Allergology International (2002) **51**: 221–231

Review Article

Allergen immunotherapy: Current and new therapeutic strategies

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Abstract

Allergen-specific immunotherapy (SIT) involves the administration of gradually increasing amounts of an allergen extract to reduce clinical symptoms of allergy. Well-controlled clinical trials have demonstrated the efficacy of SIT in the treatment of allergic diseases, including rhinoconjunctivitis and asthma, and best practice protocols have been established. Nevertheless, application of this potentially curative treatment is restricted, largely due to the risk of serious adverse events, especially in asthmatics. Although efficacy is high for venom-induced allergy, success rates for the more common aeroallergen-induced disease range from 60 to 80% depending on the allergen. The practice of SIT is currently being refined following major advances in our knowledge of basic immune mechanisms. In particular, new T cell-targeted strategies are being explored with the awareness of the pivotal role allergen-specific T cells play in initiating and regulating the immune response to allergens. Current SIT induces decreased IgE class switching and eosinophil activation by downregulating production of the T helper (Th) 2-type cytokines interleukin (IL)-4 and IL-5. Therefore, allergen preparations that have ablated IgE binding while retaining T cell reactivity should still be clinically effective but have substantially improved safety. These approaches include the use of small peptides based on dominant T cell epitopes of allergens and chemically modified or recombinant allergen molecules. Both approaches have already been tested, with promising results, in animal models; peptide immunotherapy has been shown effective in clinical trials. Defined hypoallergenic molecules or peptides offer ease of standardization in addition to efficacy and safety and will result in more widespread use of SIT in clinical practice. Elucidation of mechanisms for downregulating Th2-predominant responses to allergen by SIT will enable the development of laboratory assays for monitoring clinical efficacy.

Key words: allergen, IgE, immunotherapy, T cell.

Introduction

Allergic diseases, including asthma, rhinitis/conjunctivitis and eczema, are common and are estimated to affect up to one-quarter of the population in developed countries at some time in their lives. The allergic diseases are a class of disorders that result from interactions between an inherited genetic predisposition and the local environment. Allergy may be defined as an aberrant immune-mediated response to commonly encountered substances that are otherwise harmless. Allergic diseases mainly affect atopic individuals, who synthesize specific IgE to common environmental allergens, usually proteins or glycoproteins. These include allergens of house dust mites (HDM), grass, tree and weed pollens, domestic pets (cats and dogs) and various molds. The major perennial allergens are from HDM and cats, with pollens forming the main sources of seasonal allergens.

Immunoglobulin E is the main specific effector molecule in the immune response to allergens. Re-exposure to the relevant allergen of sensitized skin or airways of atopic subjects may induce a biphasic response, with an immediate hypersensitivity and a delayed inflammatory component. Allergen can cross-link specific IgE bound to

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Accepted for publication 10 September 2002.

high-affinity IgE receptors (Fc∈RI) on the surface of mast cells or basophils, inducing calcium flux and the subsequent release of inflammatory and vasoactive mediators, including histamine, leukotrienes and cytokines. The late-phase response predominantly results from sustained effects of mediators such as prostaglandins, leukotrienes (LT) B_4, LTC_4 and platelet-activating factor released from the recruited cellular infiltrate of eosinophils, neutrophils, macrophages and lymphocytes (for review see Kay[1]).

The $CD4^+$ T cells play a central role in orchestrating the immune response to allergen. The peripheral $CD4^+$ T cell repertoire of both atopic and non-atopic individuals includes recognition of allergens, but the nature of the immune response differs between atopic and non-atopic subjects.[2] Allergen is initially taken up by antigen-presenting cells (dendritic cells, monocytes/macrophages, B cells), processed and presented as peptides to specific antigen receptors on the T cell surface in association with major histocompatibility complex (MHC) class II molecules. In atopic individuals, the activated allergen-specific T cell secretes a T helper (Th) 2-type cytokine profile, dominated by interleukin (IL)-4, IL-5 and IL-13, and these cytokines, together with cognate B and T lymphocyte interactions, result in immunoglobulin class switching with specific IgE synthesis by B cells. In contrast, activated allergen-specific T cells from non-atopic subjects elaborate a Th1-type cytokine profile with dominant interferon (IFN)-γ secretion. The nature of the immune response is determined by factors including the genotype of the individual, antigen form and concentration, the antigen-presenting cell and the local cytokine milieu.

Conventional treatment for allergic diseases is primarily the use of non-specific pharmacotherapy, including antihistamines, β_2-adrenergic receptor agonists, leukotriene receptor antagonists, corticosteroids and, occasionally, epinephrine for symptomatic relief, and specific therapy targeting the underlying disease process.[3] Specific therapies include allergen avoidance where feasible and allergen-specific immunotherapy (SIT). A clear demonstration of symptoms on exposure to the offending allergen with documentation of allergen-specific IgE is required for recommendation of specific therapies.

Allergen-specific immunotherapy has been used in clinical practice since 1911, when it was pioneered by Noon[4] and Freeman.[5] Conventional SIT involves vaccination with increasing subcutaneous quantities of allergen in order to hyposensitize the patient and achieve both immunologic and physiologic tolerance on subsequent allergen re-exposure. Allergen-specific immunotherapy is an attractive treatment option because it may alter the natural course of allergic disease. Controlled trials have demonstrated efficacy for stinging insect allergy, allergic rhinitis/conjunctivitis and allergic asthma in appropriately selected patients.[6] Research in children has shown that SIT may prevent the development of new sensitivities in monosensitized patients[7,8] or the progression to asthma in patients with allergic rhinitis.[9,10] Currently, immunotherapy with unfractionated extracts of natural rubber latex and foods such as peanuts and shellfish has proved unsatisfactory due to the high risk of systemic adverse events and is used only for research studies. Elucidation of underlying mechanisms of SIT and of relevant antigen preparations for efficacy is required to develop more widespread use of SIT in routine clinical practice.

Allergen-specific immunotherapy: Current strategies

Allergen preparations and therapeutic regimens

The majority of allergenic extracts in current use for SIT are unfractionated alum-precipitated preparations with delayed allergen absorption to reduce adverse events particularly associated with IgE-mediated mast cell and basophil degranulation. Aqueous allergen extracts are available with higher potency due to more rapid absorption and a concomitantly higher risk of adverse events.

Allergen extract standardization is increasingly emphasized to improve the overall clinical efficacy of SIT with determination of total allergenic proteins and biological activity. Traditional unfractionated extracts were characterized to a biological standard 'Bioequivalent Allergy Units' by correlation of skin test potency compared with histamine controls using a known panel of highly sensitized subjects. However, alum-precipitated extracts cannot be accurately standardized by these methods. With the availability of improved biochemical and molecular immunologic techniques, including the generation of allergen-specific monoclonal antibodies, more importance is now placed on quantitation of the dominant allergens in therapeutic extracts; for example, measurement of the Der p 1 concentration in an HDM *Dermatophagoides pteronyssinus* extract. Clinical studies have clearly demonstrated that mixtures of unrelated allergens may be less efficacious.[11] Well-characterized allergen extracts of known potency and shelf life are recommended for both effective diagnosis and treatment regimens in

accordance with the guidelines of the World Health Organization.[6]

Traditionally, SIT involves the administration of incremental doses of allergen extract in an initial up-dosing regimen followed by maintenance treatment (reviewed by Rolland *et al*.[12]). The subcutaneous route is still the most commonly used, although sublingual therapy is increasingly popular in central Europe.[13] The different dosage schedules include standard, rush and ultra-rush regimens. Standard SIT involves weekly or two-weekly injections during the up-dosing induction phase, followed by maintenance injections at increasing intervals as tolerated, usually up to monthly intervals. Typically, subcutaneous injections are administered into the soft tissue of the upper lateral arm followed by a 45 min observation period in case of an adverse event. Rush immunotherapy over, perhaps, 5 days and ultra-rush immunotherapy over 1 day may be used in hospital-based allergy units with more rapid dose escalations at 30–60 min intervals. The immediate availability of epinephrine and adequate cardiorespiratory resuscitation facilities to treat anaphylaxis if necessary are mandatory if SIT is offered to patients as a treatment option.

Clinical efficacy

Immunotherapy for allergic disease is of proven efficacy for allergic rhinitis/conjunctivitis and for insect venom allergy. In contrast, although meta-analyses have demonstrated efficacy of SIT in asthma for HDM allergy,[14] the role of SIT for the treatment of asthma remains controversial with regard to adverse events, cost-benefits and efficacy. Insect venom allergy resulting in systemic allergic reactions is an absolute indication for SIT, with respiratory allergies providing a relative indication depending on the clinical history of the individual patient and the offending allergen.

Efficacy is judged on the basis of subjective and objective outcomes. Symptom and medication scores can be monitored by diarization for comparison between therapeutic modalities. Numerous controlled studies demonstrate high efficacy in insect venom hypersensitivity.[15] Similarly, the efficacy of SIT in seasonal allergic rhinitis is well established.[16] A meta-analysis of clinical trials of HDM SIT in selected asthmatic populations reported efficacy[17] and a recent Cochrane's analysis again demonstrated improvement in symptoms and bronchial reactivity to allergen, but no objective improvement in lung function was determined.[14] Efficacy of SIT using a standardized cat dander extract has also been reported.[18]

IgE-mediated side-effects of immunotherapy

Local reactions to conventional SIT for inhalant allergens or venom occur in 25% of patients, but are not a contraindication to therapy. Large local reactions (> 5 cm) require a dosage reduction. The estimated frequency of systemic adverse events is one event per 500 injections.[19] Typically, such reactions occur within 15–20 min, although they may occur up to 45 min after dosage administration. Deaths are infrequent and almost invariably follow a departure from recommended best practice, in particular by delayed treatment of anaphylaxis.

Laboratory assays for monitoring efficacy of immunotherapy

Currently, there are no approved laboratory assays for monitoring the clinical efficacy of SIT and treatment regimens are largely empiric. Specific IgE levels assayed by skin prick tests and radioallergosorbent test (RAST) are useful in the diagnosis of allergic disease but, as will be discussed further in the present review, are not reliable indicators of clinical improvement. Suitable assays for monitoring SIT will develop from a greater understanding of the underlying mechanisms of clinically effective SIT.

Proposed mechanisms of clinically effective allergen SIT

Many different regimens with respect to the allergen (inhalant or venom), route of administration, allergen form and concentration, adjuvant, dosing interval and duration of therapy are currently in clinical use for allergen SIT with probable differing mechanisms of action. Immunologic studies investigating the mechanisms of effective immunotherapy following parenteral administration of allergen in the human model are limited. The majority of the available data has been derived from evaluation of SIT given via the subcutaneous route, although increasing interest is focusing on sublingual and intranasal routes of administration, with efficacy reported in double-blind, placebo-controlled studies.

Because allergen-specific IgE antibodies are the hallmark of the immune response to allergens, early studies

investigating the mechanisms of SIT focused on changes in allergen-specific antibodies. Characteristically, allergen-specific IgE antibody levels increase initially, followed by a decrease several months or years after treatment and with poor correlation to the overall clinical efficacy.[20–22] These changes in specific IgE are accompanied by increases in allergen-specific IgG1 and IgG4 antibody subclasses, which have been suggested to act as 'blocking' antibodies, but, again, there has been poor correlation between the timing of these changes and of clinical efficacy.[23,24] It was suggested that IgG1 and IgG4 antibodies compete with IgE for binding sites on the allergens, with the net result of preventing allergen-induced activation of basophils and mast cells and consequent prevention of the release of inflammatory mediators.[25] However, more recent studies have suggested that these blocking antibodies may play a role by the prevention of $CD4^+$ T cell activation mediated by serum IgE-facilitated allergen presentation.[26] Prior to SIT, allergen-specific IgE facilitates antigen presentation to $CD4^+$ T cells via CD23, the low-affinity IgE receptor, with activation in response to even very low allergen concentrations. Post-SIT, in the presence of increased levels of allergen-specific IgG, there is a requirement for much higher allergen concentrations in order to induce T cell activation.

Allergen-specific immunotherapy reduces both early and late-phase responses to allergen challenge, consistent with reduced inflammatory cell activity. There are reports of reduced recruitment of eosinophils into challenge sites following SIT[27] and of reduced release of inflammatory mediators, such as eosinophil cationic protein and platelet-activating factor.[28,29] Changes in the allergen-induced cytokine profile of cells in the nasal mucosa are also seen after SIT: increased expression of mRNA for IFN-γ and IL-12 has been reported following grass pollen immunotherapy.[30]

Recent studies investigating immunologic mechanisms of SIT demonstrate convincingly that altered T cell function correlates well with clinically efficacious SIT (reviewed by Rolland and O'Hehir[31] and Akdis and Blaser[32]). Allergen-specific inhibition of T cell proliferation is observed with a trend to decreased secretion of both Th1 and Th2 cytokines. Overall, however, there tends to be a decrease in the ratio of IL4/5 to IFN-γ levels more typical of a non-atopic subject. The changes in the prototypic Th1 and Th2 cytokines are accompanied by increased CD25 surface expression on the T cells and enhanced IL-10 production[33,34] and IL-12 signaling from antigen-presenting cells.[35] With improved assay sensitivity to allow whole blood sampling, assays of T cell cytokine changes during SIT may provide future laboratory options for objectively monitoring the efficacy of SIT.

Altered T cell function following SIT

It is now clear that the nature and degree of the T cell response to environmental allergen determines whether clinical tolerance or a pathological antibody and inflammatory cell response ensues. The observed changes in allergen-specific antibody levels and inflammatory cell activity following clinically effective SIT can be accounted for by the decreased T cell proliferative and cytokine response to allergen with a shift from a predominantly Th2- to a Th1-type profile. Elucidation of the mechanisms by which T cell function is altered by allergen immunotherapy will permit refinement in allergen administration regimens to more effectively achieve this change. Proposed mechanisms include anergy or deletion of allergen-specific Th2 cells, immune deviation and expansion of a regulatory cell subset.

Anergy

T cell anergy is defined as the antigen-induced induction of specific non-responsiveness to subsequent challenge with immunogenic concentrations of antigen.[36] That T cells have been silenced and not deleted can be demonstrated by a proliferative response to IL-2. *In vitro* models have demonstrated that T cell anergy can be induced by treatment with high doses of antigen in the form of dominant epitope peptides[36] or antigen stimulation in the absence of antigen-presenting cells (i.e. costimulation).[37] T cell anergy as a mechanism for altered T cell function with SIT is feasible because doses of allergen given in immunotherapy regimens are estimated to be considerably higher than those encountered naturally. However, precise measurement of relevant environmental allergen levels is difficult.[38] Allergen is also usually administered by subcutaneous injection rather than via the mucosal route, as for natural allergen exposure, resulting in a different type and activation state of antigen-presenting cell. Thus, both high allergen dose and altered antigen presentation could predispose to anergy induction by immunotherapy.

Clear evidence for anergy induction from clinical studies of immunotherapy is difficult to obtain due to the polyclonal nature of the analyzed T cell populations. However, studies by Akdis *et al*. point to T cell anergy as

an important mechanism for a decreased Th2-type response following bee venom immunotherapy.[39] After 2 months treatment, patients showed a marked decrease in T cell proliferative response to the major bee venom allergen phospholipase A_2 (PLA_2). Production of IL-4, IL-5 and IL-13, as well as IL-2 and IFN-γ, by allergen-stimulated T cells was also reduced. There was no change in response to control antigens purified protein derivative and tetanus toxoid, demonstrating antigen specificity of the immunotherapy. Further evidence for induction of specific anergy in allergen-reactive T cells was the almost complete recovery of the allergen-induced proliferative response when IL-2 or IL-15 was added to the *in vitro* cultures.

Increased IL-10 production was shown to parallel decreased proliferative response to PLA_2 following bee venom immunotherapy in these patients and the addition of neutralizing anti-IL-10 antibody to cultures could reconstitute the proliferative and cytokine response.[33] In a similar study on insect venom immunotherapy, Bellinghausen *et al*.[34] found an increased number of IL-10-producing cells in peripheral blood after treatment and a decreased number of IL-4-producing cells. Addition of blocking anti-IL10 antibodies to cultures restored proliferative responses to allergen and enhanced allergen-induced IFN-γ production. It has been suggested that elucidation of factors that influence the production of regulatory IL-10 and, thus, induction of specific anergy and the production of local IL-2 for subsequent reactivation of anergized T cells to a Th1-biased pathway will be pivotal to the development of improved immunotherapy strategies.[40]

Apoptosis

The decreased proliferative response to allergen following immunotherapy may also be accounted for by deletion or apoptosis of allergen-specific T cells. Activation-induced cell death by apoptosis is a recognized mechanism for T cell homeostasis and may follow repeated high-dose antigen stimulation in the periphery.[41] Therefore, apoptosis of allergen-specific T cells may be expected to be a mechanism by which allergen immunotherapy downregulates the pathogenic Th2 response to allergen. It is difficult to demonstrate induction of apoptosis *in vivo*, but analysis of blood lymphocyte susceptibility to apoptosis following allergen restimulation in culture has provided one approach. In a study of rye grass- and olive tree pollen-sensitive patients treated with specific immunotherapy, apoptosis of blood lymphocytes after 4 days of culture with allergen was shown to be increased compared with a control untreated patient group using Annexin-V staining and flow cytometric analysis.[42] In a subsequent study, dual terminal deoxyribonucleotidyl transferase-mediated dUTP–digoxigenin nick end-labeling (TUNEL) and intracellular cytokine staining revealed preferential apoptosis of Th2-type cells after immunotherapy.[43] Interestingly, in a study of mitogen-treated peripheral blood T cells, those cells that had been anergized were more susceptible to Fas-mediated apoptosis,[44] suggesting that anergy and apoptosis may act in concert during immunotherapy.

Immune deviation

Whether a committed T cell can change from predominant Th2 cytokine production to Th1 is controversial. Nevertheless, model systems using allergen-specific T cell clones provide a precedent for such 'immune deviation'. Pretreatment of a HDM allergen-specific T cell clone with a high dose of specific peptide not only induced a markedly decreased proliferative response to an immunogenic concentration of allergen, but also altered the cytokine profile on rechallenge.[45] Secretion of IL-4 could not be detected, whereas IFN-γ production was uninhibited. In another model, a dose-dependent skewing of cytokine profile of PLA_2-specific T cell clones was demonstrated; higher doses favored IFN-γ production over IL-4.[46] While clinical studies consistently show decreased Th2-type cytokine profiles accompany effective immunotherapy, IFN-γ production has been reported to increase, be unchanged or decrease depending on the allergen (reviewed by Rolland and O'Hehir[31]). Whether there is altered cytokine production by committed Th2-type cells, expansion of Th1 cells or differentiation of Thp/Th0-type cells to Th1 is not clear. Several factors may influence whether naïve allergen-specific T cells differentiate into polarized Th1- or Th2-type cytokine positive cells.[47] During allergen immunotherapy, high antigen dose will favor the induction of local Th1-type cytokines and, thus, expansion of committed Th1 cells and differentiation of naïve T cells to the Th1 type. However, to date, only limited cytokine panels have been evaluated in most studies and there is ample evidence from model studies that antigen-induced changes in cytokine profiles may be more complex than simply skewing from the Th2 to the Th1 type.

Regulatory cells

Evidence for the induction of antigen-specific suppressor cells by allergen immunotherapy was presented as early as 1980.[48] However, the role of $CD8^+$ T cells in mediating clinical tolerance to allergens by production of IFN-γ remains controversial. In animal models, IFN-γ-producing $CD8^+$ cells have been implicated.[49] By producing IFN-γ, such a subset could inhibit the Th2 cell proliferative response to allergen and skew the cytokine profile of allergen-specific cells. Interestingly, when human peripheral blood cells from atopic donors are stimulated repeatedly with a high concentration of allergen in culture for 2 weeks, there is expansion of not only $CD4^+$ IFN-γ-positive cells, but also $CD8^+$ IFN-γ-positive cells (LM Gardner, unpubl. obs., 2002). Of note is the fact that despite increased production of IFN-γ by $CD4^+$ and, possibly, $CD8^+$ cells following immunotherapy, patients do not experience cell-mediated pathology at sites of allergen encounter. This observation may be explained by the simultaneous production of the anti-inflammatory cytokine IL-10, as described earlier. Interleukin-10 has been shown to be produced by $CD4^+$ T cells within a week of commencing bee venom immunotherapy.[40] The $CD4^+$ IL-10-positive phenotype is consistent with the Tr1 regulatory cell subset reported to down-regulate a pathological immune response in a murine model of colitis.[50]

Strategy for T cell-targeted immunotherapy

With the growing appreciation that clinical efficacy of SIT is associated with an altered T cell response to allergen, refined allergen preparations that specifically target allergen-specific T cells are under investigation. Because only T cell recognition of the preparation is required, IgE reactivity can be ablated with consequent improved safety, as well as efficacy, of this approach. There is a vast literature on the serologic and molecular characterization of clinically important allergens of dust mites, pollens, foods and animal dander, although 'new' allergens are still being described (e.g. for cat[51] and HDM[52]). The majority have been cloned and sequenced. Crucial information for the design of new SIT preparations is the range of dominant T cell epitopes of major allergens.

Knowledge of T cell-reactive sites of allergen molecules has been slow to emerge because T cell assays are more cumbersome and technically demanding. Subject group sizes for these studies have been necessarily smaller. T cells recognize antigen as small peptides presented on the surface of antigen-presenting cells, such as dendritic cells in the context of MHC class II molecules. Because the frequency of antigen-specific T cells in the peripheral blood is estimated to be of the order of $1 : 10^5$, the conventional approach to T cell epitope mapping is to first expand allergen-specific T cells in culture for 2–3 weeks before testing proliferative and cytokine responses to nested sets of synthetic peptides presented by autologous antigen-presenting cells. Precise identification of the core epitope within a T cell-reactive peptide requires further testing of T cell clones with truncated peptide series.[53] Critical MHC anchor and T cell receptor contact residues may also be identified using single amino acid substituted peptides.[53]

Alternatively, algorithms have been devised for predicting T cell-reactive sites of a molecule based on known MHC class II restricted peptide motifs. In the case of allergen molecules, this approach has had limited success and has not always correlated with observed responses by patient T cells.[54] Thus, validation with *in vitro* T cell assays is a prerequisite for defining clinically relevant T cell epitopes.

From studies to date, patterns of T cell reactivity to allergens are emerging. Importantly, allergens that are defined as 'major' on the basis of specific IgE reactivity are also major T cell allergens.[55] For a particular allergen, T cell epitopes appear to be scattered throughout the molecule, although regions of high frequency of reactivity can be found.[55–57] Peptides induce a similar Th2-skewed cytokine profile in atopic donor T cell cultures, as do whole allergen molecules.[58] There appears to be considerable promiscuity of binding of T cell-reactive peptides to MHC class II molecules: frequencies of peptide recognition within a population are commonly as high as 50%.[55,58] Thus, limited sets of dominant T cell epitopes can be selected for targeting a large patient pool.

Based on a knowledge of dominant T cell epitopes of an allergen, T cell-targeted strategies for improved SIT can be devised. These include T cell-reactive peptides or modified/recombinant allergen molecules that are non-IgE reactive.

Peptide immunotherapy

Short T cell-reactive peptides offer considerable advantages for SIT because they are too small to cross-link cell-bound IgE and, thus, can be given safely at high doses, with consequent improved efficacy. An alternative approach for peptide immunotherapy is to use an altered peptide ligand where a dominant T cell epitope has been modified by a single amino acid substitution at a critical T cell receptor contact residue. Altered peptide ligands for HDM and Japanese cedar pollen allergens have been found to enhance production of IFN-γ by T cells compared with the native peptide.[59–61]

Administration of peptides to downregulate established allergic responses has been tested successfully in several murine models of allergy. Intranasal administration of 100 μg of a single dominant T cell epitope peptide of the HDM allergen Der p 1 on 5 consecutive days could ablate an established T cell response.[62] Importantly, tolerance was induced to the entire Der p 1 molecule, as well as to the peptide, with evidence for intramolecular suppression by the treatment.[63] Such animal studies provided the rationale for performing clinical studies of peptide immunotherapy. The most promising of these clinical studies has been for bee venom sensitivity. Increasing doses (1–100 μg) of a mixture of three T cell epitope peptides of PLA_2 were given subcutaneously to a group of five bee venom-allergic subjects at weekly intervals for 2 months.[64] Clinical efficacy was demonstrated by subcutaneous challenge with PLA_2 without systemic allergic symptoms. Only two of the patients exhibited allergic symptoms to a subsequent bee sting challenge and these were judged much less severe than pretreatment reactions. Proliferation and production of IL-4, IL-5, IL-13 and IFN-γ were decreased in response to *in vitro* stimulation with the peptides and PLA_2 after treatment.

The results of clinical trials of cat allergen peptide immunotherapy have been mixed. An early study using two large T cell reactive peptides (27 mers) that almost spanned the chain 1 of Fel d 1 found efficacy only at the highest dose used (750 μg) and allergic symptoms were commonly reported from 10 min to 6 h after injection.[65] In a subsequent study, Haselden *et al*. used three smaller Fel d 1 peptides of 16–17 residues and demonstrated no evidence of an early IgE-mediated response following intradermal injection.[66] However, nine of 40 patients treated developed a late asthmatic reaction, apparently T cell mediated. Follow-up studies using a panel of 12 short overlapping peptides of Fel d 1 demonstrated that careful up-dosing (starting at 0.1 μg) can inhibit early and late-phase skin reactions to whole cat dander without late asthmatic reactions to the peptides.[67] This was associated with decreased peripheral blood mononuclear cell proliferation and production of IL-4, IL-13 and IFN-γ, but increased production of IL-10.

Recombinant allergens for immunotherapy

An alternative strategy for the generation of effective but hypoallergenic preparations for immunotherapy is to generate recombinant allergens that have ablated IgE reactivity but retained dominant T cell epitopes. As for peptides, these preparations would be easily standardized and could be given at high doses without risk of IgE-mediated side-effects. Recombinant allergens have the advantage over individual peptides of providing multiple T cell epitopes for targeting a larger pool of reactive T cells. However, more exhaustive testing is required to establish the relative merits of the two approaches for particular allergens. An additional intrinsic advantage of recombinant allergen molecules that do not bind IgE is that the allergen will be more likely to be taken up by phagocytosis or endocytosis by macrophages and dendritic cells and induce Th0/Th1 differentiation of allergen-specific T cells. Immunoglobulin E-facilitated uptake of natural allergen by antigen-presenting cells has been shown to drive a predominantly Th2-type response.[68] An earlier approach to generating hypoallergenic preparations, albeit with less precision, used chemical treatment, such as by aldehydes (producing modified allergens termed 'allergoids').[69,70]

Different approaches may be taken for genetically engineering hypoallergenic molecules. In the case of the major birch pollen allergen Bet v 1, naturally occurring isoforms with high T cell reactivity but low or no IgE binding have been identified. These have been sequenced, cloned and expressed in recombinant form.[71,72] Alternatively, site-directed mutagenesis could be used to disrupt known IgE-binding epitopes. Targeting disulfide bonds to disrupt conformational determinants is an obvious approach that has been successfully applied in the case of bee venom PLA_2,[68] *Parietaria* (Par j 1)[73] and dust mite (Lep d 2)[74] allergens. For other allergens, critical residues for IgE binding can be identified by screening allergen fragments or synthetic peptides with patient serum and by 'alanine scanning' of peptides. Following

this approach, hypoallergenic but T cell-reactive allergens have been generated by site-directed mutagenesis for allergens of birch (Bet v 1),[75] peanut (Ara h 3),[76] soybean (P34/Gly m Bd)[77] and latex (Hev b 5).[78]

Utilization of recombinant allergens in animal models of allergy or in clinical studies has, to date, been limited. Using a mutant protein of Der f 2 (C8/119S) with reduced IgE binding, Korematsu *et al*. found more effective hyposensitization than with native Der f 2 in an animal model of allergic bronchial asthma.[79] The mutant also induced a strong Th1-type response by cultured human T cells.

Concluding comments

Concurrent with developments in the generation of T cell-reactive hypoallergenic preparations for SIT are those to improve SIT efficacy based on insight into underlying mechanisms and factors that influence the type of cytokines produced by an allergen-activated T cell in an atopic individual. Such factors include allergen dose, form and antigen-presenting cell type. The Th1-inducing adjuvants and DNA vaccines also show promise for improved efficacy of allergen SIT.[80] However, because Th1-type cytokines may play a role in the pathogenesis of late-phase reactions to allergens and chronic allergic disease, there should be caution in merely depolarizing T cell responses. Strategies that increase the production of anti-inflammatory cytokines, such as IL-10 and transforming growth factor-β, possibly mediated by a regulatory T cell subset, are interesting new approaches. Coupled with these studies are developments in the delivery of SIT by the more practical and potentially safe intranasal or oral routes than the current subcutaneous route. Recent clinical trials of sublingual SIT are encouraging. Elucidation of mechanisms for effective SIT will lead not only to wider application of SIT in clinical practice, but also the design of reliable laboratory assays for monitoring SIT efficacy.

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