



For reprint orders, please contact: reprints@futuremedicine.com

Factors influencing the allergenicity and adjuvanticity of allergens

IgE-mediated allergic disorders affect up to 25% of the population in industrialized countries and result in a Th2-polarized immune response to innocuous environmental proteins, so-called allergens. Among a large number of proteins to which humans are exposed to, only a minute fraction are allergens. This observation suggests that allergens share special features of allergenicity (i.e., the capacity to induce the production of specific IgE antibodies in susceptible individuals). However, the question 'what makes a protein allergenic' still remains unanswered although some biochemical characteristics of allergens and their capacity to interact with the innate immune system could be associated with their allergenic potential. Allergen-specific immunotherapy aims at an alteration of the disease-eliciting immune response by repeated administration of allergens. Recently, approaches emerged to endow allergens with adjuvanticity, in particular aiming at an increase of their immunomodulatory capacity. This article summarizes factors of allergenicity and introduces recent concepts of adjuvanticity to improve allergen-specific immunotherapy.

KEYWORDS: adjuvants ■ allergen ■ allergenicity ■ allergen-specific immunotherapy ■ allergy ■ vaccine design

This article will address two 'hot topics' in allergy research, namely allergenicity and adjuvanticity of allergens, in order to reveal what makes proteins allergenic and how allergens can be modified to improve vaccines for allergen-specific immunotherapy. Allergens are harmless, nonpathogenic agents that usually do not elicit relevant immune responses in the majority of human individuals. However, in certain individuals, allergens induce the production of specific IgE antibodies that may mediate clinical reactions. The ability to induce IgE production is termed 'allergenicity'. This article will give an overview on different features of allergens that may determine their allergenicity. However, we need to point out that none of the discussed characteristics can presently be regarded as a common factor for allergenicity. Most of the so-far described characteristics also apply to various proteins that do not cause allergy. The second part of the article covering 'adjuvanticity' will introduce scientific concepts on how to modify allergens in order to increase and guide their immunological potential. In particular, the addition of intrinsic adjuvanticity to allergens by engineering recombinant allergen–adjuvant fusion proteins is a promising approach for vaccine development in allergen-specific immunotherapy.

Type I allergy

During the past decades, allergic diseases have tremendously increased and today, IgE-mediated hypersensitivity reactions of the immune system affect more than 25% of the population in industrialized countries. Atopy-prone individuals develop IgE antibodies specific for allergens. These soluble allergen-specific IgE antibodies bind to the high-affinity Fc-ε receptor (FcεRI) expressed on the surface of effector cells, such as basophils and mast cells. Upon repeated allergen encounter, surface-bound IgE antibodies on sensitized effector cells are cross-linked, resulting in activation and immediate mediator release. Among several inflammatory mediators, histamine is the main cause of the early clinical symptoms characteristic for IgE-mediated allergic manifestations (e.g., rhinitis, conjunctivitis or airway obstruction).

The development of IgE-mediated responses to allergens requires a series of molecular and cellular interactions that involve both the innate and the adaptive immune system (FIGURE 1). Allergens have to pass through the epithelial barriers in the respiratory and gastrointestinal tract or through the skin. They may also pass the conjunctival epithelium, as recently demonstrated by light and electron microscopy [1]. Then, allergens are internalized and processed

**Stephan Deiftl¹
& Barbara Bohle^{*1}**

¹Christian Doppler Laboratory for Immunomodulation, Department of Pathophysiology & Allergy Research, Center for Pathophysiology, Infectiology & Immunology, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria

^{*}Author for correspondence:

Tel.: +43 140 400 5114

Fax: +43 140 400 5130

barbara.bohle@meduniwien.ac.at

future
medicine part of fsg

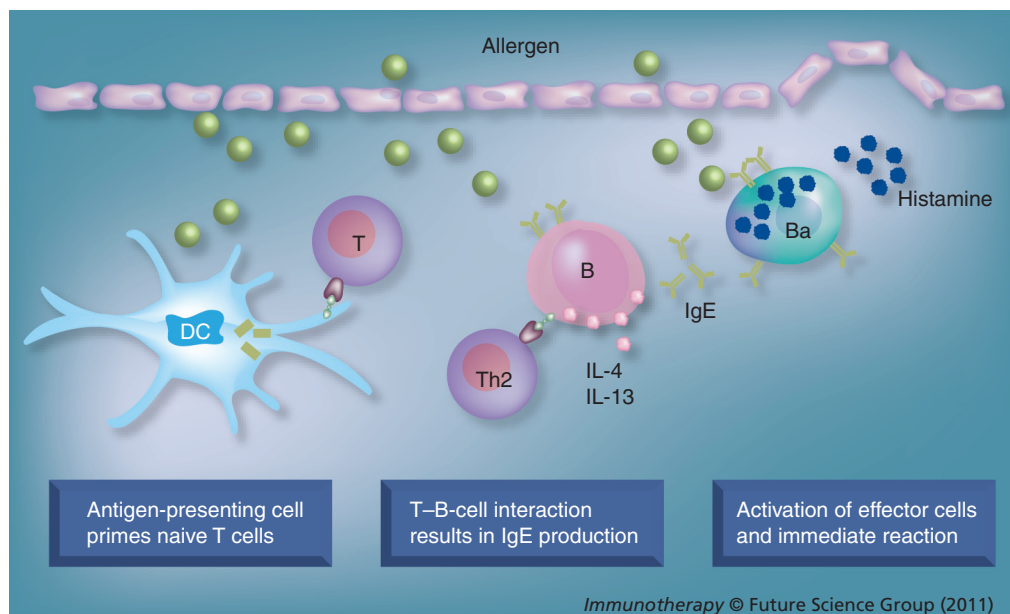


Figure 1. Development of IgE-mediated responses to allergens.

B: B cell; Ba: Basophil; DC: Dendritic cell; T: CD4⁺ T cell.

by antigen-presenting cells (APCs). Currently, dendritic cells (DC) are considered to be the most potent APCs that act as a linker of innate and adaptive immune responses. Immature DC efficiently take up and process antigens. They also express a variety of pattern-recognition receptors (PRRs) on their surface that recognize conserved pathogen-associated molecular patterns, which in turn serve as danger signals for the immune system. Upon activation via PRRs, DCs produce cytokines and upregulate the surface expression of MHC class I and II molecules and costimulatory molecules such as CD40, CD80 and CD86. Activated DCs migrate to lymph nodes where they present epitopes contained in peptides bound to MHC class II molecules to naive CD4⁺ T lymphocytes. Selected T helper cells with the T-cell receptor specific for the presented peptides (signal 1) are activated. Signal 2 is provided by costimulatory molecules on the surface of DC and necessary for full T-cell activation leading to proliferation (i.e., clonal expansion) and cytokine production. Certain cytokines (signal 3) present in the microenvironment during T-cell priming determine the differentiation of naive T cells into different effector cell subsets. Th2 cells represent one subset of allergen-specific CD4⁺ T cells that synthesize high amounts of the cytokines IL-4, IL-13 and IL-5, but little or no IFN- γ . Other subsets of CD4⁺ T cells are Th1 cells that produce high levels of the signature cytokine IFN- γ , a potent antagonist of IL-4; and CD4⁺ Treg cells. The latter actively

suppress effector T cells either by cell–cell contact or by the immunosuppressive cytokines IL-10 and TGF- β [2,3].

In atopic individuals, the inadequate balance between allergen-specific Th2 cells by Th1 and Treg cells promotes an aberrant Th2-dominated response to allergens. Allergen-specific Th2 cells have been identified in the peripheral blood and nasal mucosa of patients suffering from allergic rhinitis, as well as in the bronchial alveolar lavage fluid of allergic individuals with asthma and in skin lesions of allergic patients with atopic dermatitis [4–7]. The differentiation of Th2 cells is driven by the presence of IL-4 during their priming [8]. IL-4 is also an important growth factor for Th2 cells. Both IL-4 and IL-13 are important switch factors for B cells to produce IgE antibodies. Upon interaction with allergen-specific Th2 cells, B lymphocytes with corresponding allergen-specific B-cell receptors develop into plasma cells and produce allergen-specific IgE antibodies. As B cells express the low-affinity receptor for IgE (Fc ϵ R2, CD23) on their surface, they can act as APCs that effectively internalize allergen–IgE complexes by receptor-mediated uptake [9]. The interaction between B and T lymphocytes again promotes the induction of Th2 responses [10,11]. In addition to their pivotal role for IgE production that mediates the early inflammatory response in allergy, allergen-specific Th2 cells are also involved in late-phase responses [12,13]. Late-phase reactions are characterized by recruitment, activation and tissue infiltration of T lymphocytes, eosinophils,

basophils and neutrophils. The important role of allergen-specific Th2 cells in late-phase responses was demonstrated by the observation that intradermal administration of short, non-IgE-binding T-cell-activating peptides derived from the major cat allergen Fel d 1 elicited late asthmatic responses in cat-allergic patients [14]. Thereby, it was also demonstrated that T-cell-dependent late-phase reactions may even occur in the absence of an early IgE/mast cell-dependent response [15]. In summary, allergen-specific Th2 cells play a fundamental role in the induction and maintenance of IgE-mediated allergy. Recently, new subsets of Th cells, such as Th17 and Th9 cells, have been reported to influence airway hypersensitivity reactions and asthma in humans [16,17]. These subsets may play a role in the chronic phase of allergic inflammation and tissue remodelling.

Allergen-specific immunotherapy

Allergen-specific immunotherapy (SIT) is the only causative treatment for IgE-associated allergy. SIT consists of repeated administration of increasing doses of allergens to allergic patients with the aim of inducing clinical tolerance to natural allergen exposure [18]. Clinically, SIT improves the quality of life of treated individuals and has been shown to reduce both symptoms of allergy and medication use in controlled clinical trials at the population level in a high number of treated individuals [19,20]. SIT is associated with improved tolerance to allergen challenge, with a decrease in immediate-phase and late-phase allergic inflammation. Moreover, SIT prevents the development of new allergic sensitizations and reduces progression of allergic rhinitis to asthma. Of note, SIT modulates the allergic immune response and thereby shows long-term action that exceeds the treatment period [21,22]. Immunologically, SIT increases the ratio of Th1 to Th2 cytokines [23,24]. In addition, the disease-eliciting Th2 response is downregulated, characterized by the reduced proliferative and cytokine responses in response to allergen stimulation in SIT-treated individuals *in vitro* [23]. *In vivo*, the reduced allergen-specific T-cell response is mirrored by reduced late-phase responses [25,26]. The downregulation of the allergen-specific Th2 response has been attributed to the appearance of allergen-specific Treg cells that produce IL-10 and/or TGF- β [24,25,27–29]. Both cytokines are important switch factors for B cells and promote the production of Ig class switching to IgA, IgG₁ and IgG₄ [30,31]. Since the levels of allergen-specific IgG₄ antibodies rise dramatically during

SIT, they have been considered as ‘blocking’ antibodies that compete with IgE for allergen binding [32]. Consistent with this hypothesis, the removal of IgG₄ antibodies from sera of individuals who had received SIT resulted in an almost complete loss of the inhibition of binding of allergen–IgE complexes [28]. In summary, SIT alters the allergen-specific T-cell response from the Th2-like towards a more Th0/1-like response and induces Treg cells, which has consequences for the production of allergen-specific ‘blocking’ antibodies. Apart from this modulation of the adaptive immune response to allergens, SIT also modulates the function of APCs and effector cells [21] (e.g., reduction of the number of mast cells and their ability to release mediators). The recruitment of eosinophils and neutrophils to sites of allergen exposure is also reduced during SIT.

Allergenicity

In this article, we define allergenicity as the property of being able to induce a Th2 response and subsequent production of allergen-specific IgE antibodies. Usually, the presence of allergen-specific IgE in serum is paralleled by the property of an allergen to induce clinical reactions. However, there are circumstances where this is not the case. If IgE antibodies only react with a single epitope; no cross-linking of IgE bound to the surface of effector cells can occur. Furthermore, IgE antibodies specific for carbohydrates on glycosylated allergens exist and cause problems in *in vitro* test systems for allergy diagnosis due to broad cross-reactivity [33]. On the other hand, in many cases, IgE antibodies specific for carbohydrate determinants of allergens do not induce mediator release [34,35].

Complex allergen sources contain only a limited number of allergens that induce IgE antibodies in more than 50% of the allergic individuals, so-called ‘major’ allergens. ‘Minor’ allergens are less frequently recognized. However, the definition of major and minor allergens may differ between diverse populations of patients and should always be studied in a sufficiently high number of individuals. In view of the limited number of major allergens, one is tempted to assume that they share common characteristics that provide them with allergenic potential. Intense efforts have been made to identify common features and physicochemical parameters, such as the isoelectric point (pI value), hydrophobicity, protein stability, structural elements, molecular surface motifs, glycosylation, dimerization/oligomerization and enzymatic activity have been suggested. In addition, allergenicity

may also depend on the capacity of allergens to bind lipids or to activate the innate immune system through signaling pathways of PRR, such as Toll-like receptors (TLRs) or C-type lectin receptors. Another interesting concept suggested that a lack of homology to bacterial proteins could be a common denominator for allergenicity [36]. The sequence comparison of allergens and bacterial proteins revealed that, in contrast to randomly selected control protein sequences, allergens have no or very few bacterial homologs. Consequently, allergens might be recognized by the human immune system differently from proteins with related bacterial homologs. Some examples of features suggested to underlie the phenomenon of allergenicity will be illustrated in more detail below.

Enzymatic activity

Several allergens with cysteine and serine protease activity have been cloned from house dust mites, including Der p 1, Der p 3, Der p 6 and Der p 9, and a significant body of evidence suggests that the enzymatic activity is important for their allergenicity. For example, the cysteine protease Der p 1 has been shown to be able to cleave tight junctions, thereby facilitating allergen penetration at epithelial surfaces [37]. In addition, Der p 1 has been demonstrated to cleave various receptors on cells (i.e., the α -subunit of the IL-2 receptor [CD25] and the low-affinity IgE receptor [CD23]) [38], as well as the costimulatory molecule CD40 and C-type lectins expressed by DCs [39]. Moreover, Der p 1 can activate protease-activated receptor-2 in keratinocytes [40–42] and respiratory epithelial cells [43,44] and surfactant-associated proteins (SP-A and SP-D) in the lung [45]. All of these receptors are involved in the initiation or augmentation of Th2 responses. In *in vivo* experiments in mice, enzymatically active Der p 1 induced higher lung inflammatory responses as compared with enzymatically inactive Der p 1 [46]. Furthermore, intranasal administration of Der p 1 and the homologous protease papain induced IL-25 (also known as IL-17E), which has been identified as one of the initiators of Th2 responses, and thymic stromal lymphopoietin, an IL-7-like cytokine that promotes Th2 differentiation [47]. In synergy, the enzymatic activity of allergens in house dust mite may promote Th2 responses, IgE synthesis and lung inflammation and explain why this allergen source is a very prevalent cause of allergic sensitization and often induces allergic asthma [48,49]. Of note, Der p 1 was demonstrated to directly activate basophils to produce

IL-4, IL-5 and IL-13 [49]. This observation was further confirmed by recent data on papain, which upregulated MHC class II and costimulatory molecules on murine basophils and induced Th2 responses and IgE production in mice [50]. Thus, enzymatic activity of allergens may alter typical type 2 effector cells to relevant APCs that promote the induction of Th2 responses [51].

Together, the previously mentioned findings indicate that the enzymatic activity of allergens has an impact on the epithelial barrier function and various cell types involved in allergy. Apart from enzymatic activity of allergens, nonallergenic proteases in hazelnut and birch pollen were demonstrated to degrade human tear fluid and conjunctival cells [52]. Therefore, it was concluded that such enzymatic compounds in allergen sources may be the reason for seasonal conjunctivitis in individuals without proven allergy. However, it may also be speculated that the enzymatic activity of pollen may reduce the barrier function in other organs than the eye and thereby increase allergen uptake.

Binding of lipids

Many major allergens have been found to be lipid-binding proteins, suggesting that the accompanying lipid cargo may contribute to their allergenicity. For example, the house dust mite allergens Der f 2 and Der p 2 bind lipopolysaccharide (LPS) from bacteria because they show structural similarity with LPS-binding protein [53]. Der p 7, another house dust mite allergen, does not bind LPS but binds the bacterial lipopeptide polymyxin B with weak affinity [54]. Also, the primary amino acid structure of the group 14 allergens in house dust mite clearly defines them as lipid-binding proteins [55]. Other respiratory allergens with lipid binding capacity are Par j 1 and Par j 2, two major allergens in *Parietaria judaica*, which preferentially bind monoacylated negative lipids [56]. The group of nonspecific lipid transfer proteins (nsLTP), which are major allergens in *Rosaceae* fruits in birch-free areas in Southern Europe, were named after their capacity to possess a broad lipid-binding specificity closely related to their 3D structure [57]. Finally, the 3D structure of the major birch pollen allergen Bet v 1 revealed a solvent-accessible cavity that traverses the core of the molecule [58]. In this cavity, Bet v 1 can bind ligands such as fatty acids, flavonoids and cytokinins. By employing the fluorescent sterol dehydroergosterol, first qualitative and quantitative data on the ligand-binding properties of a major allergen have been obtained [59].

In summary, lipid binding can be noted as a very common characteristic of many important groups of allergens. Bacterial lipopeptides bound to allergens may promote Th2 immunity through activation of TLR pathways. In fact, various bacterial and mycobacterial lipopeptides can induce the release of cytokines through interactions with TLR2, which cooperates with TLR1 or TLR6. As shown for peptidoglycan and the synthetic ligand Pam3Cys, stimulation of TLR2 in the presence of allergen enhances Th2 responses in mice [60,61]. LPS from Gram-negative bacteria is recognized by TLR4 and represents a strong danger signal for the human immune system. In general, high amounts of LPS foster the induction of Th1-like responses, whereas low amounts support the induction of Th2-responses [62]. Recently, a critical involvement of TLR4 signaling in the induction of Th2 responses to inhaled antigens was reported [63].

Interestingly, it has been demonstrated that in a humid milieu such as mucosal surfaces of the human body pollen grains release pollen-associated lipid mediators in addition to allergens [64]. Pollen-derived E(1) phytosteranes were found to modulate the function of human DC in a manner that favors the polarization of Th2 cells [65–67]. Although it has not been investigated in detail yet, it may be speculated that pollen allergens with lipid-binding capacity may also bind pollen-associated lipid mediators, which may then contribute to their allergenicity.

Interaction with TLRs

The tertiary structure of group 2 allergens in house dust mites (e.g., Der p 2 and Der f 2) is homologous to MD-2. MD-2 belongs to the MD-2-related lipid-recognition (ML) domain lipid-binding family and is the LPS-binding component of the TLR4 signaling complex essential for the recognition of LPS [68]. Indeed, Der f 2 binds LPS with a nanomolar affinity in a manner similar to the binding by MD-2 [50]. Der p 2 also binds LPS although with lower affinity than MD-2 [69]. Still, Der p 2 was demonstrated to facilitate signaling through direct interactions with the TLR4 complex [70] and could reconstitute LPS-driven TLR4-signaling even in the absence of MD-2. Mirroring these properties, airway sensitization and challenge with Der p 2 led to experimental allergic asthma in wild-type and MD-2-deficient mice, but not TLR4-deficient mice. The fact that also several other allergens are members of the MD-2-like lipid-binding family may suggest that mimicking MD-2 may foster their capacity to activate innate immune responses.

Interaction with non-TLR pathogen recognition receptors

In addition to TLRs, DCs express various other PRRs including the C-type lectin receptors DC-specific ICAM-grabbing nonintegrin (DC-SIGN; CD209), dectin-1 and dectin-2. These non-TLR PRR recognize carbohydrate structures of microbiota and can modulate TLR signaling to tailor immune responses [71]. Most pathogens express carbohydrate structures (e.g., fucosylated glycans or mannose) on their surface, which can be compared with a 'sugar fingerprint' [72]. Of note, glycosylated allergens were also demonstrated to target C-type lectin receptors. The major peanut allergen Ara h 1 was shown to activate human DCs through DC-SIGNs to upregulate MHC class II and costimulatory molecules [73]. These DCs showed an increased ability to drive T-cell proliferation and induced IL-4- and IL-13-secreting Th2 cells two- to three-fold more than immature DCs. Recently, fucosylated structures on other common allergens such as Der p 2 in house dust mite or BG60 (Cyn dBG-60) in bermuda grass pollen were found to functionally interact with DC-SIGNs and its related receptor L-SIGN [74]. The mannose receptor, another C-type lectin receptor, was recently demonstrated to mediate the internalization of diverse allergens from mite (Der p 1 and Der p 2), dog (Can f 1), cockroach (Bla g 2) and peanut (Ara h 1) through their carbohydrate moieties [75]. Of note, silencing the mannose receptor expression on DCs reversed the Th2 polarization driven by exposure to Der p 1. In summary, glycosylated allergens can target C-type lectins in the innate immune system, which in turn may promote the induction of Th2-responses. This hypothesis suggests that both animal and plant carbohydrates may act as Th2 adjuvants.

Dimerization/oligomerization

Apart from the induction of IgE antibodies, an important feature of allergens is their capacity to cross-link specific IgEs bound to high-affinity receptors on the surface of effector cells resulting in the clinical manifestations of IgE-mediated hypersensitivity. In principle, at least two epitopes must be contained in an allergen to cross-link two IgE molecules. However, on the surface of small allergens, this requirement may not always be fulfilled. Another possibility may be the appearance of allergens as dimers/oligomers to induce effector cell activation. Indeed, detailed investigations of 3D structures of allergens revealed that several allergens naturally form dimers or oligomers. The major

allergen from cow's milk Bos d 5 represents a well-studied example of a transient dimer. Its dissociation constant ($K_d = 5 \mu\text{M}$) is so high that depending on its concentration Bos d 5 exists as a mixture of monomers and dimers in solution [76]. The monomeric form of Bos d 5 showed less capacity to induce histamine release from basophils [77]. Similarly, monomeric mutants of the cockroach allergen Bla g 2 induced less mediator release from mast cells than the authentic dimeric Bla g 2 [78]. Natural dimerization was also demonstrated for the major horse allergen Equ c 1 and allergens in grass pollen from *Phleum pratense* and *Dactylis glomerata*, namely Phl p 7 and the group 5 allergens [79–82]. Oligomerization was reported for polcalcins [83], which represent highly cross-reactive ubiquitous calcium-binding pollen allergens [84]. A recent analysis of 55 crystal structures of allergens showed that 80% of them existed in symmetric dimers or oligomers in the crystals and the formation of transient dimers in solution was confirmed at higher protein concentrations by native mass spectrometry [77]. Definitely, the natural occurrence of allergen dimers/oligomers provides a straightforward explanation of how these proteins can accomplish effective cross-linking of IgE and subsequent activation of effector cells. However, the importance of dimerization/oligomerization for the induction of IgE responses still remains an open issue. In this regard, conflicting results for the Bet v 1 family were obtained. One study demonstrated that dimerization is important for the allergenic reactivity of the major birch pollen allergen in mice [85] because the monomer was less capable of activating memory B cells for IgE production *in vivo*. A recent study compared the *in vivo* responses to the monomeric isoform Bet v 1a and Bet v 1d, an isoform with a high tendency to form disulfide-linked aggregates [86]. Bet v 1d activated murine DCs more efficiently than Bet v 1a, which was followed by the production of increased amounts of Th1- as well as Th2-type cytokines. Whereas both isoforms induced similar specific IgE levels, only Bet v 1d induced relevant titers of specific IgG and IgA. This finding suggested that aggregation of allergens induced protective antibody responses. Together, dimers/oligomers of allergens induce stronger immune responses as compared to monomers, possibly due to a 'particle effect' resulting in enhanced internalization by APCs. Whether these altered responses promote the induction of Th2 responses or protective responses needs to be further investigated.

Adjuvanticity

As pointed out previously, several major allergens may have special properties that contribute to their capacity to induce specific IgE responses in susceptible individuals. In SIT, crude allergen extracts are administered to allergic patients to achieve the modulation of the disease-eliciting allergic response towards a more balanced immune response. Because the administration of allergens to allergic patients can induce undesired side effects, conventional SIT is usually started with very low doses of allergen extracts. The steady increase of allergen doses during the early phase of SIT induces tolerance and allows the administration of a clinically effective maintenance dose, which is then continuously applied for 3–5 years. Still, SIT is not effective in every treated patient. To improve the curative efficacy of allergen vaccines, aluminum hydroxide (Alum) is used as adjuvant in European vaccines for subcutaneous SIT. Alum absorbs proteins and slowly releases them *in vivo*, which increases the stability of administered allergens. In addition to this antigen delivery function, Alum triggers innate immune pathways, thereby generating an immunocompetent environment at the injection site. More precisely, Alum activates a protein complex called NLPR3/inflammasome, which is required for correct processing of a number of proinflammatory cytokines [87–89]. Of note, in mouse models of allergy, allergens need to be adsorbed to Alum to achieve the induction of allergen-specific Th2 responses. This observation raised fears that Alum may also foster Th2-like responses in humans. So far, one study has addressed this issue *in vitro*. When human peripheral blood mononuclear cells (PBMCs) were cultured with allergens plus Alum, a significant decrease in both IL-5 and IL-13 production compared with allergen alone, but no changes in IFN- γ or IL-12 production or proliferative responses were observed [90]. Thus, Alum down-regulated allergen-driven Th2 responses while Th1 cytokines were unaffected, indicating that it is a reasonable adjuvant for SIT.

In view of the immunomodulation induced during SIT (i.e., the induction of Th1-like responses and Treg cells resulting in blocking antibodies), the concept emerged to employ new adjuvant substances in allergy vaccines that should particularly promote these desired immune mechanisms. Up to now, two adjuvant substances have been evaluated in clinical trials, monophosphoryl lipid (MPL)-A, a nontoxic component of LPS, and immunostimulatory sequences of bacterial DNA [91]. MPL-A, which is

commonly added to different allergen extracts, is recognized by TLR4 and induces the production of various proinflammatory and inflammatory cytokines including IL-12 and IL-18 that promote Th1 responses. Indeed, mixtures of allergen extracts and MPL-A have been shown to promote allergen-specific Th1-responses in PBMCs from allergic patients [92]. Moreover, patients treated with MPL-A-containing vaccines developed allergen-specific IgG antibodies with blocking capacity [93,94]. Immunostimulatory DNA sequences containing CpG motifs are recognized by TLR9 and induce B-cell activation, Th1 cytokines and the maturation of DCs [95–97]. Also, PBMCs from allergic individuals responded with the production of IL-12, IL-18 and IFN- γ and a reduced IgE synthesis [98]. Studies with immunostimulatory DNA sequences chemically linked to allergens have taught us that allergen–adjuvant conjugates are more effective than mixtures of both components [99]. The increased immunogenicity of allergen–adjuvant conjugates may be referred to allergen uptake and presentation by the same APC that responds to the danger signal. Thereby, the activated APC efficiently primes naive T cells in a cytokine-loaded environment that fosters their differentiation into Th1 and Treg cells, respectively. Immunostimulatory DNA sequences chemically linked to the major ragweed allergen Amb a 1 have been tested in clinical trials but are not commercially available [100].

The idea of linking adjuvants directly to allergens was successfully achieved by applying DNA technology to produce recombinant allergens. Different fusion proteins consisting of recombinant allergens and molecules with adjuvant activity were engineered [101]. For example, allergens were fused with the subunit B of cholera toxin, an efficient mucosal carrier, to support tolerance induction [102] or with diphtheria toxin protein to exert specific toxicity to cells [103]. A big advantage of recombinant fusion proteins is their constant ratio between allergen and adjuvant resulting in persistent immunological activity. Thereby, batch-to-batch variations are avoided. Moreover, some of the fusion proteins showed a reduced IgE-binding capacity as compared with the nonfused allergen. These hypoallergenic proteins should induce less IgE-mediated side effects when administered during SIT. Recently, TLR ligands were also fused with allergens and tested for their immunological features. Two examples of such recombinant allergen–adjuvant fusion proteins will be introduced in the following sections.

Allergen–S-layer fusion proteins

S-layer proteins form crystalline arrays on the cell surface of many bacteria and most archaea and have already a long tradition in vaccine development [104]. Recombinant Bet v 1, the major birch pollen allergen, was fused with the S-layer protein from the nonpathogenic bacteria *Geobacillus stearothermophilus* ATCC 12980 [105]. The resulting allergen–S-layer fusion protein induced IFN- γ and IL-10 production in PBMCs and Bet v 1-specific Th2 clones from birch pollen-allergic donors [106]. Moreover, the fusion protein displayed a reduced IgE-binding capacity and consequently a lower risk to induce adverse vaccine-related anaphylactic reactions. Similarly, a fusion protein consisting of Bet v 1 and a S-layer protein from *Bacillus sphaericus* CCM 2177 showed reduced IgE-binding and mediator-releasing capacity [107]. Because bacterial S-layer proteins target TLR2, allergen–S-layer fusion proteins induced DCs to mature and to synthesize IL-10 and IL-12 [108]. DC maturation initiated by the fusion protein promoted the differentiation of naive CD4⁺ T cells into IFN- γ -producing cells in an IL-12-dependent manner. In parallel, a substantial number of naive T cells developed into IL-10-producing Treg cells that actively suppressed anti-CD3-stimulated T cells. Together, the fusion of an allergen and a bacterial danger signal endowed the allergen with intrinsic capacity to promote the induction of allergen-specific Th1 and Treg cells. In parallel, the IgE-binding capacity of the fusion protein was clearly reduced. The combined immunomodulatory activity and reduced IgE reactivity of allergen–S-layer fusion proteins makes them promising vaccine candidates for future SIT. Recently, allergen–S-layer fusion proteins were successfully expressed in Gram-positive bacteria, thereby facilitating their production as pyrogen-free active substances for allergy vaccines [109].

Allergen–flagellin fusion proteins

The TLR5 ligand flagellin is the main constituent of motility conferring flagella of Gram-positive and negative bacteria [110]. Flagellin has been known as adjuvant for prophylactic and therapeutic immune modulation in various diseases (e.g., virus infection) [111,112]. Intranasal coadministration of flagellin with ovalbumin (OVA), a major allergen in chicken white egg, significantly suppressed OVA-induced airway hyper-responsiveness, airway eosinophilic inflammation and OVA-specific Th2-cytokine responses in OVA-sensitized mice [113]. These results indicated that flagellin can also be considered as an adjuvant for

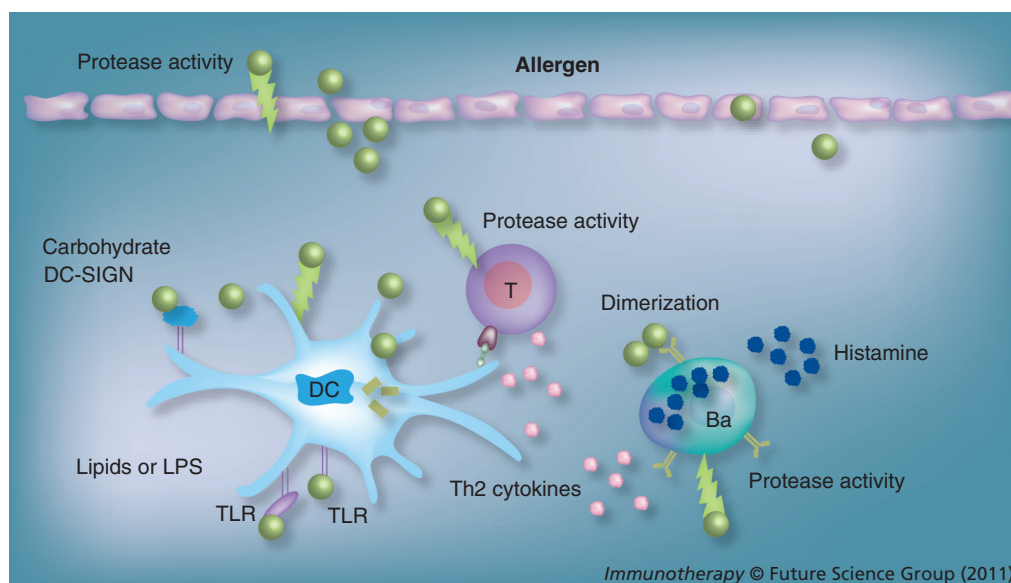


Figure 2. Characteristics of allergens associated with their capacity to induce IgE production. Enzymatic activity of allergens breaks barrier function and activates cells of the innate and adaptive immune response. Some allergens can directly interact with TLR or indirectly via bound lipids or lipopolysaccharide. Carbohydrate determinants on allergens activate C-type lectins (e.g., DC-specific ICAM-grabbing nonintegrin). Dimers of allergens promote the activation of basophils that release mediators and IL-4. Ba: Basophil; DC: Dendritic cell; LPS: Lipopolysaccharide; T: Naive CD4⁺ T cell; TLR: Toll-like receptor.

SIT. Recently, flagellin A from *Listeria monocytogenes* was genetically fused to OVA and tested in a murine *in vitro* system [114]. Compared to flagellin alone, the fusion protein induced a higher expression of maturation markers (CD40, CD69, CD80 and CD86) on myeloid DCs. Moreover, IL-6 and IL-10 production by myeloid DCs was increased when stimulated with fusion proteins in comparison to equimolar mixtures of both proteins. This observation demonstrated again that the fusion protein consisting of an allergen and a TLR ligand showed stronger immunomodulating capacity as the mixture of both components and confirmed

that the approach to endow allergens with intrinsic adjuvanticity is a promising concept for the design of vaccines for future SIT. However, up to now, none of the recombinant allergen–modulator fusion proteins have been tested in clinical trials.

In addition to ligands recognized by TLR4, TLR5 and TLR9 agonists of TLR7 (e.g., imidazoquinolines or modified adenine) have been suggested as candidate adjuvants in future allergy vaccines [115,116]. These TLR7 agonists effectively redirected Th2 responses in both, *in vitro* and *in vivo* studies. However, for all of these still scientific vaccine formulations, the potential induction of adverse Th1-mediated inflammation in humans remains an issue of concern. Thus, clinical trials in humans are needed to evaluate the *in vivo* efficacy and potential side effects of these future allergy vaccines.

Future perspective

Some biochemical features (e.g., enzymatic activity or lipid-binding capacity) and the interaction with elements of the innate immune system were suggested to underlie the phenomenon of allergenicity (FIGURE 2). However, it is difficult to judge their physiological relevance in humans and as a consequence we are still not able to fully answer the question of why particular proteins tend to act as allergens in susceptible individuals whereas others do not. Certainly, some of the mentioned characteristics may explain why certain proteins

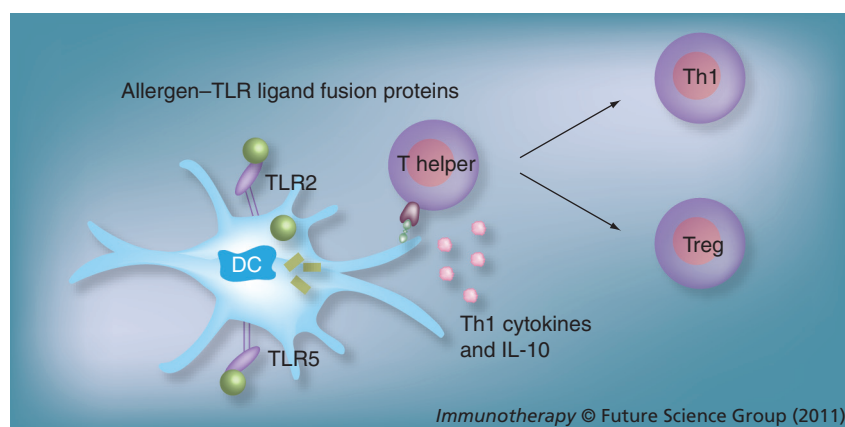


Figure 3. Creating intrinsic adjuvanticity for allergens. Allergens can be genetically fused with TLR ligands; the resulting fusion protein activates DC to promote the differentiation of allergen-specific Th1 and Treg cells. DC: Dendritic cell; TLR: Toll-like receptor.

are major allergens. Nevertheless, many of the proposed features of allergenicity are shared by minor allergens and nonallergenic proteins. More importantly, studies contradicting some concepts of allergenicity have been published. One example is the finding that a trimeric form of the birch pollen allergen is less allergenic than native Bet v 1 [117], which rebuts the concept of dimerization/oligomerization as important factor for allergenicity. Furthermore, the fact that many proteases are no allergens contradicts the concept of enzymatic activity as an important factor for allergenicity. Therefore, apart from special characteristics of individual major allergens, other aspects (e.g., their abundance and stability in the environment, the role of nonallergenic components in allergen sources as well as the route of exposure) need to be taken into account to explain what makes a protein allergenic.

Allergy is a multifactorial disorder of the immune system and in addition to genetic predisposition special characteristics of allergens certainly contribute to allergy development. Therefore, nailing down special features of allergenicity definitely helps us to understand how allergen-specific IgE responses evolve. In addition, these insights will contribute to the

improvement of therapeutic interventions for allergic disorders. Recent studies have supported the concept that adding adjuvanticity to allergens increases their immunomodulatory capacity and thereby may improve their therapeutic efficacy (FIGURE 3). In particular, recombinant fusion proteins of allergens and bacterial proteins that represent danger signals for the immune system have revealed promising results in mouse models of allergy. Furthermore, these vaccine candidates have advantages regarding production, standardization and immunological consistence and should now be tested in humans in order to prove their efficacy to cure allergic disorders.

Financial & competing interests disclosure

This work was supported by the Austrian Science fund (FWF, project F1807-B13), Biomay AG, and the Christian Doppler Research Association, Vienna, Austria. B Bohle is the head of the Christian Doppler Laboratory for Immunomodulation and a scientific advisor of Biomay. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- IgE-mediated hypersensitivity is characterized by an overboarding Th2 response affecting up to 25% of the population in industrialized countries.
- Allergenicity (i.e., the capacity to induce allergen-specific IgE antibodies in susceptible individuals) has been associated with different specific properties of major allergens.
- Enzymatic activity of allergens breaks mucosal barrier function and activates cells of the innate and adaptive immune system.
- The binding of lipids by allergens can favor Th2 immune responses by activation of the innate immune system.
- Activation of innate immune responses by allergens through triggering pattern-recognition receptors.
- Allergen-specific immunotherapy is the only causative treatment for IgE-mediated allergy that affects both the humoral as well as the cellular responses.
- Recombinant fusion proteins consisting of allergens and Toll-like receptor ligands represent promising candidates for future vaccines for allergy treatment because they bear intrinsic immunomodulatory capacity.

Bibliography

Papers of special note have been highlighted as:

■ of interest

■ of considerable interest

- 1 Renkonen J, Mattila P, Lehti S *et al.* Birch pollen allergen Bet v 1 binds to and is transported through conjunctival epithelium in allergic patients. *Allergy* 64(6), 868–875 (2009).
- 2 Jutel M, Akdis M, Budak F *et al.* IL-10 and TGF- β cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur. J. Immunol.* 33(5), 1205–1214 (2003).
- 3 Bellinghausen I, Klostermann B, Knop J, Saloga J. Human CD4⁺CD25⁺ T cells derived from the majority of atopic donors are able to suppress Th1 and Th2 cytokine production. *J. Allergy Clin. Immunol.* 111(4), 862–868 (2003).
- 4 Hamid Q, Boguniewicz M, Leung DY. Differential *in situ* cytokine gene expression in acute versus chronic atopic dermatitis. *J. Clin. Invest.* 94(2), 870–876 (1994).
- 5 Parronchi P, Manetti R, Simonelli C *et al.* Cytokine production by allergen (Der p1)-specific CD4⁺ T cell clones derived from a patient with severe atopic disease. *Int. J. Clin. Lab. Res.* 21(2), 186–189 (1991).
- 6 Bodey KJ, Semper AE, Redington AE *et al.* Cytokine profiles of BAL T cells and T-cell clones obtained from human asthmatic airways after local allergen challenge. *Allergy* 54(10), 1083–1093 (1999).
- 7 Schulten V, Radakovics A, Hartz C *et al.* Characterization of the allergic T-cell response to Pru p 3, the nonspecific lipid transfer protein in peach. *J. Allergy Clin. Immunol.* 124(1), 100–107 (2009).
- 8 Swain SL, Weinberg AD, English M, Huston G. IL-4 directs the development of Th2-like helper effectors. *J. Immunol.* 145(11), 3796–3806 (1990).

- 9 van Neerven RJ, Knol EF, Ejrnaes A, Wurtzen PA. IgE-mediated allergen presentation and blocking antibodies: regulation of T-cell activation in allergy. *Int. Arch. Allergy Immunol.* 141(2), 119–129 (2006).
- 10 Secrist H, DeKruyff RH, Umetsu DT. Interleukin 4 production by CD4⁺ T cells from allergic individuals is modulated by antigen concentration and antigen-presenting cell type. *J. Exp. Med.* 181(3), 1081–1089 (1995).
- 11 Lindell DM, Berlin AA, Schaller MA, Lukacs NW. B cell antigen presentation promotes Th2 responses and immunopathology during chronic allergic lung disease. *PLoS One* 3(9), E3129 (2008).
- 12 Durham SR. Mechanisms of mucosal inflammation in the nose and lungs. *Clin. Exp. Allergy* 28(Suppl. 2), 11–16 (1998).
- 13 Okada M, Terui T, Honda M *et al.* Cutaneous late phase reaction in adult atopic dermatitis patients with high serum IgE antibody to *Dermatophagoides farinae*: correlation with IL-5 production by allergen-stimulated peripheral blood mononuclear cells. *J. Dermatol. Sci.* 29(2), 73–84 (2002).
- 14 Haselden BM, Kay AB, Larche M. Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reactions. *J. Exp. Med.* 189(12), 1885–1894 (1999).
- **Highlights the important role of T helper cells in late asthmatic reactions even independent from early IgE-mediated allergic reactions.**
- 15 Larche M, Haselden BM, Oldfield WL *et al.* Mechanisms of T cell peptide epitope-dependent late asthmatic reactions. *Int. Arch. Allergy Immunol.* 124(1–3), 272–275 (2001).
- 16 Soroosh P, Doherty TA. Th9 and allergic disease. *Immunology* 127(4), 450–458 (2009).
- 17 Cosmi L, Liotta F, Maggi E, Romagnani S, Annunziato F. Th17 cells: new players in asthma pathogenesis. *Allergy* DOI: 10.1111/j.1398-9995.2011.02576.x (2011) (Epub ahead of print).
- 18 Bousquet J, Lockey R, Malling HJ *et al.* Allergen immunotherapy: therapeutic vaccines for allergic diseases. World Health Organization. American academy of Allergy, Asthma and Immunology. *Ann. Allergy Asthma Immunol.* 81(5 Pt 1), 401–405 (1998).
- 19 Pipet A, Botturi K, Pinot D, Vervloet D, Magnan A. Allergen-specific immunotherapy in allergic rhinitis and asthma. Mechanisms and proof of efficacy. *Respir. Med.* 103(6), 800–812 (2009).
- 20 Calderon MA, Casale TB, Togias A *et al.* Allergen-specific immunotherapy for respiratory allergies: from meta-analysis to registration and beyond. *J. Allergy Clin. Immunol.* 127(1), 30–38 (2011).
- 21 Larche M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. *Nat. Rev. Immunol.* 6(10), 761–771 (2006).
- **Educative review on the immune mechanisms of allergen-specific immunotherapy.**
- 22 James LK, Shamji MH, Walker SM *et al.* Long-term tolerance after allergen immunotherapy is accompanied by selective persistence of blocking antibodies. *J. Allergy Clin. Immunol.* 127(2), 509–516.e5 (2011).
- 23 Ebner C, Siemann U, Bohle B *et al.* Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from Th2 to Th1 in T-cell clones specific for Phl p 1, a major grass pollen allergen. *Clin. Exp. Allergy* 27(9), 1007–1015 (1997).
- 24 Mobs C, Slotosch C, Löffler H *et al.* Birch pollen immunotherapy leads to differential induction of regulatory T cells and delayed helper T cell immune deviation. *J. Immunol.* 184(4), 2194–2203 (2010).
- 25 Gardner LM, Thien FC, Douglass JA, Rolland JM, O’Hehir RE. Induction of T ‘regulatory’ cells by standardized house dust mite immunotherapy: an increase in CD4⁺ CD25⁺ interleukin-10⁺ T cells expressing peripheral tissue trafficking markers. *Clin. Exp. Allergy* 34(8), 1209–1219 (2004).
- 26 Durham SR. The inflammatory nature of allergic disease. *Clin. Exp. Allergy* 28(Suppl. 6), 20–24 (1998).
- 27 Akdis CA, Blaser K. IL-10-induced anergy in peripheral T cell and reactivation by microenvironmental cytokines: two key steps in specific immunotherapy. *FASEB J.* 13(6), 603–609 (1999).
- 28 Nouri-Aria KT, Wachholz PA, Francis JN *et al.* Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. *J. Immunol.* 172(5), 3252–3259 (2004).
- 29 Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT. Grass pollen immunotherapy induces Foxp3-expressing CD4⁺ CD25⁺ cells in the nasal mucosa. *J. Allergy Clin. Immunol.* 121(6), 1467–1472, 1472.E1 (2008).
- 30 Francis JN, James LK, Paraskevopoulos G *et al.* Grass pollen immunotherapy: IL-10 induction and suppression of late responses precedes IgG₄ inhibitory antibody activity. *J. Allergy Clin. Immunol.* 121(5), 1120–1125 e1122 (2008).
- 31 Pilette C, Nouri-Aria KT, Jacobson MR *et al.* Grass pollen immunotherapy induces an allergen-specific IgA₂ antibody response associated with mucosal TGF- β expression. *J. Immunol.* 178(7), 4658–4666 (2007).
- 32 Wurtzen PA, Lund G, Lund K *et al.* A double-blind placebo-controlled birch allergy vaccination study II: correlation between inhibition of IgE binding, histamine release and facilitated allergen presentation. *Clin. Exp. Allergy* 38(8), 1290–1301 (2008).
- 33 Mari A, Iacovacci P, Afferni C *et al.* Specific IgE to cross-reactive carbohydrate determinants strongly affect the *in vitro* diagnosis of allergic diseases. *J. Allergy Clin. Immunol.* 103(6), 1005–1011 (1999).
- 34 Mari A. IgE to cross-reactive carbohydrate determinants: analysis of the distribution and appraisal of the *in vivo* and *in vitro* reactivity. *Int. Arch. Allergy Immunol.* 129(4), 286–295 (2002).
- 35 Foetisch K, Westphal S, Lauer I *et al.* Biological activity of IgE specific for cross-reactive carbohydrate determinants. *J. Allergy Clin. Immunol.* 111(4), 889–896 (2003).
- 36 Emanuelsson C, Spangfort MD. Allergens as eukaryotic proteins lacking bacterial homologues. *Mol. Immunol.* 44(12), 3256–3260 (2007).
- 37 Wan H, Winton HL, Soeller C *et al.* Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. *J. Clin. Invest.* 104(1), 123–133 (1999).
- 38 Shakib F, Schulz O, Sewell H. A mite subversive: cleavage of CD23 and CD25 by Der p 1 enhances allergenicity. *Immunol. Today.* 19(7), 313–316 (1998).
- 39 Furmonaviciene R, Ghaemmaghami AM, Boyd SE *et al.* The protease allergen Der p 1 cleaves cell surface DC-SIGN and DC-SIGNR: experimental analysis of *in silico* substrate identification and implications in allergic responses. *Clin. Exp. Allergy* 37(2), 231–242 (2007).
- 40 Ebeling C, Lam T, Gordon JR, Hollenberg MD, Vliagoftis H. Proteinase-activated receptor-2 promotes allergic sensitization to an inhaled antigen through a TNF-mediated pathway. *J. Immunol.* 179(5), 2910–2917 (2007).
- 41 Jeong SK, Kim HJ, Youm JK *et al.* Mite and cockroach allergens activate protease-activated receptor 2 and delay epidermal permeability barrier recovery. *J. Invest. Dermatol.* 128(8), 1930–1939 (2008).
- 42 Kato T, Takai T, Fujimura T *et al.* Mite serine protease activates protease-activated receptor-2 and induces cytokine release in human keratinocytes. *Allergy* 64(9), 1366–1374 (2009).

- 43 Asokanathan N, Graham PT, Stewart DJ *et al.* House dust mite allergens induce proinflammatory cytokines from respiratory epithelial cells: the cysteine protease allergen, Der p 1, activates protease-activated receptor (PAR)-2 and inactivates PAR-1. *J. Immunol.* 169(8), 4572–4578 (2002).
- 44 Adam E, Hansen KK, Astudillo Fernandez O *et al.* The house dust mite allergen Der p 1, unlike Der p 3, stimulates the expression of interleukin-8 in human airway epithelial cells via a proteinase-activated receptor-2-independent mechanism. *J. Biol. Chem.* 281(11), 6910–6923 (2006).
- 45 Deb R, Shakib F, Reid K, Clark H. Major house dust mite allergens *Dermatophagoides pteronyssinus* 1 and *Dermatophagoides farinae* 1 degrade and inactivate lung surfactant proteins A and D. *J. Biol. Chem.* 282(51), 36808–36819 (2007).
- 46 Gough L, Campbell E, Bayley D, Van Heeke G, Shakib F. Proteolytic activity of the house dust mite allergen Der p 1 enhances allergenicity in a mouse inhalation model. *Clin. Exp. Allergy* 33(8), 1159–1163 (2003).
- 47 Yu HS, Angkasekwinai P, Chang SH, Chung Y, Dong C. Protease allergens induce the expression of IL-25 via Erk and p38 MAPK pathway. *J. Korean Med. Sci.* 25(6), 829–834 (2010).
- 48 Chapman MD, Wunschmann S, Pomes A. Proteases as Th2 adjuvants. *Curr. Allergy Asthma Rep.* 7(5), 363–367 (2007).
- 49 Phillips C, Coward WR, Pritchard DI, Hewitt CR. Basophils express a type 2 cytokine profile on exposure to proteases from helminths and house dust mites. *J. Leukoc. Biol.* 73(1), 165–171 (2003).
- 50 Sokol CL, Chu NQ, Yu S *et al.* Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nat. Immunol.* 10(7), 713–720 (2009).
- 51 Sokol CL, Medzhitov R. Role of basophils in the initiation of Th2 responses. *Curr. Opin. Immunol.* 22(1), 73–77 (2010).
- 52 Rabensteiner DF, Spreitzhofer E, Trummer G *et al.* Pollen enzymes degrade human tear fluid and conjunctival cells: an approach to understanding seasonal non-allergic conjunctivitis. *Dev. Ophthalmol.* 45, 83–92 (2010).
- 53 Ichikawa S, Takai T, Yashiki T *et al.* Lipopolysaccharide binding of the mite allergen Der f 2. *Genes Cells* 14(9), 1055–1065 (2009).
- 54 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.* 125(4), 909–917.E4 (2010).
- 55 Epton MJ, Dilworth RJ, Smith W, Thomas WR. Sensitisation to the lipid-binding apolipoprotein allergen Der p 14 and the peptide Mag-1. *Int. Arch. Allergy Immunol.* 124(1–3), 57–60 (2001).
- 56 Gonzalez-Rioja R, Asturias JA, Martinez A, Goni FM, Viguera AR. Par j 1 and Par j 2, the two major allergens in *Parietaria judaica*, bind preferentially to monoacylated negative lipids. *FEBS J.* 276(6), 1762–1775 (2009).
- 57 Salcedo G, Sanchez-Monge R, Barber D, Diaz-Perales A. Plant non-specific lipid transfer proteins: an interface between plant defence and human allergy. *Biochim. Biophys. Acta* 1771(6), 781–791 (2007).
- 58 Spangfort MD, Mirza O, Ipsen H *et al.* Dominating IgE-binding epitope of Bet v 1, the major allergen of birch pollen, characterized by x-ray crystallography and site-directed mutagenesis. *J. Immunol.* 171(6), 3084–3090 (2003).
- 59 Mogensen JE, Wimmer R, Larsen JN, Spangfort MD, Otzen DE. The major birch allergen, Bet v 1, shows affinity for a broad spectrum of physiological ligands. *J. Biol. Chem.* 277(26), 23684–23692 (2002).
- 60 Chisholm D, Libet L, Hayashi T, Horner AA. Airway peptidoglycan and immunostimulatory DNA exposures have divergent effects on the development of airway allergen hypersensitivities. *J. Allergy Clin. Immunol.* 113(3), 448–454 (2004).
- 61 Redecke V, Hacker H, Datta SK *et al.* Cutting edge: activation of Toll-like receptor 2 induces a Th2 immune response and promotes experimental asthma. *J. Immunol.* 172(5), 2739–2743 (2004).
- 62 Eisenbarth SC, Piggott DA, Huleatt JW *et al.* Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J. Exp. Med.* 196(12), 1645–1651 (2002).
- 63 Tan AM, Chen HC, Pochard P *et al.* TLR4 signaling in stromal cells is critical for the initiation of allergic Th2 responses to inhaled antigen. *J. Immunol.* 184(7), 3535–3544 (2010).
- 64 Gilles S, Mariani V, Bryce M *et al.* Pollen allergens do not come alone: pollen associated lipid mediators (PALMS) shift the human immune systems towards a Th2-dominated response. *Allergy Asthma Clin. Immunol.* 5(1), 3 (2009).
- 65 Traidl-Hoffmann C, Mariani V, Hochrein H *et al.* Pollen-associated phytoprostanes inhibit dendritic cell interleukin-12 production and augment T helper type 2 cell polarization. *J. Exp. Med.* 201(4), 627–636 (2005).
- Suggests a possible role for nonallergenic components in pollen for allergenicity.
- 66 Gilles S, Mariani V, Bryce M *et al.* Pollen-derived E1-phytoprostanes signal via PPAR- γ and NF- κ B-dependent mechanisms. *J. Immunol.* 182(11), 6653–6658 (2009).
- 67 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* 40(2), 269–278 (2010).
- 68 Gruber A, Mancek M, Wagner H, Kirschning CJ, Jerala R. Structural model of MD-2 and functional role of its basic amino acid clusters involved in cellular lipopolysaccharide recognition. *J. Biol. Chem.* 279(27), 28475–28482 (2004).
- 69 Keber MM, Gradaris H, Jerala R. MD-2 and Der p 2 – a tale of two cousins or distant relatives? *J. Endotoxin Res.* 11(3), 186–192 (2005).
- 70 Trompette A, Divanovic S, Visintin A *et al.* Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. *Nature* 457(7229), 585–588 (2009).
- 71 van Kooyk Y, Engering A, Lekkerkerker AN, Ludwig IS, Geijtenbeek TB. Pathogens use carbohydrates to escape immunity induced by dendritic cells. *Curr. Opin. Immunol.* 16(4), 488–493 (2004).
- 72 den Dunnen J, Gringhuis SI, Geijtenbeek TB. Dusting the sugar fingerprint: C-type lectin signaling in adaptive immunity. *Immunol. Lett.* 128(1), 12–16 (2010).
- 73 Shreffler WG, Castro RR, Kucuk ZY *et al.* The major glycoprotein allergen from *Arachis hypogaea*, Ara h 1, is a ligand of dendritic cell-specific ICAM-grabbing nonintegrin and acts as a Th2 adjuvant *in vitro*. *J. Immunol.* 177(6), 3677–3685 (2006).
- 74 Hsu SC, Chen CH, Tsai SH *et al.* Functional interaction of common allergens and a C-type lectin receptor, dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN), on human dendritic cells. *J. Biol. Chem.* 285(11), 7903–7910 (2010).
- 75 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.* 185(3), 1522–1531 (2010).
- 76 Niemi M, Jylha S, Laukkanen ML *et al.* Molecular interactions between a recombinant IgE antibody and the β -lactoglobulin allergen. *Structure* 15(11), 1413–1421 (2007).
- 77 Rouvinen J, Janis J, Laukkanen ML *et al.* Transient dimers of allergens. *PLoS One* 5(2), E9037 (2010).

- 78 Li M, Gustchina A, Alexandratos J *et al.* Crystal structure of a dimerized cockroach allergen Bla g 2 complexed with a monoclonal antibody. *J. Biol. Chem.* 283(33), 22806–22814 (2008).
- 79 Lascombe MB, Gregoire C, Poncet P *et al.* Crystal structure of the allergen Equ c 1. A dimeric lipocalin with restricted IgE-reactive epitopes. *J. Biol. Chem.* 275(28), 21572–21577 (2000).
- 80 Verdino P, Westritschnig K, Valenta R, Keller W. The cross-reactive calcium-binding pollen allergen, Phl p 7, reveals a novel dimer assembly. *EMBO J.* 21(19), 5007–5016 (2002).
- 81 Rajashankar K, Bufe A, Weber W *et al.* Structure of the functional domain of the major grass-pollen allergen Phlp 5b. *Acta Crystallogr. D Biol. Crystallogr.* 58(Pt 7), 1175–1181 (2002).
- 82 van Oort E, Dieker MC, de Heer PG *et al.* The major grass pollen group 5 allergen from *Dactylis glomerata* and its C-terminal split product both behave as dimers: implications for allergen standardization. *Int. Arch. Allergy Immunol.* 136(2), 113–122 (2005).
- 83 Magler I, Nuss D, Hauser M, Ferreira F, Brandstetter H. Molecular metamorphosis in polcalcin allergens by EF-hand rearrangements and domain swapping. *FEBS J.* 277(12), 2598–2610 (2010).
- 84 Wopfner N, Dissertori O, Ferreira F, Lackner P. Calcium-binding proteins and their role in allergic diseases. *Immunol. Allergy Clin. North Am.* 27(1), 29–44 (2007).
- 85 Scholl I, Kalkura N, Shedziankova Y *et al.* Dimerization of the major birch pollen allergen Bet v 1 is important for its *in vivo* IgE-cross-linking potential in mice. *J. Immunol.* 175(10), 6645–6650 (2005).
- 86 Zaborsky N, Brunner M, Wallner M *et al.* Antigen aggregation decides the fate of the allergic immune response. *J. Immunol.* 184(2), 725–735 (2010).
- 87 Eisenbarth SC, Colegio OR, O'Connor W, Sutterwala FS, Flavell RA. Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. *Nature* 453(7198), 1122–1126 (2008).
- 88 Kool M, Petrilli V, De Smedt T *et al.* Cutting edge: alum adjuvant stimulates inflammatory dendritic cells through activation of the NALP3 inflammasome. *J. Immunol.* 181(6), 3755–3759 (2008).
- 89 Franchi L, Nunez G. The Nlrp3 inflammasome is critical for aluminium hydroxide-mediated IL-1 β secretion but dispensable for adjuvant activity. *Eur. J. Immunol.* 38(8), 2085–2089 (2008).
- 90 Wilcock LK, Francis JN, Durham SR. Aluminium hydroxide down-regulates T helper 2 responses by allergen-stimulated human peripheral blood mononuclear cells. *Clin. Exp. Allergy* 34(9), 1373–1378 (2004).
- **First study to address the effects of aluminum hydroxide on human cells.**
- 91 Casale TB, Stokes JR. Future forms of immunotherapy. *J. Allergy Clin. Immunol.* 127(1), 8–15; quiz 16–17 (2011).
- 92 Puggioni F, Durham SR, Francis JN. Monophosphoryl lipid A (MPL) promotes allergen-induced immune deviation in favour of Th1 responses. *Allergy* 60(5), 678–684 (2005).
- 93 Mothes N, Heinzkill M, Drachenberg KJ *et al.* Allergen-specific immunotherapy with a monophosphoryl lipid A-adjuvanted vaccine: reduced seasonally boosted immunoglobulin E production and inhibition of basophil histamine release by therapy-induced blocking antibodies. *Clin. Exp. Allergy* 33(9), 1198–1208 (2003).
- 94 Rosewich M, Schulze J, Eickmeier O *et al.* Tolerance induction after specific immunotherapy with pollen allergoids adjuvanted by monophosphoryl lipid A in children. *Clin. Exp. Immunol.* 160(3), 403–410 (2010).
- 95 Krieg AM, Yi AK, Matson S *et al.* CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature* 374(6522), 546–549 (1995).
- 96 Klinman DM, Yi AK, Beaucage SL, Conover J, Krieg AM. CpG motifs present in bacteria DNA rapidly induce lymphocytes to secrete interleukin 6, interleukin 12, and interferon γ . *Proc. Natl Acad. Sci. USA* 93(7), 2879–2883 (1996).
- 97 Hartmann G, Weiner GJ, Krieg AM. CpG DNA: a potent signal for growth, activation, and maturation of human dendritic cells. *Proc. Natl Acad. Sci. USA* 96(16), 9305–9310 (1999).
- 98 Bohle B, Jahn-Schmid B, Maurer D, Kraft D, Ebner C. Oligodeoxynucleotides containing CpG motifs induce IL-12, IL-18 and IFN- γ production in cells from allergic individuals and inhibit IgE synthesis *in vitro*. *Eur. J. Immunol.* 29(7), 2344–2353 (1999).
- 99 Tighe H, Takabayashi K, Schwartz D *et al.* Conjugation of immunostimulatory DNA to the short ragweed allergen Amb a 1 enhances its immunogenicity and reduces its allergenicity. *J. Allergy Clin. Immunol.* 106(1 Pt 1), 124–134 (2000).
- 100 Creticos PS, Schroeder JT, Hamilton RG *et al.* Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. *N. Engl. J. Med.* 355(14), 1445–1455 (2006).
- 101 Mutschlechner S, Deiff S, Bohle B. Genetic allergen modification in the development of novel approaches to specific immunotherapy. *Clin. Exp. Allergy* 39(11), 1635–1642 (2009).
- 102 Bublin M, Hoflehner E, Wagner B *et al.* Use of a genetic cholera toxin B subunit/allergen fusion molecule as mucosal delivery system with immunosuppressive activity against Th2 immune responses. *Vaccine* 25(50), 8395–8404 (2007).
- 103 Lee BK, Yoo YG, Lee WY *et al.* Ovalbumin fused with diphtheria toxin protects mice from ovalbumin induced anaphylactic shock. *Yonsei Med. J.* 42(1), 91–105 (2001).
- 104 Sleytr UB, Bayley H, Sara M *et al.* Applications of S-layers. *FEMS Microbiol. Rev.* 20(1–2), 151–175 (1997).
- 105 Breitwieser A, Egelseer EM, Moll D *et al.* A recombinant bacterial cell surface (S-layer)-major birch pollen allergen-fusion protein (rSbsC/Bet v1) maintains the ability to self-assemble into regularly structured monomolecular lattices and the functionality of the allergen. *Protein Eng.* 15(3), 243–249 (2002).
- 106 Bohle B, Breitwieser A, Zwolfer B *et al.* A novel approach to specific allergy treatment: the recombinant fusion protein of a bacterial cell surface (S-layer) protein and the major birch pollen allergen Bet v 1 (rSbsC-Bet v 1) combines reduced allergenicity with immunomodulating capacity. *J. Immunol.* 172(11), 6642–6648 (2004).
- 107 Gerstmayr M, Ilk N, Jahn-Schmid B, Sleytr UB, Bohle B. Natural self-assembly of allergen-S-layer fusion proteins is no prerequisite for reduced allergenicity and T cell stimulatory capacity. *Int. Arch. Allergy Immunol.* 149(3), 231–238 (2009).
- 108 Gerstmayr M, Ilk N, Schabussova I *et al.* A novel approach to specific allergy treatment: the recombinant allergen-S-layer fusion protein rSbsC-Bet v 1 matures dendritic cells that prime Th0/Th1 and IL-10-producing regulatory T cells. *J. Immunol.* 179(11), 7270–7275 (2007).
- 109 Ilk N, Schumi CT, Bohle B, Egelseer EM, Sleytr UB. Expression of an endotoxin-free S-layer/allergen fusion protein in gram-positive *Bacillus subtilis* 1012 for the potential application as vaccines for immunotherapy of atopic allergy. *Microb. Cell Fact.* 10(1), 6 (2011).
- 110 Hayashi F, Smith KD, Ozinsky A *et al.* The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 410(6832), 1099–1103 (2001).
- 111 Huleatt JW, Nakaar V, Desai P *et al.* Potent immunogenicity and efficacy of a universal influenza vaccine candidate comprising a recombinant fusion protein linking influenza M2e to the TLR5 ligand flagellin. *Vaccine* 26(2), 201–214 (2008).

- 112 McDonald WF, Huleatt JW, Foellmer HG *et al.* A West Nile virus recombinant protein vaccine that coactivates innate and adaptive immunity. *J. Infect. Dis.* 195(11), 1607–1617 (2007).
- 113 Lee SE, Koh YI, Kim MK *et al.* Inhibition of airway allergic disease by co-administration of flagellin with allergen. *J. Clin. Immunol.* 28(2), 157–165 (2008).
- 114 Schulke S, Waibler Z, Mende MS *et al.* Fusion protein of TLR5-ligand and allergen potentiates activation and IL-10 secretion in murine myeloid DC. *Mol. Immunol.* 48(1–3), 341–350 (2010).
- 115 Fili L, Ferri S, Guarna F *et al.* Redirection of allergen-specific TH2 responses by a modified adenine through Toll-like receptor 7 interaction and IL-12/IFN release. *J. Allergy Clin. Immunol.* 118(2), 511–517 (2006).
- 116 Vultaggio A, Nencini F, Fitch PM *et al.* Modified adenine (9-benzyl-2-butoxy-8-hydroxyadenine) redirects Th2-mediated murine lung inflammation by triggering TLR7. *J. Immunol.* 182(2), 880–889 (2009).
- 117 Vrtala S, Hirtenlehner K, Susani M *et al.* Genetic engineering of a hypoallergenic trimer of the major birch pollen allergen Bet v 1. *FASEB J.* 15(11), 2045–2047 (2001).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.