poulsen FM *et al.* X-ray and NMR structure of Bet v 1, the origin of birch pollen allergy. *Nat Struct Biol* 1996; 3:1040–5.
- 139 Kaiser L, Velickovic TC, Badia-Martinez D *et al.* Structural characterization of the tetrameric form of the major cat allergen Fel d 1. *J Mol Biol* 2007; 370:714–27.
- 140 Saarelainen S, Rytönen-Nissinen M, Rouvinen J *et al.* Animal-derived lipocalin allergens exhibit immunoglobulin E cross-reactivity. *Clin Exp Allergy* 2008; 38:374–81.
- 141 Tan YW, Chan SL, Ong TC *et al.* Structures of two major allergens, Bla g 4 and Per a 4, from cockroaches and their IgE binding epitopes. *J Biol Chem* 2009; 284:3148–57.
- 142 Mueller GA, Gosavi RA, Krahn JM *et al.* Der p 5 crystal structure provides insight into the group 5 dust mite allergens. *J Biol Chem* 2010; 285:25394–401.
- 143 Mueller GA, Edwards LL, Aloor JJ *et al.* The structure of the dust mite allergen Der p 7 reveals similarities to innate immune proteins. *J Allergy Clin Immunol* 2010; 125:909–17.
- 144 Shen HD, Tam MF, Huang CH *et al.* Homology modeling and monoclonal antibody binding of the Der f 7 dust mite allergen. *Immunol Cell Biol* 2011; 89:225–30.
- 145 Yennawar NH, Li LC, Dudzinski DM, Tabuchi A, Cosgrove DJ. Crystal structure and activities of EXPB1 (Zea m 1), a beta-expansin and group-1 pollen allergen from maize. *Proc Natl Acad Sci* 2006; 103:14664–71.
- 146 Shani N, Shani Z, Shoseyov O, Mruwat R, Shoseyov D. Oxidized cellulose binding to allergens with a carbohydrate-binding module attenuates allergic reactions. *J Immunol* 2011; 186:1240–7.
- 147 Seyedarabi A, To TT, Ali S *et al.* Structural insights into substrate specificity and the anti beta-elimination mechanism of pectate lyase. *Biochem* 2010; 49:539–46.
- 148 Liu Z, Bhattacharyya S, Ning B *et al.* Plant-expressed recombinant mountain cedar allergen Jun a 1 is allergenic and has limited pectate lyase activity. *Int Arch Allergy Immunol* 2010; 153:347–58.
- 149 Tan AM, Chen HC, Pochard P, Eisenbarth SC, Herrick CA, Bottomly HK. TLR4 signaling in stromal cells is critical for the initiation of allergic Th2 responses to inhaled antigen. *J Immunol* 2010; 184:3535–44.
- 150 McFadden JP, Dearman RJ, White JM, Basketter DA, Kimber I. The Hapten-Atopy hypothesis II: the 'cutaneous hapten paradox'. *Clin Exp Allergy* 2011; 41:327–37.
- 151 Gringhuis SI, den Dunnen J, Litjens M, van der Vlist M, Geijtenbeek TB. Carbohydrate-specific signaling through the DC-SIGN signalosome tailors immunity to Mycobacterium tuberculosis, HIV-1 and Helicobacter pylori. *Nature Immunol* 2009; 10:1081–8.
- 152 Gazi U, Martinez-Pomares L. Influence of the mannose receptor in host immune responses. *Immunobiol* 2009; 214:554–61.
- 153 Emara M, Royer PJ, Mahdavi J, Shakib F, Ghaemmaghami AM. Retagging identifies dendritic cell-specific intercellular adhesion molecule-3 (ICAM3)-grabbing non-integrin (DC-SIGN) protein as a novel receptor for a major allergen from house dust mite. *J Biol Chem* 2012; 287:5756–63.
- 154 Epton MJ, Smith W, Hales BJ, Hazell L, Thompson PJ, Thomas WR. Non-allergenic antigen in allergic sensitization: responses to the mite ferritin heavy chain antigen by allergic and non-allergic subjects. *Clin Exp Allergy* 2002; 32:1341–7.
- 155 O'Neil SE, Heinrich TK, Hales BJ *et al.* The chitinase allergens Der p 15 and Der p 18 from Dermatophagoides pteronyssinus. *Clin Exp Allergy* 2006; 36:831–9.
- 156 Hales BJ, Elliot CE, Chai LY *et al.* Quantitation of IgE binding to the chitinase and chitinase-like house dust mite allergens Der p 15 and Der p 18 compared to the major and mid-range allergens. *Int Arch Allergy Immunol* 2012; 160:233–40.
- 157 Platts-Mills TA, Woodfolk JA. Allergens and their role in the allergic immune response. *Immunol Rev* 2011; 242:51–68.
- 158 Oseroff C, Sidney J, Kotturi MF *et al.* Molecular determinants of T cell epitope recognition to the common Timothy grass allergen. *J Immunol* 2010; 185:943–55.
- 159 Brown JS, Wilson WE, Grant LD. Dosimetric comparisons of particle deposition and retention in rats and humans. *Inhal Toxicol* 2005; 17:355–85.