

Immunological mechanisms of allergen-specific immunotherapy

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Abstract | Allergen-specific immunotherapy has been carried out for almost a century and remains one of the few antigen-specific treatments for inflammatory diseases. The mechanisms by which allergen-specific immunotherapy exerts its effects include the modulation of both T-cell and B-cell responses to allergen. There is a strong rationale for improving the efficacy of allergen-specific immunotherapy by reducing the incidence and severity of adverse reactions mediated by IgE. Approaches to address this problem include the use of modified allergens, novel adjuvants and alternative routes of administration. This article reviews the development of allergen-specific immunotherapy, our current understanding of its mechanisms of action and its future prospects.

One hundred years ago, von Pirquet¹ introduced the term allergy to distinguish immune responses that are harmful to the host from a physiological state of protective immunity. The most common form of allergy — IgE-mediated hypersensitivity — affects more than 25% of the population in industrialized countries². The allergic response is directed against various environmental proteins (known as allergens) and manifests clinically as allergic rhinitis (more commonly known as hay fever), allergic asthma, food allergy, allergic skin inflammation, ocular allergy and/or anaphylaxis. Allergic inflammation and reactions to allergen challenge can be local (that is, within the target organ), as is the case for allergic rhinitis and allergic asthma, or systemic, as is the case for anaphylaxis. The aetiology of allergic immune responses is complex and has been shown to be influenced by several factors, including genetic susceptibility, route of exposure, dose of the allergen and, in some cases, structural characteristics of the allergen³.

In 1921, Prausnitz and Küstner⁴ showed that allergen-specific sensitivity could be transferred to a non-allergic person by injection of serum. It was not until 1966–1967 that the serum factor was identified as IgE^{5,6} (TIMELINE). During sensitization to allergen, priming of allergen-specific (CD4⁺) T helper 2 (T_H2) cells results in the production of T_H2 cytokines (such as interleukin-4 (IL-4) and IL-13), which are responsible for class switching to the ε immunoglobulin heavy chain, allowing IgE production by B cells (FIG. 1a). IgE sensitizes mast cells and basophils by binding the high-affinity receptor for IgE (FcεRI), which is expressed at the surface of these cells.

On crosslinking of the IgE–FcεRI complexes by allergen, mast cells and basophils degranulate, releasing vasoactive amines (mainly histamine), lipid mediators (prostaglandins and cysteinyl leukotrienes), chemokines and other cytokines, all of which characterize the immediate phase of the allergic reaction (FIG. 1b). IgE also binds FcεRI at the surface of dendritic cells (DCs) and monocytes, as well as the low-affinity receptor for IgE, FcεRII (also known as CD23), at the surface of B cells. This process increases the uptake of allergen by these antigen-presenting cells (APCs) and the subsequent presentation of allergen-derived peptides to specific CD4⁺ T cells, which drive the late phase of the allergic reaction⁷.

In vivo data supporting a role for IgE in increasing the responses of T cells to allergen come from observations that treatment with IgE-specific antibody considerably reduces allergen-induced late-phase responses in both the airways⁸ and the skin⁹. The production of IL-4, IL-5, IL-9 and IL-13 by allergen-specific T_H2 cells also contributes to the development, survival and recruitment of eosinophils, as well as the differentiation of mast cells and the hypersecretion of mucus^{10,11}. Production of interferon-γ (IFNγ) and tumour-necrosis factor, together with expression of CD95 ligand (also known as FAS ligand), by T_H1 cells leads to the apoptosis of epithelial cells and compromises barrier function of epithelial cells in the lungs and the skin¹² (FIG. 1c).

Allergen-specific immunotherapy (SIT) involves repeated administration of the sensitizing allergen (usually by subcutaneous injection or, more recently, by sublingual application). SIT was first reported at the

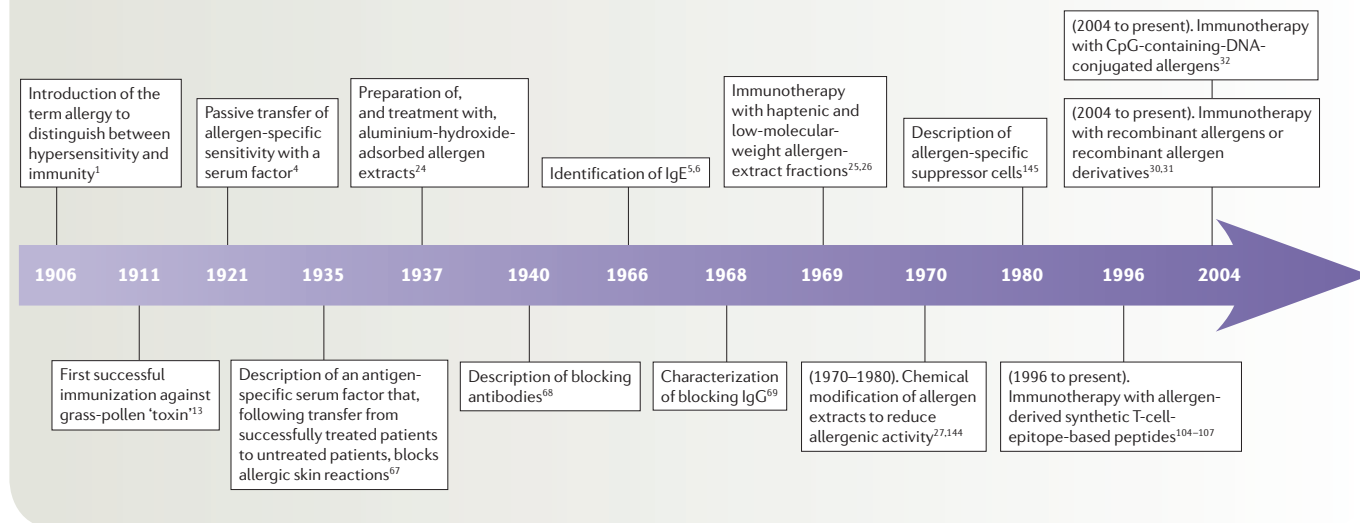
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Timeline | Steps in the development of allergen-specific immunotherapy



Hypersensitivity

An exaggerated and inappropriate immune response to an antigen or allergen. In the context of allergic diseases, this includes production of allergen-specific IgE to environmental or self antigens.

Class switching

The somatic-recombination process by which the class of immunoglobulin is switched from IgM to IgG, IgA or IgE.

Immediate phase of the allergic reaction

(Also known as the early phase). The biological and clinical consequences that occur within the first hour of crosslinking of IgE–FcεRI (high-affinity receptor for IgE) complexes at the surface of mast cells and/or basophils by allergens. The clinical manifestations are characterized by tissue-specific effects: for example, constriction of the large airways in asthma, and wheal-and-flare reactions in the skin. Generalized symptoms in multiple target organs can include oedema and pruritus (itching). Systemic manifestations can include angio-oedema, urticaria and, in severe cases, vascular collapse (anaphylaxis).

beginning of the last century¹³ and has been shown to be a robust and clinically effective allergen-specific form of treatment that induces active immunity to the allergen (FIG. 2). SIT is disease modifying, rather than palliative, and has a duration of action that exceeds the treatment period^{14,15}. It has been shown to prevent the onset of new sensitizations to different allergens¹⁶ and to reduce the development of asthma in patients with allergic rhinitis caused by inhaled allergens¹⁷. SIT improves the quality of life of the treated individuals, through the reduction of symptoms and medication usage¹⁸. More specifically, it has been shown to reduce seasonal increases in specific IgE¹⁹ and in the nonspecific airway hyper-reactivity that occurs in individuals with asthma¹⁸. Bronchial responses to an inhaled allergen challenge²⁰, and late-phase responses to an allergen challenge in the skin²¹ or nasal mucosae²², are also reduced. Here, we review the development of SIT, the immunological mechanisms of action of SIT and the novel approaches that are underway to improve its safety and efficacy.

Development of SIT

Noon¹³ carried out the first study of active immunization to prevent allergy to grass pollen, using subcutaneous injection of grass-pollen extract (TIMELINE). Pollens were thought to contain undefined ‘toxins’ that caused allergic symptoms, and it was thought that injection of small doses of pollen extract would induce antitoxins and ameliorate disease. Larger clinical trials that were carried out by Freeman and Noon²³ showed an improvement in symptoms that was sustained for 1 year after treatment.

To increase the efficacy of SIT and to reduce its side-effects (including local allergic reactions, urticaria, asthma and frequent anaphylaxis), allergen extracts were adsorbed to adjuvants, mainly in an attempt to generate a slow-release depot of allergen²⁴. In addition, attempts were made to decrease the allergenic activity of the allergen extracts, either by cleaving them to generate short peptides or by chemically modifying them (by treatment

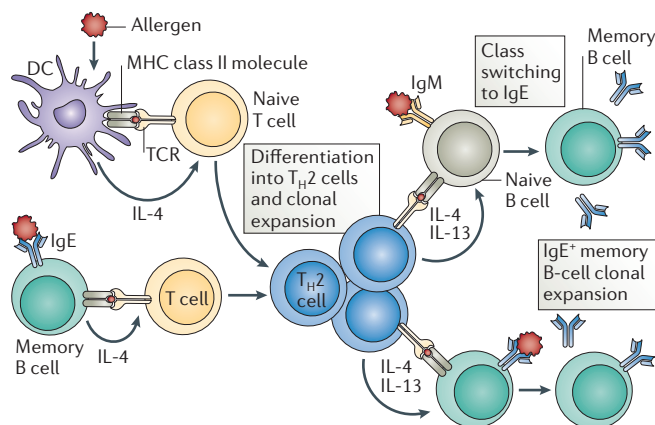
with aldehydes)^{25–27}. In the 1980s, recombinant-DNA technology and molecular characterization of allergens provided gene and amino-acid sequences from which synthetic-peptide epitopes and recombinant allergens were derived for therapy²⁸. During the past decade, clinical trials of SIT have been carried out using synthetic peptides that contain T-cell epitopes²⁹, purified recombinant allergen derivatives that have been modified by genetic engineering to reduce their allergenic activity³⁰, recombinant ‘native’ allergens (which retain the sequence and conformation of the natural molecule)³¹ and purified allergens coupled to immunostimulatory synthetic oligodeoxynucleotides that contain CpG motifs³². These approaches are discussed further later.

Mechanisms of SIT

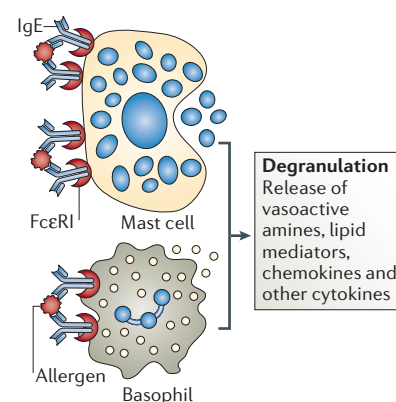
The mechanisms by which SIT mediates its anti-inflammatory effects remain incompletely defined because of the use of heterogeneous allergen preparations, treatment protocols, administration routes and outcome measures in different studies. However, several common features emerge from the multiple studies that show that SIT modifies the responses of APCs, T cells and B cells (FIG. 2), as well as both the number and the function of effector cells that mediate the allergic response. For example, the number of T_H2 cells and eosinophils is reduced at sites of allergen challenge following SIT^{21,22}. Furthermore, SIT reduces the seasonal increase in the number of basophils³³ and eosinophils^{33,34} in the mucosae, as well as the number of mast cells in the skin³⁵ and the IgE-mediated release of histamine by basophils³⁶.

Modulation of APC function following SIT. APCs, particularly DCs, control both peripheral tolerance and immunity through the interpretation of environmental signals that are associated with antigen encounter (such as pathogen-associated molecular patterns). The tolerogenic function of DCs depends on the maturation status

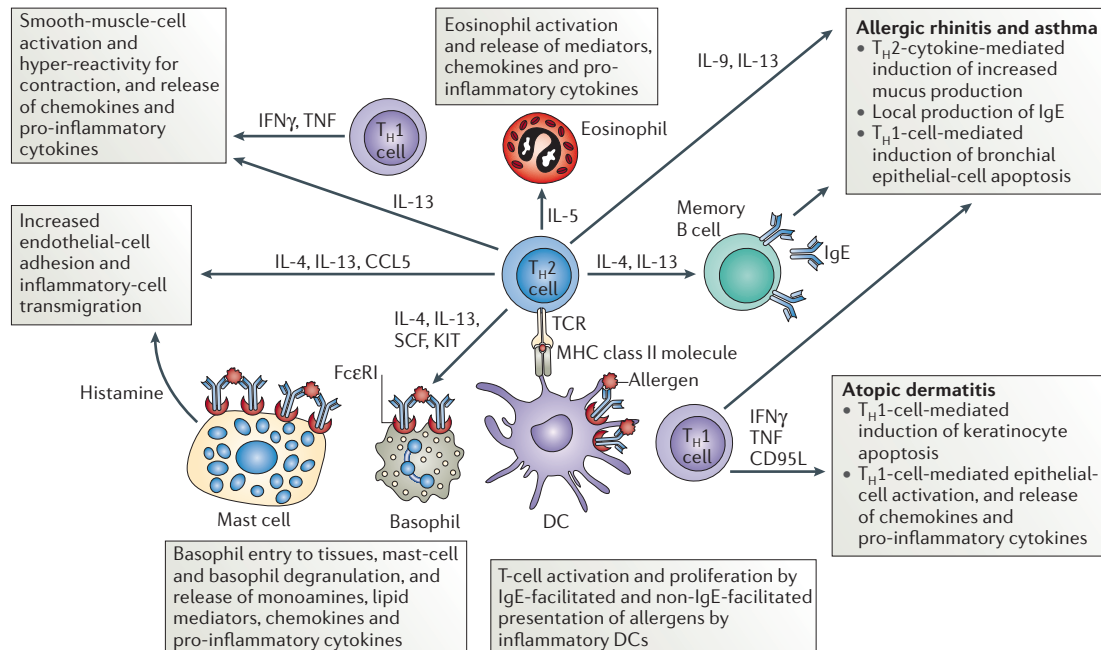
a Sensitization and memory induction



b Immediate phase: type 1 reaction



c Late phase: allergic inflammation



Late phase of the allergic reaction

Clinical manifestations can be measurable (visible) two or more hours after allergen exposure but might appear much later. These manifestations peak at 6–9 hours after allergen exposure and have resolved by 24–48 hours. Reactions are characterized by oedema and the infiltration of T helper 2 cells and eosinophils. Tissue reactions are characterized by oedema, pain, warmth and erythema (redness). Reactions in the lungs are characterized by airway narrowing and mucus hypersecretion.

CpG motif

A deoxycytosine–deoxyguanosine sequence. Such sequences are prevalent in bacterial DNA but are rare in mammalian DNA. Unmethylated CpG is endocytosed by cells of the innate immune system and interacts with Toll-like receptor 9, activating a signalling cascade that results in the production of pro-inflammatory cytokines.

Figure 1 | Mechanisms of allergic reactions. **a** | Sensitization to allergens and development of specific B-cell and T-cell memory. Differentiation and clonal expansion of allergen-specific T helper 2 (T_H2) cells leads to the production of cytokines (interleukin-4 (IL-4) and IL-13), which induce immunoglobulin class switching to IgE and clonal expansion of naive and IgE⁺ memory B-cell populations. IgE at the surface of allergen-specific IgE⁺ B cells and other IgE-sensitized antigen-presenting cells facilitates antigen presentation. T-cell activation in the presence of IL-4 increases differentiation into T_H2 cells. **b** | Type 1 hypersensitivity reaction (immediate phase of the allergic reaction). Crosslinking of mast-cell and basophil cell-surface FcεRI (high-affinity receptor for IgE)-bound IgE by allergens leads to the release of vasoactive amines (such as histamine), lipid mediators (such as prostaglandin D, platelet-activating factor, leukotriene C_4 (LTC₄), LTD₄ and LTE₄), chemokines (CXC-chemokine ligand 8 (CXCL8), CXCL10, CC-chemokine ligand 2 (CCL2), CCL4 and CCL5) and other cytokines (such as IL-4, IL-5 and IL-13), and to the immediate symptoms of allergic disease. **c** | Allergic inflammation (late phase of the allergic reaction). Following migration to sites of allergen exposure under the influence of chemokines and other cytokines, allergen-specific T cells are reactivated and clonally expand. Local IgE-facilitated antigen presentation by dendritic cells (DCs) increases T-cell activation. Local IgE production is seen in allergic rhinitis and asthma but not in allergic skin inflammation (the main example of which is atopic dermatitis). Eosinophils are one of the main inflammatory cells (constituting up to 50% of the cellular infiltrate) in the lungs of asthmatic individuals but not in the skin of those with atopic dermatitis (1–2% of the cellular infiltrate). T_H1 cells, which produce interferon- γ (IFN γ) and tumour-necrosis factor (TNF), contribute to the activation and apoptosis of keratinocytes (in the skin), bronchial epithelial cells and pulmonary smooth-muscle cells. Activation of mast cells and basophils, which release histamine, chemokines and other cytokines, also contributes to the late-phase allergic reaction. CD95L, CD95 ligand; SCF, stem-cell factor (also known as KIT ligand); TCR, T-cell receptor.

Plasmacytoid DC

An immature dendritic cell (DC) with a morphology that resembles that of a plasma cell. Plasmacytoid DCs produce type I interferons (that is, interferon- α and interferon- β) in response to viral infection.

T_H0 cells

Precursors of T helper 1 (T_H1) cells and T_H2 cells. T_H0 cells produce both interferon- γ and interleukin-4. They have the capacity to become T_H1 cells and/or T_H2 cells.

and the activation status of the cell, in addition to the cell lineage (for example, myeloid DCs versus plasmacytoid DCs), all of which can be influenced by immunomodulatory agents such as adjuvants. DCs in the airways control the pulmonary immune response and determine tolerance and immunity to newly encountered antigens. These DCs are distributed in an interdigitating network throughout the airways, capturing allergens and migrating to the T-cell area of the mediastinal lymph nodes within 12 hours of exposure to allergen³⁷. In the absence of pro-inflammatory signals, as is the case in SIT, airway DCs have a partially mature phenotype and express a range of co-stimulatory molecules that is intermediate between that of immature and mature DCs, resulting in tolerogenic interaction with lymph-node T cells³⁸. Several studies support a role for DCs in the induction of T cells with a regulatory phenotype and function, particularly IL-10-secreting regulatory T cells (T regulatory 1 (T_R1) cells). Such regulatory T cells might be important mediators of the beneficial action of SIT. Repeated stimulation of T cells with immature DCs results in the generation of non-proliferating T_R1-like cells³⁹. And antigen presentation by partially mature airway DCs that express IL-10 induces the formation of T_R1-like cells, which inhibit

subsequent inflammatory responses⁴⁰. Experiments involving the depletion and adoptive transfer of pulmonary plasmacytoid DCs have shown that these cells have a central role in protection against sensitization to allergen and development of asthma in a mouse model⁴¹. And, finally, in clinical trials, SIT has been shown to increase the production of IL-10 by APCs, including B cells, monocytes and macrophages^{42,43}, a phenomenon that might lead to increased generation of IL-10-secreting T_R1-like cells.

Modulation of T-cell responses following SIT. CD4⁺ T cells from healthy individuals with no IgE response to allergen recognize the same T-cell epitopes as do CD4⁺ T cells from allergic individuals^{44–46}. Therefore, differences in the quality of the response to allergens — for example, T_H1-cell responses versus T_H2-cell responses — are likely to contribute to development of allergic diseases. Recent data indicate that active regulation might be an essential mechanism for both inducing and maintaining peripheral tolerance to allergens. Analysis of T-cell responses to food allergens and airborne allergens showed that allergens induce T_H1-, T_H2 and T_R1-cell responses⁴⁷. The ratio of allergen-specific IL-10-secreting cells to IFN γ -secreting cells and IL-4-secreting cells determines the development of a healthy or a pathogenic immune response: having a small number of regulatory T cells and a large number of T_H2 cells results in an allergic response⁴⁷. Recent data indicate that the activity of both allergen-specific IL-10-secreting T_R1-like cells and CD4⁺CD25⁺ regulatory T (T_{Reg}) cells is compromised in allergic disease but can be boosted by SIT^{42,47–50}.

SIT has been shown to modify T-cell responses to allergen in several ways, including the following: by increasing the allergen-induced ratio of T_H1 cytokines to T_H2 cytokines^{21,51,52}, by inducing epitope-specific T-cell anergy that can be blocked by neutralization of IL-10 (REF. 53), by generating allergen-specific regulatory T cells that can suppress the responses of effector T cells following delivery of either whole allergen extracts or synthetic peptides that contain or consist of a T-cell epitope^{49,50}, and by increasing the production of cytokines with regulatory activity (FIG. 3). Induction of mRNA that encodes IL-10 and increased production of IL-10 protein have been reported to occur in both the blood and the tissues following SIT^{42,43,49,54–58}. Increased production of transforming growth factor- β (TGF β) has also been reported following SIT and has been shown to contribute to regulatory T-cell function, because the addition of neutralizing TGF β -specific antibodies to T-cell cultures was found to inhibit the regulatory function of these T cells⁴⁹. IL-10 has been shown to modulate the function of effector cells in allergic responses. For example, IL-10 inhibited IgE-dependent activation of human mast cells⁵⁹ and suppressed production of IL-5 by resting human T_H0 cells and T_H2 cells⁶⁰. Furthermore, IL-10 inhibited production of granulocyte/macrophage colony-stimulating factor and expression of CD40 by activated eosinophils, and it increased eosinophil cell death⁶¹. In addition, specific induction of T_H1-cell responses, rather than

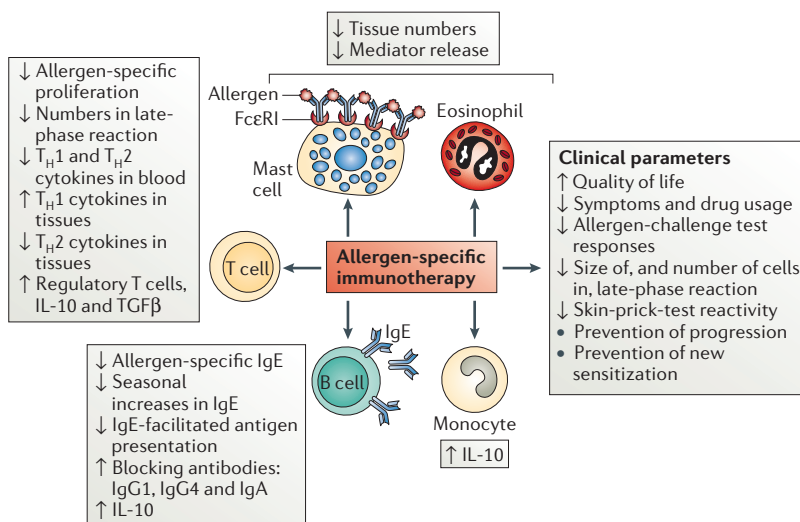


Figure 2 | Effects of allergen-specific immunotherapy on clinical and experimental immune parameters. Allergen-specific immunotherapy (SIT) improves the quality of life of treated individuals and has been shown to reduce both symptoms of allergy and medication use in controlled clinical trials. SIT is associated with improved tolerance to allergen challenge, with a decrease in immediate-phase and late-phase allergic inflammation. SIT prevents the development of new allergic sensitizations and reduces progression of allergic rhinitis to asthma. SIT is disease modifying and has a duration of effect beyond the treatment period. SIT also modifies cellular and humoral responses to allergen. The ratio of T helper 1 (T_H1) cytokines to T_H2 cytokines is increased following SIT, and functional regulatory T cells are induced. The production of interleukin-10 (IL-10) by monocytes, macrophages, B cells and T cells is increased. The expression of transforming growth factor- β (TGF β) is increased and, together with IL-10, TGF β might contribute to regulatory T-cell function and immunoglobulin class switching to IgA, IgG1 and IgG4. These immunoglobulins compete with IgE for allergen binding, decreasing the allergen capture and presentation that is facilitated by IgE in complex with the high-affinity receptor for IgE (Fc ϵ RI) or the low-affinity receptor for IgE (Fc ϵ RII). In addition, SIT reduces the number of mast cells and the ability of mast cells to release mediators. The recruitment of eosinophils and neutrophils to sites of allergen exposure is also reduced.

Skin-prick test

If allergen-specific IgE is present in an individual, then the introduction of tiny amounts of allergen into the epidermis by a prick or scratch induces the degranulation of mast cells. This results in a wheal-and-flare reaction, the size of which is used as a measure of the sensitivity to allergen of an individual.

regulatory responses, has also been achieved using T_H1 -cell-inducing adjuvants, such as microbial components that stimulate APCs through Toll-like receptor (TLR) activation^{32,62,63}.

Humoral and cellular features that are similar to those induced by SIT can also result from naturally occurring immunomodulation. For example, many bee-keepers, despite being frequently stung and having a positive skin-prick test and IgE specific for bee venom, do not develop allergic symptoms when stung⁶⁴. Protection seems to be mediated through the induction of bee-venom-specific IgG following bee stings. Long-term exposure to bee stings drives the IgG response from a mainly IgG1 response to an IgG4 response⁶⁵. Studies of the immune response of cohorts of bee-keepers have indicated that natural systemic exposure to large doses of the protein components of venom resembles SIT with venom, because both SIT and natural exposure lead to modulation of peripheral T-cell responses through the generation of allergen-specific IL-10-secreting T cells and the increased

synthesis of IL-10 by monocytes and B cells⁴². Similar induction of IL-10 production and protective allergen-specific IgG4 production has been observed in children who have been exposed to large concentrations of animal dander⁶⁶.

Modulation of antibody responses following SIT. In a series of elegant experiments, Cooke *et al.*⁶⁷ showed that serum from patients treated using SIT contained allergen-specific factors that prevented immediate allergic skin inflammation. These factors were identified to be allergen-specific IgG molecules that competed with IgE for the binding of allergen and were therefore termed blocking antibodies^{68,69}. However, the relationship between the efficacy of SIT and the induction of allergen-specific IgG remains a controversial issue, with serum concentrations of allergen-specific IgG correlating with clinical improvement in some studies but not in others^{70–72}. But functional activity, rather than the absolute amount of antibody, might be a more appropriate measure and seems to correlate more closely with clinical parameters⁴³. Moreover, antibody responses that are induced during SIT are functionally heterogeneous, perhaps accounting for the conflicting data with respect to the protective effects of IgG^{73,74}. Allergen-specific IgG might be directed against the same epitopes as allergen-specific IgE, resulting in direct competition for allergen binding and a 'blocking' effect. By contrast, induction of IgG specific for other epitopes might result in a failure of the IgG response to compete with IgE, even when IgG is present in molar excess. Furthermore, some IgG specificities might amplify the crosslinking of allergen–IgE–FcεRI complexes, leading to increased effector-cell function⁷⁴.

Nevertheless, IgG that is induced by SIT or by natural allergen exposure has been shown to have numerous effects on cells that are involved in allergic responses and to reduce allergic inflammation. For example, SIT-induced IgG has been shown to reduce the IgE-mediated degranulation of mast cells and basophils in an allergen-specific manner, leading to a reduction in allergic inflammation^{63,73,75}. IgG that is induced by treatment with hypoallergenic recombinant allergens has been found in respiratory secretions and has been shown to reduce acute respiratory symptoms of allergic disease⁷⁶. Allergen-specific IgG that is induced following seasonal exposure to allergens has been associated with attenuation of the increase in serum IgE^{30,63,77}. Other mechanisms of action of SIT-induced IgG include inhibition of IgE-facilitated allergen presentation to T cells, possibly decreasing late-phase reactions to allergen⁷⁸, and reduction in the number of allergen-specific memory B cells that have undergone class switching to IgE (through preventing the activation signals that are required for affinity maturation, memory induction and differentiation)⁷⁹.

Analysis of the IgG isotypes that are induced by SIT has shown that specific increases in IgG1 and, in particular, in IgG4 occur, with the concentrations increasing 10–100-fold; much smaller responses are seen for IgG2 (REFS 30,31,76). Consistent with these findings, removal of IgG4 activity from the sera of individuals who had

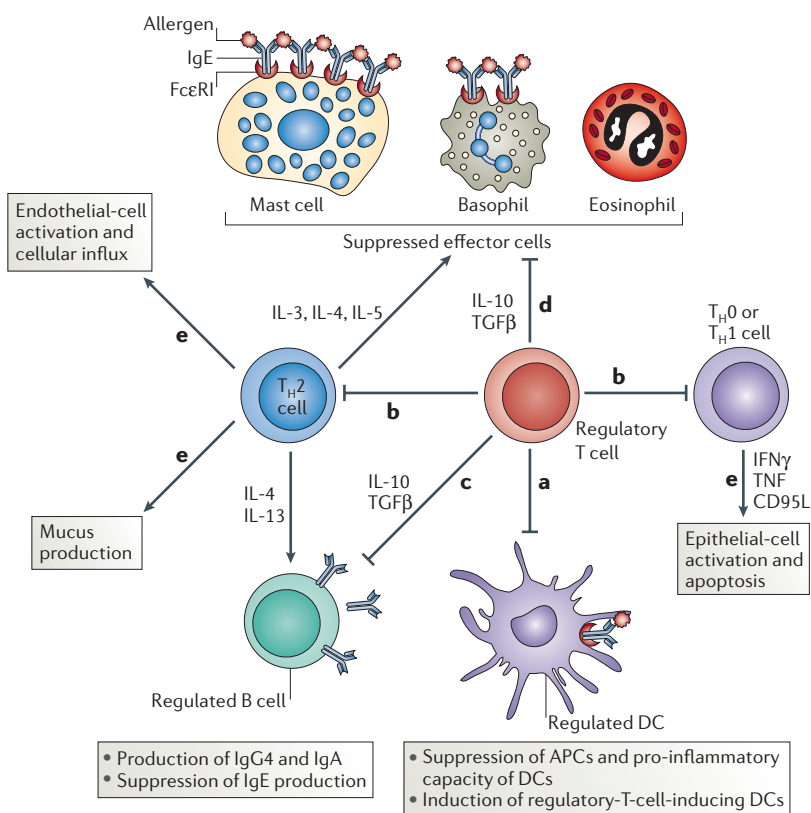


Figure 3 | Proposed role of regulatory T cells and cytokines in allergen-specific immunotherapy. Regulatory T cells and regulatory cytokines — such as interleukin-10 (IL-10) and transforming growth factor-β (TGFβ) — might contribute to the control of allergen-induced immune responses in five main ways: suppression of antigen-presenting cells (APCs) that support the generation of T helper 2 (T_H2) cells and T_H1 cells (a); direct suppression of T_H2 cells and T_H1 cells (b); suppression of allergen-specific IgE production, and induction of allergen-specific IgG4 and/or IgA production (c); and suppression of mast cells, basophils and eosinophils (d). In addition, indirect inhibition of T_H2 -cell-associated phenomena (such as mucus production, and endothelial-cell activation and cellular influx) and T_H1 -cell-associated phenomena (such as epithelial-cell activation and apoptosis) is observed (e). CD95L, CD95 ligand; DC, dendritic cell; FcεRI, high-affinity receptor for IgE; IFNγ, interferon-γ; TNF, tumour-necrosis factor.

received SIT resulted in an almost complete loss of the inhibition of binding of allergen–IgE complexes to FcεRII at the surface of B-cell lines (an assay that is used to measure the blocking activity of IgG)⁴³. This dominant role for IgG4 might result from the unique structural features of the hinge region of IgG4; these features result in a lower affinity than other isotypes for certain Fcγ receptors (FcγRs; which bind IgG) and in the ability to separate and re-pair (leading to bispecific antibodies that are functionally monomeric; reviewed in REF. 80). Furthermore, IgG4 does not fix complement and can inhibit immune-complex formation by other isotypes, giving IgG4 anti-inflammatory characteristics. Indeed, the regulatory cytokine IL-10 is implicated in the differential regulation of IgE and IgG4 production following IL-4- and IL-13-dependent class switching^{42,81}. Although not directly a class-switching factor for IgG4 (REF. 82), IL-10 increases the production of IgG4 while preventing IL-4-mediated class switching to IgE⁸¹. By contrast, IL-10 has been shown to induce direct switching of immunoglobulin classes expressed by naive B cells to IgG1 (REF. 82), possibly accounting for the considerable quantities of IgG1 that have been reported following SIT. Interestingly, IFNγ has been shown to suppress the production of IgG1 and to increase the production of IgG2 (REF. 83). The dominance of IgG1 over IgG2 after SIT indicates that the action of IFNγ might be tempered by other cytokines in the milieu such as IL-4 and IL-10.

In addition to IgG, recent studies have also provided evidence for increases in the amount of TGFβ-driven allergen-specific IgA following SIT, indicating that other antibody classes might contribute to clinical efficacy⁴⁹.

Current developments in SIT

Historically, variability in safety and clinical efficacy has limited the widespread application of SIT. Many strategies have been adopted in an attempt to standardize practice, including better characterization of the active ingredients through measurement of protein content, determination of the biological activity, estimation of the main allergens that make up the preparation, and the production of pure allergen molecules with the aid of recombinant-DNA technology⁸⁴.

Recombinant allergens and allergen derivatives. For most important allergens, cDNAs have now been isolated and expressed as recombinant proteins (reviewed in REFS 85–87). Defined molecules have been used to establish diagnostic tests for determining and monitoring the molecular reactivity profiles of patients to allergens and for developing improved forms of treatment⁸⁴. For example, reactivity profiles of individuals have been defined by using recombinant-allergen microarrays, and these profiles allow SIT to be tailored to individual needs⁸⁵. Recombinant allergens have also been used successfully for diagnostic *in vivo* provocation testing, in which allergens are administered to the skin, conjunctiva and nasal mucosa (reviewed in REF. 86).

At present, several treatment strategies that involve recombinant allergens or engineered allergen derivatives are being pursued in preclinical studies and clinical

trials. The use of recombinant ‘native’ allergens (which retain the sequence and conformation of the natural molecule)³¹ has advantages over the use of natural allergen extracts, because recombinant ‘native’ allergens contain defined amounts of the active ingredient and can be formulated in a standardized manner. However, the use of recombinant ‘native’ allergens retains the risk of adverse allergic events as a consequence of reactivity with IgE. Furthermore, certain allergens are poorly immunogenic and induce only small amounts of protective IgG. To address these issues of immunogenicity and allergenicity, recombinant allergens have been modified *in vitro* to create hypoallergenic molecules that have reduced reactivity with IgE, in common with some naturally occurring polymorphisms^{88–94}. Introduction of multiple mutations into cysteine residues in the latex allergen Hev b 6.01 resulted in mutants that showed less binding, or no binding, to IgE in the sera of individuals who are allergic to latex⁹⁵. Evaluation of the allergenicity of a genetically engineered, hypoallergenic mutant of the apple protein Mal d 1 showed reduced release of histamine by basophils, decreased responses to skin-prick tests and reduced allergenicity, in a double-blinded, placebo-controlled food-challenge study⁹⁶. Recombinant-allergen oligomers (such as genetically engineered recombinant proteins that are expressed as dimers and trimers and have reduced reactivity with IgE) have been produced by expressing several copies of the cDNA that encodes one particular allergen, or the cDNA that encodes several allergens and/or allergen epitopes, in one expression vector^{30,97–99}. When used for therapy, a recombinant trimer of the main birch-pollen allergen, Bet v 1, had less reactivity with IgE in skin-prick tests and was more immunogenic (induced more IgG production) than placebo, inducing a T_H1-cell-biased immune response *in vitro* and *in vivo*^{30,100}. Fragments of recombinant Bet v 1 polypeptide were similarly hypoallergenic but were less immunogenic than the recombinant trimer when administered therapeutically³⁰.

Recombinant hybrid allergen proteins have also been created for use as combination vaccines^{101,102}. These molecules consist of the most important epitopes or polypeptides of one or more allergens, but some of these molecules lack the IgE reactivity of the native molecules^{101,102}. Hybrid allergen proteins contain a range of T-cell epitopes that is similar to complex allergens but in a single molecule, with the immunogenicity of the components being increased as a result of their presence in one molecule, in contrast to separate molecules. A hybrid allergen protein consisting of three major bee-venom allergens in one molecule was shown to be markedly hypoallergenic in human skin-prick tests and could also prevent allergic sensitization to bee venom in a mouse model¹⁰¹. Hybrid allergen proteins generated by the fusion of allergens with human FcγRI have also been reported to inhibit allergen-induced degranulation of basophils and mast cells, by co-crosslinking of FcεRI and FcγRs and by activation of inhibitory intracellular-signalling pathways. This is a novel approach to negatively regulate mast-cell and basophil degranulation in an allergen-specific manner. Furthermore, co-ligation of allergen-specific membrane

Hinge region

The sequence of amino acids, which is often rich in cysteine and proline residues, that is present in the constant region of immunoglobulin heavy chains. It provides increased molecular flexibility. This region might be involved in the disulphide bonds that link adjacent immunoglobulin heavy chains.

Allergen microarray

Similar to a DNA microarray. High-density arrays of individual allergen proteins or extracts spotted onto a solid phase (usually glass). The arrays can be incubated with small volumes of serum from patients, and the binding of IgE to the arrayed allergens can be determined and this information used for diagnostic purposes.

immunoglobulin with FcγRs might also downregulate B-cell responses to allergen¹⁰³. Until further trials have been carried out (particularly of hypoallergenic isoforms and mutants), it is unclear which of these approaches will be the safest and the most efficacious.

T-cell-epitope-containing peptide approaches. Peptides that consist of the amino-acid sequences of allergen T-cell epitopes retain the ability to immunomodulate T cells, but these short linear molecules have less ability to crosslink IgE and activate effector cells than do the corresponding intact allergens. Synthetic-peptide-based vaccines derived from the major cat allergen, Fel d 1, and from bee-venom phospholipase A₂ (Api m 1) have been developed and clinically evaluated^{29,58,104–107}. Early studies of peptide-based SIT for individuals who are allergic to cat allergens showed moderate clinical efficacy but also showed frequent adverse events that might have been associated, in some cases, with residual tertiary structure in the peptides leading to crosslinking of IgE. Other symptoms were probably the result of transient activation of effector T cells in the target organ, resulting in airway narrowing¹⁰⁸. More recent studies that used mixtures containing more peptides, which consisted of shorter sequences, showed the following outcomes: downregulation of systemic T_H1- and T_H2-cell responses to allergen, together with concomitant induction of IL-10 production^{58,106}; induction of regulatory T cells⁵⁰; reduction of immediate- and late-phase allergic skin reactions following allergen challenge^{106,109}; and reduction in late-phase asthmatic responses to inhaled allergen challenge, and improved nasal symptoms after nasal provocation¹¹⁰. Peptide-based SIT for individuals who are allergic to bee venom was associated with protection against cutaneous allergen challenge and partial protection against live bee-sting challenge¹⁰⁵. Evidence was also provided that allergen-specific IgG is produced^{58,105,107}. The use of short peptides and small (<50 μg) doses might be optimal for both safety and efficacy, although further clinical trials, testing larger groups, are needed.

B-cell-epitope-containing peptide approaches. Low-molecular-weight fractions of allergen extracts and drug-derived haptens have been used in SIT^{26,58,111}. More recently, hapten-like peptides have been identified within major allergens, and it has been proposed that these molecules could be used for SIT¹¹². Synthetic peptides with the potential to induce the production of blocking IgG have been defined from IgE-binding epitope-mapping data¹¹³ and from analysis of the three-dimensional structure of an allergen¹¹⁴. Vaccination of animals with such peptides induced allergen-specific IgG that was able to block patient-derived IgE from binding to allergens *in vitro* and to prevent allergen-induced degranulation of basophils¹¹⁵. Phage-display technology has been used to isolate random peptides that are recognized by allergen-specific IgE, and subsequent molecular modelling showed that these peptides correspond to IgE-binding epitopes on the allergen surface^{116,117}. Such epitope mimics (known as mimotopes) could partially inhibit the high-affinity interaction between allergen and specific IgE¹¹⁶.

Conjugated molecules, adjuvants and modes of application. Targeting TLRs with TLR ligands linked to allergens has been proposed as a strategy for modulating immune responses to allergens, with the aim of inducing a shift in the balance of the T_H1- and T_H2-cell responses. Synthetic DNA sequences that contain CpG motifs have been covalently linked to Amb a 1, the major ragweed allergen, to generate immunostimulatory allergen conjugates^{32,118}. In addition to interaction with TLR9, covalent linkage of CpG-containing DNA sequences reduced the allergenicity of Amb a 1 in skin-prick tests, probably through steric hindrance of the access of IgE to the allergen. Treatment of individuals who are allergic to ragweed with the CpG-containing-DNA-allergen complexes resulted in marked increases in allergen-specific T_H1-cell responses in the peripheral blood, and these responses were characterized by increased production of IFNγ, CXC-chemokine ligand 9 (CXCL9) and CXCL10, and decreased production of IL-5, CC-chemokine ligand 17 (CCL17) and CCL22 (REFS 32,118).

Monophosphoryl lipid A, a bacterial cell-wall component that binds TLR4, has been evaluated as an adjuvant for use in SIT. Clinical trials showed improved symptom and medication scores, together with reduced seasonal increases in IgE and an increase in IgG1 and IgG4 that resulted in blocking of allergen-induced degranulation of basophils⁶².

Other adjuvants, such as aluminium hydroxide, are in use in SIT. Paradoxically, aluminium hydroxide is also widely used to increase allergic sensitization in mouse models of allergic disease, so its use in the treatment of allergic disease in humans seems counter-intuitive. However, recent studies have indicated that this adjuvant downregulates human T_H2-cell responses to allergens *in vitro*¹¹⁹. Carbohydrate-based particles (CBPs) could provide an alternative to aluminium hydroxide and have the advantage of a defined coupling chemistry. CBP-coupled grass-pollen allergen induced robust IgG responses in an experimental model of allergic sensitization, without inducing tissue destruction or systemic release of allergens. Instead, a local immune response was generated at the site of administration, thereby rendering systemic anaphylactic reactions less likely¹²⁰. However, CBPs have yet to be evaluated in human trials of SIT.

Several other approaches are also under investigation. Lectins have been used to target allergen directly to α-L-fucose moieties on microfold (M) cells, which are present in mouse intestines, and this strategy results in sustained release of allergen into the draining lymphatic vessels and in an increase in allergen-specific IgG2a¹²¹. A recombinant fusion protein consisting of Bet v 1 and a bacterial cell-surface protein has been shown to induce T_H1-cell responses in mice and in *in vitro*-cultured human cells¹²². Lipopeptides derived from lipoproteins of Gram-negative bacteria activate TLR2, and they promote production of the T_H1 cytokines IFNγ and IL-10 (but not T_H2 cytokines) by human T cells in response to the bee-venom allergen Api m 1 (REF. 123). Transgenic rice that expresses allergen-derived T-cell epitopes has also been evaluated in a mouse model for its potential as an edible vaccine, and it was found to induce oral

Phage-display technology

A technology for displaying a protein on the surface of a bacteriophage that contains the gene(s) encoding the displayed protein(s), thereby physically linking the genotype and phenotype.

Microfold cells

(M cells). Specialized epithelial cells that deliver antigens from the gut lumen directly to intraepithelial lymphocytes and to subepithelial lymphoid tissues, using transepithelial vesicular transport.

tolerance¹²⁴. Finally, mice that are allergic to peanuts have been treated by rectal administration of heat-killed *Escherichia coli* that expresses peanut allergens. This treatment resulted in an increase in the amount of T_H1 cytokines and a decrease in the amount of T_H2 cytokines, together with a reduction in the amount of allergen-specific IgE and in allergic symptoms following subsequent challenge with peanut allergens¹²⁵.

At present, owing to the limited number of clinical trials of adjuvanted preparations and conjugated molecules, the benefit of these approaches over the current methods of SIT is not clear. Clinical trials of these approaches (which are at preclinical stages, at present) are required before objective evaluations can be made.

DNA vaccination. As an alternative to the administration of allergens or allergen derivatives, vaccination with allergen-encoding DNA has been proposed as a strategy for SIT^{126,127}. In addition to injection of plasmid DNA, the oral delivery of genes, using chitosan–DNA nanoparticles, has been described¹²⁸. Vaccination with allergen-encoding DNA seemed to induce an allergen-specific T_H1 -cell response and, when administered prophylactically in mouse models, prevented allergic sensitization. However, systemic production of allergen by transfected cells has the potential to cause anaphylaxis¹²⁹. One possibility to reduce allergenicity would be to use DNA that encodes hypoallergenic allergen derivatives. Finally, the targeting of DNA vaccination directly to DCs, and the use of replicon-based DNA vaccines that induce allergen-specific T_H1 -cell responses when delivered at low doses, is being evaluated in mice¹³⁰.

Sublingual versus subcutaneous delivery for SIT

Recently, interest has focused on the delivery of allergen orally, particularly by sublingual administration. The first reports of mucosal SIT were made more than 60 years ago^{131,132}, and in the past 25 years, numerous clinical trials have been carried out to study sublingual SIT (reviewed in REF. 133). A meta-analysis of the double-blinded, placebo-controlled trials that have been carried out in the past decade has shown that sublingual SIT is clinically efficacious, although the treatment benefit is about half that achieved with subcutaneous SIT at present¹³⁴. In common with SIT administered by subcutaneous injection, sublingual administration of allergen has been shown to reduce the development of asthma in children with allergic rhinitis. The immunological mechanisms of sublingual SIT seem to be similar to those of subcutaneous SIT, although the magnitude of the change in most parameters is more moderate. Moreover, a considerable number of studies have failed to detect systemic immunological changes. This might be a consequence of the different doses of allergen that were administered in different studies or of the development of more localized immunological changes. The limited mechanistic data relating to sublingual SIT have recently been reviewed in depth¹³⁵. Most studies using sublingual SIT have reported increased amounts of serum IgG4 (REF. 134), with some evidence of increased amounts of IgA¹³⁶. However, decreases in the ratio of IgE to IgG4

following SIT have not been consistently observed¹³⁷. The correlation of immunological parameters with clinical parameters has been observed in one study¹³⁸ but not in another¹³⁹. Reports of the effect of sublingual SIT on T-cell responses are also mixed. Reduced proliferation of peripheral-blood T cells in response to allergen was reported in one study¹⁴⁰, although no changes in the cytokine-secretion patterns were observed for T-cell clones derived before and after SIT¹⁴⁰. However, other studies have failed to detect a change in T-cell parameters^{137,138}. Sublingual SIT also reduces the number and the size of inflammatory-cell infiltrates in allergen-challenged tissues¹⁴¹. Following sublingual SIT and allergen challenge, the amount of serum eosinophil cationic protein (also known as RNase3) was reduced, together with the number of eosinophils and neutrophils in the conjunctiva and the expression of intercellular adhesion molecule 1 (ICAM1; a marker of allergic inflammation) by epithelial cells¹⁴¹. Similar changes in eosinophils, neutrophils and ICAM1 expression were observed in the nasal mucosa following nasal challenge¹⁴².

Although sublingual SIT is clearly efficacious and is associated with a favourable safety profile, efficacy is lower than that of subcutaneous SIT. The better safety profile of sublingual SIT might be associated with specific anatomical features of the oral mucosa, such as the presence of fewer mast cells, although it might equally be attributable to the delivery of smaller immunologically active doses of allergen (despite the larger dose that is administered). So future studies that use larger doses of allergen might achieve equivalent clinical efficacy but at the expense of the favourable safety profile.

Future directions

The immunological mechanisms of SIT are becoming increasingly well defined. The modulation of APC, T-cell and B-cell responses to allergen has been described in numerous studies. Successful SIT is associated with several features, including increases in allergen-specific serum antibodies (particularly IgG1 and IgG4 and, to a lesser extent, IgA). The proliferative responses of T cells to allergens are reduced, and cytokine-secretion profiles are modified, resulting in an increased ratio of T_H1 -cell responses to T_H2 -cell responses and in the induction of functional regulatory T cells. Regulatory T-cell function and changes in serum-antibody profiles seem to be associated with expression of IL-10 and TGF β . In the future, therapeutic strategies will attempt to harness such mechanisms to optimize clinical efficacy. For example, adjuvants that favour the induction of regulatory cytokines might be identified. Or pharmacotherapy could be combined with SIT to induce optimal clonal expansion of regulatory T cells¹⁴³.

With most of the important allergens now identified and with their T-cell and B-cell epitopes characterized, the use of defined allergen molecules and allergen derivatives will clarify the heterogeneous data relating to mechanisms and clinical efficacy that were obtained in earlier studies using crude allergen extracts. Hypoallergenic allergens and allergen derivatives that have reduced interaction with IgE might improve the

Chitosan–DNA nanoparticle
A small (150–300 nm) particle that is formed by mixing plasmid DNA (in this case, encoding allergen) with the high-molecular-weight molecule chitosan, a biodegradable polysaccharide derived from crustaceans. Chitosan has been used extensively for drug delivery and increases molecular transport across the mucosal epithelial-cell barrier.

Replicon-based DNA vaccine

A vaccine that is based on DNA molecules that can replicate autonomously (for example, plasmids and phage).

safety of SIT. However, loss of IgE binding might reduce the uptake of allergen by tolerogenic DCs that present FcεRI-bound IgE. Furthermore, in the absence of epitopes that are recognized by IgE, the induction of competitive antibody classes of the same specificity might be less efficient.

Optimization of the sublingual approach for SIT will result in improved clinical efficacy, but this might come at the price of reduced safety. Further developments in adjuvant technology and improvements in our understanding of how to optimize the routes and schedules of administration will also help to improve

the safety, efficacy and delivery of SIT. In the future, SIT will take advantage of molecularly defined diagnosis, such as using allergen microarrays, to address the needs of individual patients and their particular clinical manifestations. Finally, in light of the increasing prevalence of allergic diseases, population studies might identify susceptible individuals, together with the most relevant allergen molecules from which to formulate prophylactic strategies based on vaccination and/or tolerance induction. Ultimately, prophylaxis might be used to prevent allergic diseases, similar to the current strategy of vaccination against infectious diseases.

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Competing interests statement

The authors declare **competing financial interests**: see web version for details.

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