

The molecular basis of allergenicity

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Allergens are mostly innocuous antigens that elicit powerful T helper cell type 2 (Th2) responses leading to hyper-immunoglobulin E (IgE) production and allergy. Research carried out over several years has highlighted the possible role of the inherent protease activity, surface features and glycosylation patterns of allergens in the engagement of a Th2 signalling pathway. It is thought that allergens possess common features and patterns that enable them to be recognized by innate immune defences as Th2-inducing antigens. These events are further amplified by proteolytically active allergens through digestion of cell surface molecules involved in regulating innate and adaptive immune functions, favouring Th2 responses. A greater understanding of the molecular features that make proteins allergenic will help define new therapeutic targets aimed at blocking allergen recognition and protease activity.

Introduction

Type I hypersensitivity, or allergy, is the clinical manifestation of an immune response against foreign protein molecules, commonly known as allergens, which are potent inducers of immunoglobulin E (IgE) synthesis [1,2]. Allergic reactions consist of a series of events that start with recognition of the native allergen structure by antigen presenting cells, such as dendritic cells (DC), and culminates in IgE antibody production, mast cell sensitization and triggering (Figure 1). Although a large number of allergens have been characterized in recent years, there is still uncertainty about the structural, functional or biochemical features that these molecules have in common, and that could explain their ability to provoke powerful IgE antibody responses. It has previously been suggested that allergenicity might be because of an intrinsic biochemical property of the molecule, and on that basis allergens were classified into a few broad biochemical groups including enzymes and enzyme inhibitors, transport proteins, regulatory proteins and allergens with biochemical activities that do not fit into any of the previous categories [3]. However, it is difficult to imagine how such relatively diverse biochemical activities could all lead to the polarization of T helper (Th) cells towards Th2 and subsequent IgE synthesis. There is, therefore, considerable interest in defining the unique features that render particular proteins allergenic, as an important first step in any attempt to understand the nature of the initial trigger for allergic responses and the subsequent development of effective intervention strategies.

Studies carried out over the past few years have defined three important intrinsic features that might, either singly or collectively, render proteins allergenic, and these are, (i) protease activity, (ii) surface features and (iii) glycosylation patterns. Much of these advances have been made possible by structural studies (i.e. crystallization) and the use of modern computational methods, in conjunction with empirical analysis, which have helped define new immunologically relevant substrate targets for proteolytically active allergens and identified surface features that could represent allergen-specific molecular patterns. This review is an attempt to draw together the different molecular attributes of allergens, excluding small molecules and haptens, into a single unifying mechanism that leads to Th2 responses and allergy.

As will become evident later, the powerful role of allergens, which are usually innocuous inhaled (e.g. mite allergens) or ingested (e.g. peanut allergens) molecules, in initiating Th2 responses resides in their ability to target the innate defences of the body (mainly DC), which then sets the stage for further amplification and perpetuation of the pro-allergic cascade via allergen-modified T cells, B cells and possibly regulatory T (Treg) cells. Thus, allergens seem to employ a distinct set of mechanisms that uniquely endow them with the ability to bypass the tolerogenic fate normally exhibited by most other non-allergenic proteins coming into contact with the immune system via the mucosa. Collectively, these observations represent an important conceptual advance in our understanding of how Th2 responses are initiated and maintained by allergens and highlight the role of intrinsic properties of allergens, other than their immunogenic non-self structural presentation, in driving immune responses.

Protease activity of allergens

The incidence of allergic conditions such as asthma has reached epidemic proportions throughout the world [2]. The allergens that cause these inflammatory reactions are proteins that elicit powerful IgE antibody responses, but what is it that makes these innocuous molecules provoke a highly deleterious immune response, and which from an evolutionary point of view provides no survival advantage? For certain allergens, it has been suggested that properties other than immunogenic non-self structural presentation could contribute to their allergenicity (e.g. enzymatic activity) [4,5]. This is best exemplified by the archetypal allergen *Dermatophagoides pteronyssinus* 1 (Der p 1) from the house dust mite species *D. pteronyssinus*. This protein is a cysteine protease and, in addition to being intrinsically immunogenic, has been shown via its proteolytic activity to target cells of the innate and adaptive immune systems

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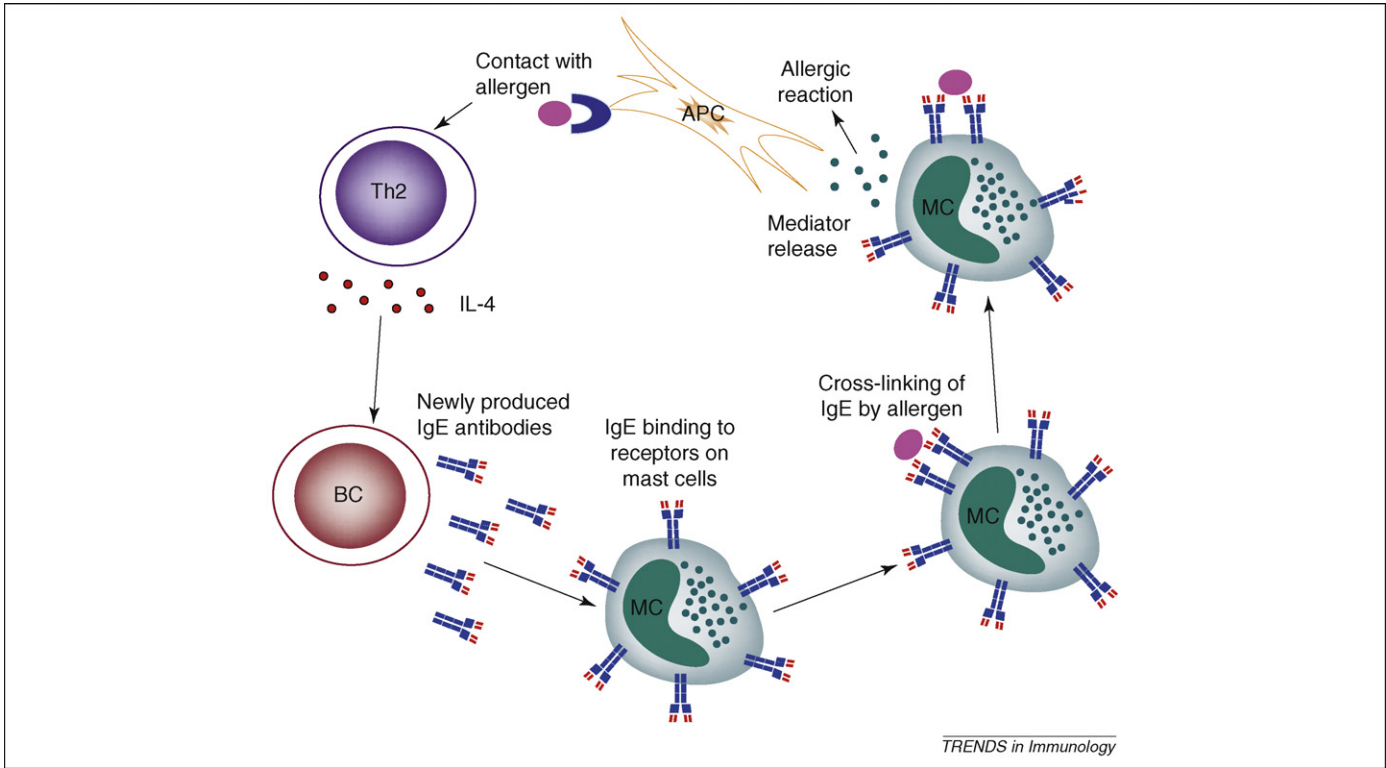


Figure 1. The classic pathway for the allergic cascade. Allergen recognition and processing by antigen presenting cells (APC) leads to Th2 polarization, IL-4 production and subsequent switching to IgE synthesis by B cells (BC). The binding of secreted IgE to mast cells (MC) via specific Fc receptors (i.e. FcεRI) and subsequent cross linking of receptor-bound IgE by allergen triggers the release of mediators and the onset of allergy.

(Table 1), and in doing so subvert the regulatory processes controlling IgE synthesis, thereby favouring hyper-IgE production and allergy [4,5] (Figure 2).

Der p 1 is found in mite faecal pellets, which because of their small size (10–20 μm) become easily airborne and inhaled. The proteolytic activity of Der p 1 in the human host is likely to be influenced by several factors including concentration in lung tissues, pH optimum, stability and susceptibility to inactivation by anti-Der p 1 antibodies [5]. This most clinically important allergen accounts for ~20% of the total protein content of the faecal pellets (i.e. 0.1 ng/

pellet) [6], and there is no doubt that chronic exposure [7], which is characteristic of dust mite sensitivity, would result in the cumulative build up and continuous replenishment of very high concentrations of Der p 1 in lung tissues (i.e. possibly approaching the microgram range). The activation state of the cysteine protease activity of Der p 1 is likely to be maintained by the high concentrations of glutathione (which acts as a reducing agent for Cysteine in the Der p 1 active site) found in airway lining fluid [8,9]. Der p 1 is also optimally active at neutral pH [10] and is resistant to autoproteolysis at body temperature. We also

Table 1. Immunologically relevant substrate targets of the protease allergen Der p 1

Target molecule	Cellular location	Cleavage site	Immunological effect	Refs
SP-A	Lung epithelium	Multiple	Blockade of anti-allergic effect	[12]
SP-D	Lung epithelium	Multiple	Blockade of anti-allergic effect	[12]
Occludin	Lung epithelium	Unknown	Increased permeability	[21]
Protein ZO-1	Lung epithelium	Unknown	Increased permeability	[21]
α1-antitrypsin	Lung epithelium	D30-A31	Upset protease-antiprotease	[24,25]
		D36-T37		
		E378-A379		
Unknown	Lung epithelium	Unknown	Increased IL-8 release	[72]
Unknown	Mast cell and basophil	Unknown	Mediator release	[28]
CD40	Dendritic cells	Unknown	Diminished IL-12 and thiol secretion	[39,40]
DC-SIGN	Dendritic cells	A87-V88	Loss of ICAM-3 binding	[41]
		A110-V111		
		A248-V249		
DC-SIGNR	Lymph node endothelium	A99-V100	Loss of ICAM-3 binding	[41]
		A122-V123		
		A260-F261		
CD25	T cells	Unknown	Less IFN-γ and more IL-4 production	[44,45]
CD23	B cells	S155-S156	Upset IgE regulation	[24,36,54]
		E298-S299		
Unknown	Keratinocyte	Unknown	Increased IL-8 and GM-CSF release	[69,70]
Unknown	Airway smooth muscle	Unknown	Enhanced constrictor response	[33]

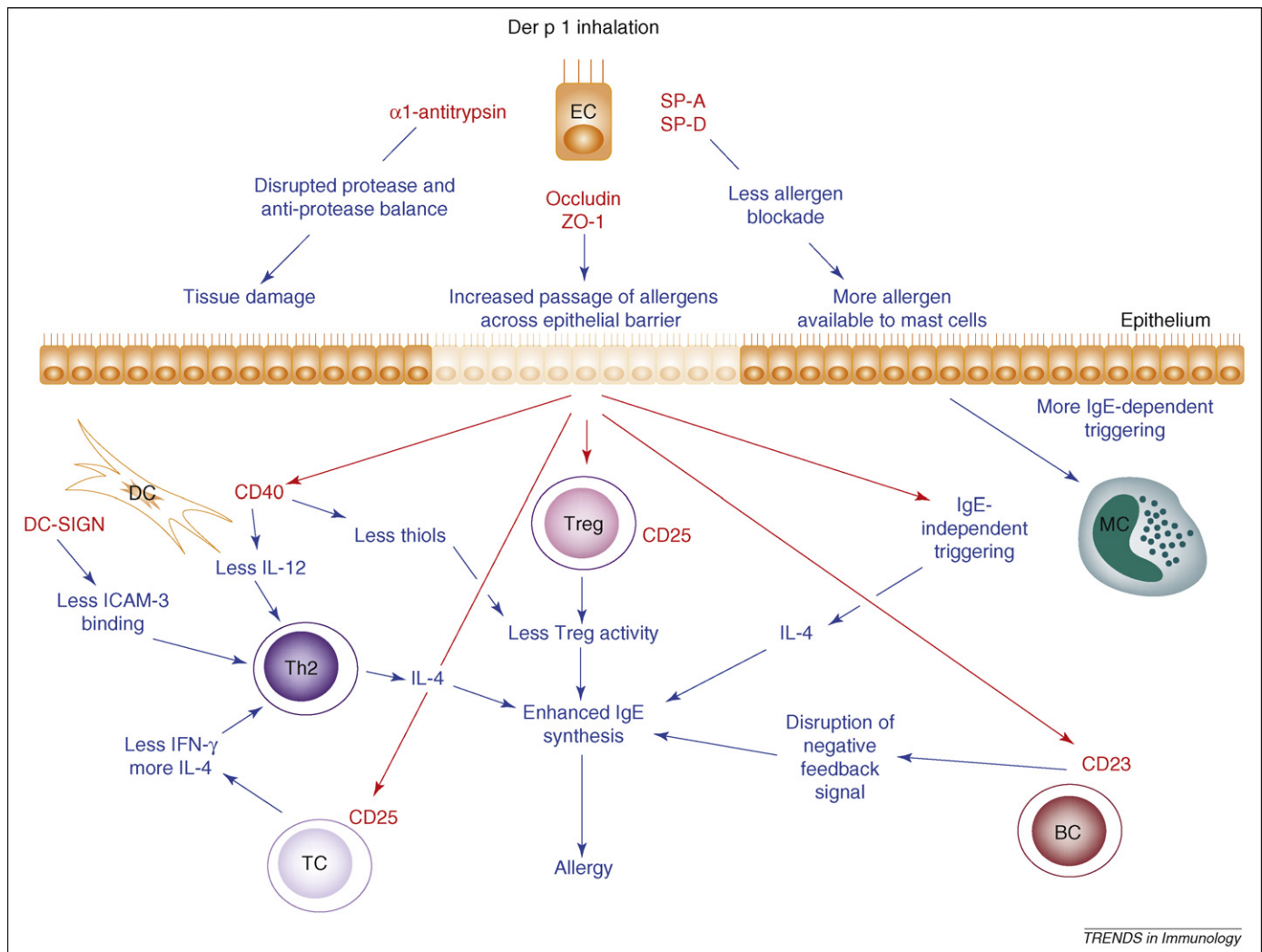


Figure 2. The protease activity of the mite allergen Der p 1 subverts the regulatory process controlling IgE synthesis, favouring hyper-IgE production and allergy. Proteolytic digestion of epithelial cell (EC) surface molecules by inhaled Der p 1 disrupts the epithelial barrier, bringing Der p 1, and potentially other allergens, into contact with dendritic cells (DC), mast cells (MC), T cells (TC), T regulatory cells (Treg) and B cells (BC). Proteolytic digestion of surface molecules on these target cells leads to uncontrolled IgE production and allergy. Proteolytic targets and processes are indicated in red and their immunological consequences in blue.

have evidence that a substantial component of the human IgE anti-Der p 1 response is directed against a region of Der p 1 (Leu147-Gln160) that is remote from its enzyme active site [11] and that Der p 1 retains a substantial amount of its cysteine protease activity even when complexed with antibody [5]. Collectively, these observations indicate that the respiratory mucosa is exposed to high concentrations of Der p 1, the proteolytic activity of which is well adapted to the local tissue microenvironment.

The innate immune system

Recent work has demonstrated that the cysteine protease activity of two important mite allergens, Der p 1 and *D. farinae* 1 (Der f 1), degrades and inactivates the lung surfactant proteins (SP) -A and -D [12], which are calcium-dependent carbohydrate-binding proteins with multiple innate immune functions, such as bacterial agglutination and modulation of leukocyte functions [13,14]. More importantly, in mouse models of house dust mite [15,16] and *Aspergillus fumigatus* [17] allergy, SP-A and SP-D have been shown to have anti-allergic effects, the underlying mechanism for which involves binding to

inhaled allergens, stopping them from reaching free and high-affinity receptor (FcεRI)-bound IgE and resulting in inhibition of mast cell activation [18–20]. Thus, allergen-driven degradation and consequent inactivation of SP-A and SP-D is likely to interfere with their anti-allergic effects. The protective effect of SP-A and SP-D is thought to be mediated by binding to the glycan moieties of allergens, in a calcium-dependent fashion, through their carbohydrate recognition domains (CRDs), with consequent blockade of allergen-triggered mast cell and basophil degranulation [18–20].

Der p 1 has been shown to facilitate its own passage across the epithelial barrier by digesting occludin and ZO-1 (components of intercellular tight junctions) [21,22] and disruption of cellular architecture [23], thereby increasing the permeability of the bronchial mucosa to macromolecules. Furthermore, we [24] and others [25] have shown that Der p 1 completely cleaves α1-antitrypsin (an anti-protease) the function of which is to protect the respiratory mucosa against serine proteases, an activity exhibited by at least two other clinically relevant mite allergens (i.e. Der p 3 and Der p 9) [26]. Thus, the inactivation of α1-anti-

trypsin by Der p 1 is likely to upset the local protease-antiprotease balance and enhance the activity of endogenous serine proteases, especially those from neutrophils (elastase and cathepsin G), leading to further tissue damage. Therefore, these observations offer a plausible mechanism whereby Der p 1 would facilitate its own passage, and that of other allergens (i.e. a bystander effect), across the epithelium and gain access to cells of the immune system. In addition to exposure via inhalation, some allergens are known to breach the skin barrier function and this is likely to provide a portal of entry for such allergens leading to skin inflammation. It is, therefore, interesting in this connection that a recombinant Der f 1 preparation exhibited a protease-dependent ability to disrupt the barrier function of the skin in nude mice [27], a finding with obvious clinical implications within the context of allergen-driven dermatological disease.

Previous work has shown that Der p 1 induces mast cell and basophil degranulation and stimulates interleukin-4 (IL-4) synthesis and secretion by these cells independently of IgE and FcεRI cross-linking [28]. These actions of Der p 1, which were mediated by its proteolytic activity, could induce IgE production because mast cells and basophils support IgE synthesis by secreting IL-4 and IL-13 and provide contact-mediated help for B cells through interactions of CD40 with CD40 ligand [29,30]. IgE-independent mast cell and basophil activation might be mediated by protease-activated receptors (PARs), and this could be the underlying mechanism for the induction of bee venom phospholipase A2-specific IgE in mice given intraperitoneal injections of enzymatically active phospholipase A2 [31]. These observations indicate that direct mast cell degranulation by enzymatically active allergens might create an IL-4 enriched environment and this will help trigger an IgE response at the onset of an immune response.

The cysteine proteases Der f 1 and papain (another cysteine protease allergen commonly used as a tenderiser in the meat industry) have also been shown to use their protease activity to induce the release of pro-inflammatory mediators from eosinophils, possibly via a PAR₂-independent mechanism [32], and this is interesting given the important role of eosinophils in the onset of inflammatory changes in the lungs of allergic asthma patients. Within the context of allergic lung pathology, Der p 1 was shown to induce changes in airway smooth muscle responses, which were associated with mitogen-activated protein kinase signalling pathways, and again this was because of its cysteine protease activity [33].

As indicated earlier, some of the more salient cellular targets of Der p 1, particularly those on DC (e.g. DC-SIGN), were initially identified using new *in silico* methods which utilize knowledge of the protease specificity of Der p 1 to predict potential substrates within the human proteome [34]. Much of this effort was based on data derived from the use of synthetic peptide substrates [10] and substrate libraries, in conjunction with functional proteomic micro-arrays [35], which showed that Der p 1, unlike papain, has a distinct secondary substrate specificity, in which cleavage is influenced by the amino acid residue in the P₂ position (Figure 3). This, together with the characteriz-

ation of cleavage sites within protein substrates such as human α1-antitrypsin [25], CD23 [36], the insulin β-chain [37] and the Der p 1 pro-polypeptide sequence [38], indicates that Der p 1 has a preference for small hydrophobic residues (e.g. Valine or Alanine) in the P₂ position, charged residues (e.g. Glutamine or Arginine) in the P₁ position and small hydrophilic or hydrophobic residues (e.g. Serine or Alanine) in the P₁' position (Figure 3). Given their exquisite accuracy, it is impossible to overestimate the importance of such computational methods [34], as powerful timesaving tools, in gaining a global view of the full range of potentially relevant immunological substrates of Der p 1 that could eventually be corroborated by empirical analysis.

The demonstration of the cleavage of CD40 on human monocyte-derived DC by Der p 1 [39] provided the first insight into how Der p 1 might influence the behaviour of these cells, such that they support Th2 responses (Figure 2). Thus, DC that have been matured in the presence of proteolytically active Der p 1 produced significantly less of the pivotal Th1 differentiating cytokine IL-12 (p70, the biologically active form), compared with those that have been matured in the presence of proteolytically inactive Der p 1 [39]. The suppression of IL-12 production was because of the cleavage of CD40 by the protease activity of Der p 1, hence rendering the DC less responsive to stimulation through the CD40L-CD40 signalling pathway, which is important for IL-12 production and Th1 differentiation [39]. Furthermore, DC that have been matured in the presence of proteolytically active Der p 1 induced the production of significantly less interferon gamma (IFN-γ) and more IL-4 by naïve CD4 T cells, compared to DC that have been matured in the presence of proteolytically inactive Der p 1. Another serious consequence of Der p 1-induced degradation of CD40 is reduced production of extracellular thiols by DC, and this could further polarize naïve T cells towards Th2 because thiols seem to be more important for the generation of Th1 and Tc1 (type 1 cytotoxic T cells), than Th2 and Tc2 cells [40]. These observations clearly demonstrate how a proteolytically active allergen such as Der p 1 directly conditions DC such that they support Th2 responses.

In silico digestion of human DC surface proteins, using the prediction of protease specificity (PoPS) bioinformatics tool [34], in conjunction with cellular *in vitro* analysis and cleavage site determination, has revealed two further Der

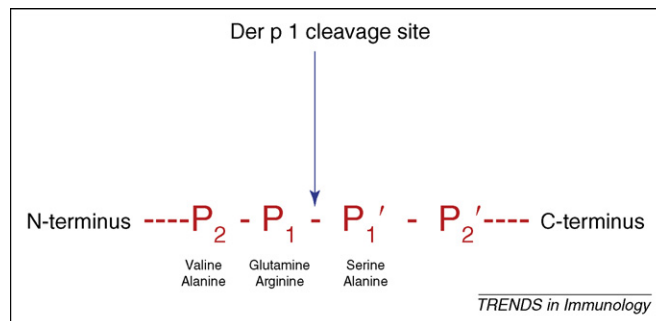


Figure 3. Cleavage site specificity (i.e. amino acid preference) of the cysteine protease allergen Der p 1. Data were derived from the use of synthetic peptides, substrate libraries and characterization of cleavage sites within known protein substrates.

p 1 targets, DC-SIGN (CD209) and DC-SIGNR (CD299 and also known as L-SIGN), two C-type lectins implicated mostly in pathogen trafficking [41]. DC-SIGN and DC-SIGNR are closely related transmembrane receptors expressed within immunological compartments such as DC and macrophage subsets (DC-SIGN) and liver sinusoid and lymph node endothelium (DC-SIGNR). *In vitro* treatment of DC-SIGN and DC-SIGNR-expressing cells with Der p 1 led to loss of detectable surface DC-SIGN and DC-SIGNR [41]. Digestion of purified soluble recombinant DC-SIGN and DC-SIGNR, followed by N-terminal sequencing and mass spectrometry, indicated in each case one main cleavage site and several minor sites, the former correlating with Der p 1 enzymology and the folded state of the substrate proteins. Loss of DC-SIGN from the cell surface led to reduced binding of intercellular adhesion molecule-3 (ICAM-3), an endogenous DC-SIGN ligand expressed on naïve T cells that is thought to be involved in Th1 cytokine signalling [42,43].

Clearly, Der p 1-induced degradation of CD40 [39] and DC-SIGN [41], and the knock-on effects on downstream events within T cells, provide compelling evidence for the role of the protease activity of Der p 1 in influencing the decision making process of DC. It is becoming increasingly evident that DC functions are strongly influenced by the total pattern of signals transduced by the array of receptors they possess. It is, therefore, highly likely that modification of the expression of DC surface proteins, such as CD40 [39] and DC-SIGN [41], via exogenous proteases could have the potential to dramatically affect the control of immune responses in tissue areas of high protease allergen exposure. More generally, allergen-driven proteolysis of proteins on epithelial cells [12,21–25], mast cells, basophils [28], eosinophils [32] and DC [39–41] represents a powerful mechanism by which enzymatically active allergens influence the innate immune system, and in doing so bias the adaptive immune response in favour of Th2.

The adaptive immune system

Der p 1 proteolytically cleaves the α chain of the IL-2 receptor (CD25) on human T cells [44], and as a result, these T cells show a marked decrease in proliferation and IFN- γ production in response to stimulation by anti-CD3 antibody (Figure 2). In subsequent work [45], we demonstrated that the proteolytic activity of Der p 1 directly biases human CD4 and CD8 T cells towards a type 2 cytokine profile, characterized by higher IL-4 and lower IFN- γ production. T cells that have been in contact with proteolytically active Der p 1 (and have hence lost a significant proportion of their surface CD25 [44,45]), subsequently washed extensively and then co-cultured with autologous B cells, show significant augmentation of IgE synthesis caused by diminished IFN- γ production [46], which is reminiscent of what we see in allergic individuals. These data provide further compelling evidence for the role of the proteolytic activity of Der p 1 in creating a micro-environment conducive for IgE synthesis. Clearly, the proteolytic degradation of CD25 on Treg cells, which also require IL-2 stimulation for their growth in the periphery [47], could potentially lead to further amplification of the pro-allergic cascade.

Within the context of Treg cells, a recent study indicated that a Der p 1-induced depletion of thiol production by DC, after CD40 cleavage, could possibly lead to down-regulation of Treg-cell activity because thiols seemed to induce the Treg-cell phenotype (i.e. TGF- β 1 producing CD25^{high} FOXP3⁺ T cells) in naïve human T cells [40], a population which has been shown to suppress immune responses *in vitro* [47] and *in vivo* [48]. If such thiol-induced CD25^{high} FOXP3⁺ Treg cells can be shown to inhibit T-cell proliferation, the suppression of their activity by the protease action of Der p 1 could interfere with the control of IgE-mediated airway hyper responsiveness by mucosal Treg cells [48].

Recent evidence indicates that the significant increase in the incidence of allergy over the past few decades can be attributed to a decrease in microbial burden during childhood, the so-called 'hygiene hypothesis' [49–52], and this can be interpreted on the basis of two immunological mechanisms which might operate synergistically. The first is based on lack of immune deviation from Th2 to Th1 cells, and the second on reduced Treg-cell activity [53]. Thus, the earlier mentioned observations provide new insights as to how Der p 1, via its proteolytic activity, could perpetuate these deleterious processes and in doing so evoke exuberant IgE responses, which are characteristic of allergic responses.

The low-affinity receptor for IgE, also called CD23, was the first immunologically relevant cell surface molecule shown to be degraded by Der p 1 [24,54] and in many ways acted as the gateway for the subsequent revelation that CD25 [44,45], CD40 [39], DC-SIGN and DC-SIGNR [41] and SP-A and SP-D [12] are further legitimate targets for the proteolytic activity of Der p 1. It has been shown that Der p 1 cleaves CD23 from the surface of cultured human B cells [24,54]. A more detailed investigation of CD23 cleavage by Der p 1 used both a recombinant 40 kilodalton (kDa) protein, representing the entire extracellular portion of human CD23 (Asp48-Ser321), and B-cell surface-expressed CD23 [36]. This study found that Der p 1 cleaves CD23 at two sites (Ser155-Ser156 and Glu298-Ser299) to produce a 143 residue 17 kDa fragment, commonly known as monomeric derCD23 [55,56] and containing the lectin domain and part of the C-terminal tail [36]. B-cell membrane-bound CD23 is a negative regulator of IgE synthesis [1,57], and the implication of the Der p 1-induced cleavage of CD23 is that it could potentially upregulate IgE synthesis by disrupting the negative feedback signal, which is thought to be delivered by IgE binding to membrane CD23. Interestingly, recent work has shown that monomeric derCD23 inhibits IgE synthesis in human B cells after IgE heavy chain switching [56].

An interesting line of evidence with regard to possible lectin involvement in allergy is that within the human genome, the DC-SIGN and DC-SIGNR genes map to a cluster on chromosome 19p13, adjacent to CD23 [58]. Structurally, all three proteins are type II transmembrane proteins and possess domains belonging to the C-type lectin family. It has been speculated that DC-SIGN, DC-SIGNR and CD23 have evolved from an ancestral gene involved in the regulation of Th2-type immunity. In keeping with this notion, DC-SIGN expression on DC and CD23

expression on B cells are known to be upregulated by the two canonical Th2 cytokines, IL-13 [59] and IL-4 [57], respectively.

The natural progression from the human *in vitro* experiments discussed earlier was to demonstrate the role of the cysteine protease activity of Der p 1 in enhancing IgE production in an animal model. In a series of experiments, conducted in CBA/J [60,61] and Balb/c [62] mice, it became clear that exposure of these animals to proteolytically active Der p 1, via the intraperitoneal [60,61] or inhalation [62] routes, led to a significant and selective increase in total and Der p 1-specific IgE, compared to animals exposed to Der p 1 that had been rendered proteolytically inactive by prior treatment with the cysteine protease inhibitor, E-64. A more recent series of experiments using recombinant rather than native Der p 1, provided further evidence for the role of the protease activity of Der p 1 in eliciting IgE, but in this case also immunoglobulin G (IgG), antibody responses in mice [63]. The exact mechanism by which protease allergens, such as Der p 1, favour Th2 responses and IgE production in mouse models, and the initial source of Th2-inducing cytokines, remained unresolved until recently. However, a very recently published study using mice [64] has clearly demonstrated that basophils are activated and recruited to the draining lymph nodes in response to challenge with the cysteine protease allergen papain, and that basophils are the accessory cells required for Th2 responses after exposure to protease allergens. The Th2-inducing cytokines produced by basophils included IL-4 and thymic stromal lymphopoietin, which are known to support Th2 differentiation [64].

Co-injection of ovalbumin, as a bystander antigen, with proteolytically active Der p 1 led to a significant and selective increase in ovalbumin-specific IgE, compared to animals given ovalbumin in conjunction with proteolytically inactive Der p 1 [61]. These observations clearly demonstrate the adjuvant effect of the protease activity of Der p 1, which not only enhances IgE antibody responses to itself but also to other allergens present in the micro-environment. Therefore, Der p 1 might play a central role in conditioning the microenvironment within target tissues to one that is pro-allergic, and thus aids in the initiation and propagation of the allergic cascade.

The important role played by endogenous proteases, such as matrix metalloproteases, in innate and adaptive immune responses is well known [65] and many such proteases, particularly serine proteases, induce their effects via PARs [66]. This, therefore, raises the possibility that exogenous protease allergens might work through a similar mechanism, and this is certainly true for Der p 1 [67] and the two mite serine proteases, Der p 3 and Der p 9 [68], which are known to target PAR₂ for the induction of pro-inflammatory mediators from respiratory epithelial cells [65,67]. A similar PAR-mediated mechanism might also be responsible for the ability of Der p 1 and Der f 1 to induce the release of IL-8 and granulocyte-macrophage colony stimulating factor (GM-CSF) from keratinocytes [69,70], and for the ability of Asp f 13, the serine protease allergen from *A. fumigatus*, to induce inflammatory airway responses [71]. However, the cysteine protease allergen Der p 1 remains relatively unique (even when compared to

the mite serine protease allergens Der p 3 and Der p 9) in its additional ability to directly influence innate and adaptive immune responses through cleavage of cell surface molecules such as SP-A and SP-D [12], occludin [21] and α 1-antitrypsin [24,25] on epithelial cells, CD40 [39] and DC-SIGN [41] on DC, CD25 on T cells [44,45] and CD23 on B cells [24,36,54]. Another reported function of the protease activity of Der p 1 (but not Der p 3) that is also PAR₂-independent, is its ability to stimulate the release of IL-8 from human airway epithelial cells [72].

Surface features of allergens

The identification of common functional and structural features among allergens is of considerable importance to the basic understanding of allergic sensitization and to the development of effective immune-intervention strategies. The previous section highlighted the role of the cysteine protease activity of the mite allergen Der p 1 in subverting the regulatory process controlling Th2 responses and IgE synthesis.

The grass pollen allergen *Phleum pratense* 1 (Phl p 1) also exhibits cysteine protease activity and is a remote homologue of cysteine proteases of the papain family [73]. Interestingly, however, Phl p 1 does not possess the characteristic catalytic triad found in the enzyme active site of Der p 1 (Cys34, His170 and Asn190) [38]. This, therefore, prompted the search for other remote homologues of Der p 1, which have sequences similar to those surrounding the catalytic cysteine residue, Cys34, in Der p 1 (i.e. Met19-Leu48). This 30 amino acid stretch of Der p 1 forms the interdomain groove, with an α -helix on one side and an anti-parallel β sheet on the other [74,75], and is the most conserved sequence in Der p 1 and other cysteine proteases. Sequence comparisons using PROSEARCH, a search tool based on amino acid composition rather than order, and studies of available 3D structures revealed that this structural α - β motif is also present in allergens of diverse sources, including those not known to have protease activity [76]. This, therefore, raises the possibility that the groove (i.e. the α - β motif) could provide functions beyond accommodating the protease active site such as, for example, recognition by cells of the innate immune system.

Other studies have used different algorithms to try to predict what might be considered as unique molecular characteristics of allergens [77,78], but these have only searched for sequence motifs, rather than common surface features. An earlier study indicated that most allergens could be classified into four structural families, but again it fell short of demonstrating any common surface features [79]. A more recent *in silico* study of IgE cross-reactivity concluded that most plant food allergens belong to a small number of structural families [80], but clearly any discussion of cross-reactive IgE epitopes is beyond the scope of this section that is primarily concerned with allergen-specific surface features recognizable by innate immune cells.

Analysis of the surface of diverse allergens, using bioinformatic approaches, could still prove to be rewarding because this might reveal common structural features enabling them to be recognized as Th2-inducing antigens by antigen presenting cells, such as DC. Thus, using the

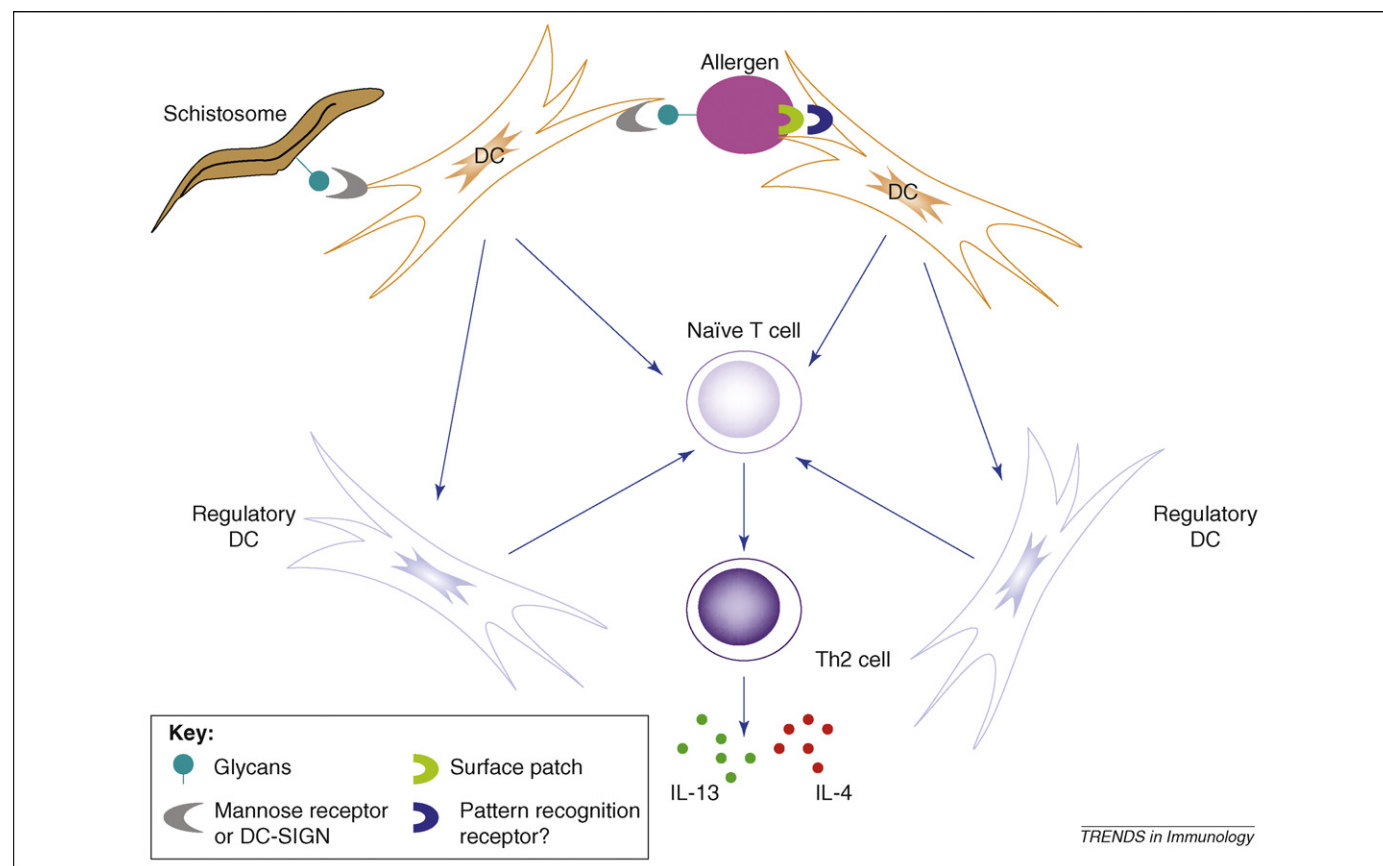


Figure 4. Surface patches and glycans on allergens engage allergen-specific pattern recognition receptors or C-type lectins (mannose receptor and DC-SIGN) on DC, respectively, leading to Th2 responses and/or the generation of a 'regulatory' DC that supports a Th2 signalling pathway. It is thought that glycans on schistosome helminth parasites are also recognized by C-type lectins on DC, enabling them to use a similar mechanism for stimulating Th2 responses.

ConSurf server [81] to search for allergen-specific motifs, enabled the identification of residue conservation patterns in the homologues of Ara t 8 (plant profilin), Act c 1 (actinidin), Bet v 1 (plant pathogenesis-related protein) and Ves v 5 (venom allergen) [82]. The results show the presence of allergen-specific patches consisting of an unusually high proportion of surface-exposed hydrophobic residues, which is in keeping with the notion that the innate immune system might have evolved to detect hydrophobic parts of immunogenic proteins [83]. These authors [83] have argued that what makes a protein recognizable as an allergen by antigen presenting cells is its hydrophobic nature, as illustrated by allergens such as lipocalins, lipid transfer proteins and seed storage proteins [83]. The hydrophobic parts of such proteins are normally buried, but they can become exposed upon unfolding, such as after the loss of a specific ligand that is integrated into their 3D structure. The relevance to allergen recognition and Th2 responses of the hydrophobic patches identified will clearly need to be validated by mutagenesis experiments, in conjunction with studying downstream DC and T-cell responses (Figure 4). Clearly, any future bioinformatic studies of the kind described here should perhaps focus on allergens and non-allergens that come into contact with the immune system via the mucosa because this will probably better explain the non-tolerogenic nature of allergens compared to non-allergens entering through this route.

Glycosylation patterns of allergens

The search for common allergenic features recognizable by innate immune cells, such as DC, will no doubt be facilitated, and indeed simplified, by identifying the cell surface receptor(s) involved. The mannose receptor (CD206), an endocytic C-type lectin involved in the internalization of specific sugar-bearing antigens, is so far the only known receptor involved in the uptake of Der p 1 by DC. In line with a previous report [84], we have demonstrated that the mannose receptor ligand, mannan, inhibits the internalization of Der p 1 and that targeted downregulation of mannose receptor expression on human DC is associated with diminished Der p 1 uptake by these cells. We, therefore, believe that the mannose receptor binds directly to Der p 1 and that this interaction requires the presence of carbohydrates in Der p 1 and is probably mediated by the carbohydrate-recognition domains of the mannose receptor. The biological relevance of these observations to downstream events could potentially be established by studying T-cell responses induced by mannose receptor-deficient, compared to mannose receptor-sufficient, DC in response to Der p 1. Such data would clearly be in agreement with a previous study showing that engagement of the mannose receptor by selected ligands on human DC leads to the induction of 'regulatory' DC favouring Th2 polarization [85].

The earlier mentioned observations are clearly in line with the demonstration that glycan structures present in the peanut glycoallergen Ara h 1 have Th2 inducing

characteristics [86]. Thus, native, but not deglycosylated, Ara h 1 was shown to activate human monocyte-derived DC and induce IL-4 and IL-13 secreting Th2 cells [86]. The internalization of Ara h 1 was calcium and glycan-dependent and was mediated by DC-SIGN [86]. Interestingly, non-mammalian glycans from helminthic parasites are also known to have Th2 stimulating properties. For instance, schistosome antigens have been shown to bind to C-type lectins on DC, including DC-SIGN and mannose receptor, such that the DC are conditioned to support a Th2 response [87].

Collectively, these observations seem to indicate that carbohydrates displayed by glycoallergens (and some helminths) could have unique structures and/or orientations and that recognition by the mannose receptor (Der p 1) [84] and DC-SIGN (Ara h 1) [86] on DC would set the stage for Th2 polarization and allergy (Figure 4). This is clearly an area of great interest and promise in trying to better understand how allergen-stimulated DC elicit Th2 responses and how this process might be targeted by novel therapeutics [88].

Conclusions

Allergic diseases are complex conditions with strong environmental and genetic components [89]. The clinical manifestation of allergy in the increasing number of individuals who are susceptible is clearly driven by recurrent exposure and repeated 'neo-sensitisation' to allergens. Allergens are innocuous antigens and are powerful inducers of Th2 responses and hyper-IgE production. As has become clear from this review, allergens are most likely recognized by innate immune cells through common molecular features, potentially their glycosylation pattern and/or surface characteristics, which enable them to engage an undesirable Th2 signalling pathway. However, an increasing number of allergens are also proteases, and it is this intrinsic activity that seems to drive the most exuberant allergic responses, through wholesale digestion of cell surface molecules involved in the homeostatic control of innate, Th1, Th2 and IgE responses, as key events in the amplification of the allergic cascade. However, the fact remains that other allergens might still elicit strong Th2 responses without being proteases, and this is certainly true for cockroach allergens, which are an important cause of asthma in North America [90].

It will hopefully have become evident from this review that computer aided prediction can provide vital shortcuts to identifying allergenic properties that could ultimately be tested experimentally. However, despite the many successful examples cited here, it is also important to highlight some of the limitations of *in silico* approaches, particularly within the context of predicting protease cleavage sites. One such limitation is the apparent discrepancy seen in some cases between predicted and empirical Der p 1 cleavage, which might well have been caused by occlusion of cleavage sites because of ordered secondary structure in the target protein [41]. This, therefore, highlights the need for better and more sophisticated *in silico* tools for unravelling biological mechanisms.

Knowledge of the fundamental mechanisms underlying allergic sensitization is becoming clearer and the next few

years should, therefore, see a better understanding of the special molecular features exhibited by allergens, their cell surface receptors and the signalling molecules involved. It is hoped that these efforts will help define new ways of effectively blocking allergic sensitization at the point of initial contact with the immune system.

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