

SHORT ANALYTICAL REVIEW

Allergen Immunotherapy: Novel Approaches in the Management of Allergic Diseases and Asthma

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Currently available pharmacotherapies for allergic diseases and asthma, which are serious public health problems, are aimed primarily at neutralizing effector molecules and inflammatory mediators such as histamine and leukotrienes or at inhibiting the function of inflammatory cells such as eosinophils and Th2 lymphocytes. While this approach is effective in controlling symptoms, these therapies have a limited capacity to alter the natural course of allergic diseases and asthma, and discontinuation of medications results in the redevelopment of symptoms on reexposure to the offending allergens. In contrast, immune-based allergen immunotherapies modify and correct the underlying pathological immune responses in allergy and asthma in an antigen-specific manner. These immunotherapies replicate the regulatory processes that occur in nonallergic individuals and allow patients to tolerate exposure to allergens. Current and future methodologies for immunotherapy involve immunization with allergen, modified allergen, peptides of allergen, cDNA of allergen, with adjuvants, including immunostimulatory DNA sequences, cytokines, and bacterial products such as *Listeria monocytogenes*. This form of therapy can provide a long-lasting cure for allergic diseases without the need for continuous therapeutic intervention and without causing generalized immunosuppression or immune augmentation.

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INTRODUCTION

Allergic diseases and asthma are serious public health problems, which have doubled in prevalence in industrialized countries over the last 2 decades. Such disorders are characterized by pathological immune responses directed against ubiquitous and generally innocuous environmental antigens, which sensitize and cause disease in atopic but not nonatopic individuals. The pathological features of allergic diseases and asthma include the production of allergen-specific IgE, which results in immediate phase responses, with mast cell and basophil degranulation and the release of

mediators, including histamine, proteases, cytokines, and arachadonic acid products, including leukotrienes. In addition, immediate phase responses are usually followed by the development of late phase reactions and eosinophilic inflammatory processes driven by the production by Th2 lymphocytes of IL-4, IL-5, IL-9, and IL-13. Although asthma can occur in the absence of IgE-mediated allergic responses, the risk of developing asthma is directly associated with elevated serum IgE levels and with the development of sensitization to environmental allergens (1, 2).

Currently available therapies for allergic disease and asthma are aimed primarily at neutralizing effector molecules and inflammatory mediators, such as histamine and leukotrienes, or inhibiting the function of inflammatory cells, such as eosinophils and Th2 lymphocytes, by treatments with anti-histamines, leukotriene antagonists, and corticosteroids. In addition, potential future therapies, with anti-IgE mAb (3), cyclosporin/FK506 (4), soluble IL-4 receptors (5), anti-IL-5 mAb (6), soluble IL-13 receptors (7), focus on neutralization of the cytokines, chemokines, or adhesion molecules important in amplifying the allergic inflammatory cascade. These therapies, particularly corticosteroids, are effective in reducing allergic inflammatory processes and reducing the clinical symptoms of allergic disease and asthma. However, while this approach with medications is effective in controlling symptoms, in all instances, discontinuation of these medications results in the redevelopment of symptoms on reexposure to the offending allergens. Thus these therapies have a limited capacity to alter the natural course of allergic diseases and asthma, and patients must rely on maintenance anti-inflammatory medications because these therapies do not correct the underlying immunological processes and because the environmental factors that cause and precipitate these diseases are not eliminated. Moreover, the most effective current therapy, i.e., inhaled corticosteroids, has significant side effects (e.g., growth suppression in children and glaucoma and cataracts after prolonged use) and may, with use over extended periods of time, indi-

rectly increase the severity of allergic disease, by inhibiting protective immunity by reducing IL-12 production in antigen presenting cells, and enhancing Th2 cytokine synthesis in T cells (8–10).

In contrast to pharmacologic therapies that focus on symptom reduction, the goals of immune-based therapies for allergic disease are to modify and correct the underlying pathological immune responses in allergy and asthma. A major feature of this approach is the modification of immune responses in an antigen-specific manner, made possible by the fact that the specific antigens that cause allergic diseases (e.g., environmental allergens, such as plant pollens and dust mite allergens) have been identified. Antigen-specific immunotherapies have the potential to induce protective immune responses in allergic diseases, and these protective responses may be reinforced by natural exposure to the allergen, thereby providing long-lasting cures for these problems without the need for continuous therapeutic intervention and without causing generalized immunosuppression or immune augmentation. In this review we discuss the specific goals, immunological principles, and novel methodologies of immunotherapy for the treatment of allergic diseases and asthma.

PROTECTIVE IMMUNITY IN ALLERGIC DISEASE AND ASTHMA

While it is clear that Th2-driven allergen-specific immune responses are responsible for the development of the pathology in allergic disease and asthma, the specific features of immune responses that protect *nonatopic* individuals from the development of allergic diseases are poorly understood (Table 1). Historically, investigators attributed the lack of symptoms in nonallergic individuals to the lack of allergen-specific immune responses, due either to lack of sufficient previous exposure to allergen or to favorable immune response genes that prevented recognition of allergens. However, the clinical experience is that nonatopic individuals are indeed exposed to a very broad number of allergens in quantities sufficient to induce immune responses. Indeed, T cell proliferative responses to common allergens occur in both atopic and nonatopic populations, and proliferative responses to allergens are present in neonates at high or low risk for the development of atopy (11). Moreover, MHC markers have not been linked in any significant way to the development of allergic disorders. Immunological control of allergy is thus considered related to broader regulatory mechanisms, where nonallergic individuals develop immune responses that actively protect against allergy or asthma.

There has been much recent interest in the role of allergen-specific Th1 cells, which produce high levels of IFN- γ , as regulators of allergic disease and asthma.

Th1 cells cross-regulate Th2 cells by inhibiting the development and proliferation of Th2 cells (12), and IgE production is reciprocally regulated by IL-4 and IFN- γ . Thus, many investigators conclude that Th1 cells protect against allergy and asthma. In this model, protection from allergy is due to the development of a nonallergic form of immunity, involving inhibitory allergen-specific Th1 cells, rather than the lack of immune responsiveness to allergens. A significant number of data support the idea that Th1 cells protect against allergy and asthma, but much of it is indirect. Allergen-specific T cell clones derived from the peripheral blood of nonallergic individuals have been shown to produce Th1 cytokines (13, 14). In addition, we and others have shown that individuals predisposed toward the production of Th1 cytokines had a reduced likelihood of developing allergic disease. Thus, individuals with strong delayed type hypersensitivity to *Mycobacteria tuberculosis* after immunization with BCG had a reduced likelihood of developing allergy or asthma (15). Furthermore, neonates who subsequently developed allergic disease had cord mononuclear cells with a reduced capacity to produce IFN- γ (16). In addition, patients with multiple sclerosis, an autoimmune disease characterized by the overproduction of Th1 cytokines in myelin-specific T cells and by an increased capacity of monocytes to produce IL-12, were also protected from the development of allergy (17, 18). Similarly, patients with other autoimmune diseases, such as rheumatoid arthritis or type 1 diabetes mellitus, appeared to be protected from the development of allergy (19, 20).

Other studies that support the role of Th1-biased responses in protection against allergy and asthma include the demonstration that administration of IL-12 intratracheally, or of IL-12 plus IL-18, inhibited allergen-induced airway inflammation and hyperreactivity (21, 22). Both IL-12 and IL-18 are potent inducers of IFN- γ production and together appear capable of preventing the development of Th2-biased inflammatory responses in the lung. IL-18 has the additional capacity to stimulate the development of CD8 cells, suggesting that IFN- γ producing CD8 cells may be important in regulating Th2-mediated diseases, as has been previously suggested (23). Although IL-18 in the absence of IFN- γ can promote eosinophilia and Th2 cytokine production (24–26) and IgE production (27), in the presence of IL-12, which enhances the expression of IL-18 receptors, IL-18 greatly increases IFN- γ production in T cells (28, 29).

While these studies indicate that Th1 cells may be involved in negatively regulating allergic disease, other observations suggest that IFN- γ may contribute to the development of airway inflammation. Allergen-specific T cells in the peripheral blood and the lungs of patients with asthma also produce IFN- γ , and classical Th1 cells, when adoptively transferred into mice, do

TABLE 1

Clinical Manifestations of Th1 vs Th2 Responses Associated with Asthma and Allergy

Characteristic	Atopic individual	Nonatopic individual
Serum markers	↑ IgE, ↑ PGE ₂ , ↓ cAMP	↓ IgE; normal PGE ₂ and cAMP
Allergen-induced cytokine production (13, 14)	↑ IL-4, ↑ IL-5, ↑ IL-13, ↓ IFN- γ ; possible ↓ IL-10, ↓ TGF- β	Possible ↑ IFN- γ , IL-2
T cell proliferative response to allergen	Increased	Present but low; regulatory cells may proliferate poorly to allergen
Cord blood IFN- γ (16)	↓ In neonates with subsequent atopy	Normal in neonates with no subsequent atopy
Immunization/infections (15)	↓ DHT to <i>M. tuberculosis</i>	↓ DHT to <i>M. tuberculosis</i>
Associated disorders (16, 17, 19, 20)	↓ Incidence autoimmune disease	

not counterbalance effector Th2 cells, but rather codominantly generate inflammatory responses in the lungs (30–33). Thus, it is unlikely that large numbers of Th1 cells function normally in the lung mucosa itself, since Th1 cells exacerbate inflammation and cause tissue damage (30). Th1 cells, however, may inhibit the development of Th2-dominated immune responses and might do so in peripheral lymphoid organs, such as lymph node and spleen, when present early during the development of an immune response, by inhibiting the development of Th2 effector cells from naïve memory cells.

The allergen-specific immune responses in nonallergic individuals that inhibit the development of allergic symptoms may involve Th1-like cells as well as other immunoregulatory cells that greatly limit inflammatory responses. In contrast to the significant inflammation found in the lungs of patients with asthma, the airways of nonallergic subjects are characterized by the absence of inflammatory changes, including the absence of Th1 cells, suggesting that immunoregulatory cells, distinct from Th1 cells, for example, producing regulatory cytokines such as IL-10 (32, 34, 35) and TGF- β (Th3 cytokines) (31, 36), or CD8 T cells (37, 38) and $\gamma\delta$ T cells (39), suppress allergic inflammation. Thus administration into the lungs of IL-10, a cytokine with potent anti-inflammatory and immunoregulatory activity (34, 40), using replication-deficient adenovirus vectors in a murine model of airway hyperreactivity during the time of mucosal sensitization, abrogated both the cellular and physiological recall responses *in vivo*. These studies suggest that regulatory cytokines, such as IL-10, may critically regulate the development of asthma, although some studies suggest that IL-10 may exacerbate allergen-induced airway responses (41).

TGF- β has been shown previously to be a key immunoregulatory factor in the development of unresponsiveness to antigens in the gastrointestinal tract, an anatomic site that is closely related developmentally to the respiratory tract. Several investigators demonstrated that TGF- β producing Th3 cells and Tr1 cells inhibited experimental colitis (42, 43) and TGF- β induced by oral tolerance inhibited subsequent tracheal

eosinophilia (44). Furthermore, TGF- β production controlled Th2-driven inflammation in chemical-induced autoimmunity (42). Moreover, we have demonstrated that ovalbumin (OVA)-specific Th cells engineered to express latent transforming growth factor β 1 (TGF- β) abolished airway hyperreactivity induced by OVA-specific Th2 effector cells in SCID and BALB/c mice (31). The inhibitory effect of TGF- β secreting T cells was antigen-specific and was reversed by neutralization of TGF- β . Moreover, airway inflammation, as measured by eosinophils in bronchoalveolar lavage fluid or by examination of lung histology, was greatly reduced by the transfer of TGF- β 1 secreting T cells, and no increase in respiratory fibrosis was observed. Finally, blockade of TGF- β signaling enhanced antigen-induced airway inflammation (45). These studies, as well as those showing that TGF- β regulates Th1-driven autoimmune diseases, such as EAE, uveoretinitis, and collagen-induced arthritis (46–51), indicate that TGF- β inhibits both Th2- and Th1-driven inflammation. Thus, T cells activated to produce TGF- β may be critical in regulating allergen-induced airway hyperreactivity, asthma, and allergic diseases.

CONVENTIONAL ALLERGEN IMMUNOTHERAPY, MECHANISMS OF ACTION

Conventional allergen immunotherapy, or allergy shots, appears to induce protective immunity in patients with allergic disease. It is performed by the subcutaneous injection of increasing quantities of allergen protein, has been in use for the treatment of allergic disease for over 80 years, and is effective in greatly reducing clinical symptoms of allergy and asthma. During conventional immunotherapy for allergic rhinitis or allergic asthma, the injections are increased to a maximally tolerated level, generally 10⁵ times the initial dose, and equivalent to 2 to 100 μ g of the major allergen. Clinical benefit occurs after several months of injections for inhalant allergy, and progressive reduction of symptoms occurs over the first 3 to 4 years of injection therapy. In patients with *Hymenoptera* venom (bee sting) anaphylaxis, allergen immunotherapy has the potential for curing this disease, since

TABLE 2
Immunological Effects of Conventional
Allergen Immunotherapy

Pre-immunotherapy	Post-immunotherapy
Positive immediate skin test	↓ Immediate skin test reactivity
↑ Allergen-specific IgE	↓ Allergen-specific IgE
↓ Allergen-specific IgG	↑ Allergen-specific IgG
↓ IgG4	↑ IgG4
↑ Allergen-specific IL-4, ↑ IL-5, ↓ IFN- γ	↓ Allergen-specific IL-4, ↓ IL-5, ↑ IFN- γ
↓ Allergen-specific IL-10	↑ Allergen-specific IL-10
↑ Allergen-specific T cell proliferation to allergen	↓ Allergen-specific T cell proliferation

patients who have completed a course of therapy can tolerate bee stings received years after the cessation of therapy. In patients with allergic rhinitis and asthma, completion of a course of conventional allergen immunotherapy allows patients to tolerate exposure to significant quantities of allergen with minimal symptoms (52, 53), and clinical improvement is sustained following discontinuation of immunotherapy after 3 to 4 years of injections in most patients (54). Moreover, treatment of children with pollen sensitization prevents the usual progression from allergic rhinitis to asthma (55). Thus conventional allergen immunotherapy, in contrast to other pharmacologic therapies, appears to modify the natural course of atopic disorders and induce at least partial cure, presumably by altering the underlying immune responses. However, the specific immunological mechanisms that are altered by allergen immunotherapy are incompletely understood (Table 2).

It is known that conventional allergen immunotherapy substantially reduces IgE-mediated allergic responses and allergic sensitivity in an antigen-specific fashion and is associated with an increase in antigen-specific IgG and IgG4 antibody, a long-term reduction in IgE antibody, and a diminution of immediate skin test reactivity. Initially, investigators focused on the importance of induction of "blocking" IgG antibodies, which were thought to bind to exogenous allergen, impeding exposure of allergen to allergen-specific IgE on the surface of mast cells or basophils. With improved understanding of the importance of CD4 T cells in the pathogenesis of allergic disease and asthma, it has become clear that allergen immunotherapy is associated with immune deviation, with a reduction in the function of allergen-specific Th2 cells (anergy?) and with the development of allergen-specific IFN- γ producing cells (56–58). Moreover, failure of conventional allergen immunotherapy is associated with the failure to reduce allergen-specific IL-4 production (59).

The induction of IL-10 production has also been associated in some instances with successful immunotherapy, and it is likely that IL-10 can contribute to

tolerance to antigen by its immunoregulatory effects on Th1 and Th2 cells. IL-10 production has been observed during bee venom immunotherapy in activated allergen-specific CD4⁺ CD25⁺ T cells, and neutralization of IL-10 resulted in restoration of *in vitro* proliferation and cytokine production (60). Whether other immunologic mechanisms also occur following successful immunotherapy, e.g., induction of CD8 regulatory cells (37), is not yet clear. Although now considered an epiphenomenon, induction of IgG may still play a role in immunotherapy, as it has been recently shown that IgG induced by immunotherapy inhibits serum-facilitated antigen presentation to T cells, resulting in a requirement for a significantly higher antigen concentration to activate antigen-specific T cells (61).

NOVEL METHODOLOGIES FOR ALLERGEN IMMUNOTHERAPY

Although conventional allergen immunotherapy effectively controls symptoms in most patients even after the discontinuation of therapy, the process requires nearly 100 injections, over 3–5 years, and in addition, is associated with frequent unpredicted allergic reactions, including anaphylaxis. Administration of multiple subcutaneous injections is difficult and traumatic in the young pediatric population, arguably the population most likely to benefit from immune modulation. Moreover, immunotherapy is not universally successful in the treatment of allergic rhinitis and asthma and is particularly problematic in patients with multiple allergen sensitization. Standardization of allergen extracts has also been a problem, as the allergens used in conventional immunotherapy have been generally extracted from animals, plants, and insects and as such are crude or only partially purified. Thus, safer, more effective, methodologies for delivering immunotherapy are required, and major efforts have been made in this regard over the past 5 years. These efforts include the study of vaccines containing modified protein allergens, peptide allergens, cDNA of allergens, use of immunostimulatory sequences (ISS) of DNA as adjuvant, the fusion of Th1 promoting cytokines such as IL-12 and IL-18 to allergen, and coadministration of allergen and adjuvants such as *Listeria monocytogenes* (Table 3).

Modified Allergens

Approaches to reduce allergenicity and retain immunogenicity of antigens have been undertaken to improve the efficiency and reduce the risk of serious side effects of immunotherapy. Theoretically, such proteins of high immunogenicity with reduced allergenicity can be given in high doses without risk of IgE-mediated immediate reactions, because they have a reduced capacity to trigger degranulation in mast cells, yet are

TABLE 3
Novel Approaches to Immunotherapy

Type of immunotherapy	Observed effects	Possible mechanism of action	Potential advantages	Potential disadvantages/problems	Human use
Sublingual immunotherapy (SLIT) (65, 66)	Clinical improvement ↓ IgE Th2 → Th1 responses	Antigen-specific T cell deletion/apoptosis IL-10/TGF- β induction (67) ? Oral tolerance	No injections ↓ Anaphalaxis and brochospasm	Large allergen doses required	Yes
Modified allergen (59, 60)	↓ IgE Th2 → Th1 responses	Reduced allergenicity ↓ Mast cell degranulation	Safer Larger allergen doses possible	Difficulty with stability and manufacture	Human trials
Peptide (61, 63)	Clinical improvement Reversal of airway hyperactivity (murine)	Functional CD4 T cell inactivation/anergy	↓ Allergenicity (?)	Unexpected late phase side effects (63, 64) Anti-peptide IgE develops	Human trials
ISS + allergen ISS-allergen (83, 84)	Clinical improvement Reversal of airway hyperactivity (murine)	Induction of Th1 responses Antigen-specific CD8 cells	↑ Immunogenicity (86)	Antigen-nonspecific responses Toxic Th1 side effects	Human trials
Cytokine + allergen, fusion protein (e.g., OVA/IL-12) (79)	Reversal of airway hyperactivity (murine) Th2 → Th1 responses	Induction of Th1-type responses	↓ "Bystander" cytokine side effects Allergen specific	May produce too vigorous Th1 response	No
Adjuvants + allergen, e.g., heat-killed <i>Listeria</i> (37, 87), <i>M. tuberculosis</i> (88)	Prevention/reversal of airway hyperactivity (murine) Th2 → Th1 responses ↓ IgE	Induction of regulatory cells (TGF- β /IL-10) CD8 T cells IL-12/IL-18	Very potent effects Regulatory cells limit Th1 effects		No
DNA vaccination (74, 76, 78)	Reversal of airway hyperactivity (murine)	Induction of Th1 responses Antigen-specific CD8 cells		? Route of administration	No

capable of inducing IFN- γ production in T cells (62). Initially, investigators focused on generating polymers by treatment of allergen with urea, formaldehyde, glutaraldehyde, or polyethylene glycol. Studies in both humans and in mice demonstrated that polymerized allergens induced immune deviation/modulation, converted allergen-specific IL-4 producing Th2 lymphocytes into allergen-specific Th1 cells, and reduced allergen-specific IgE (63). However, significant difficulty in producing uniform lots of these modified proteins reduced commercial interest in this idea.

Recombinant allergens with cysteine residues removed by site-directed mutagenesis have been examined as possible candidates for immunotherapy. These modified allergens do not possess disulfide bonds and therefore lack normal secondary/tertiary structure and have 100-fold reduced IgE binding activity (64). These modified allergens, however, retain their T cell epitopes and immunogenicity, are capable of stimulating T cells and inducing immune deviation, and are

currently being studied for their effectiveness as therapy in allergic individuals.

Peptide Immunotherapy

Another approach to immunotherapy involves the use of peptide fragments of allergens. Since T cells recognize peptide fragments of antigens or allergens, generally 10–15 amino acid residues in length, such peptides, which lack tertiary structure and the capacity to bind to allergen-specific IgE and trigger mast cell degranulation, can be administered to patients without risk of immediate reactions. As a result, peptide fragments of allergens can be administered in much larger doses than is possible with conventional allergen and may still affect antigen-specific T cells (65–67). These peptides, when administered *in vivo*, activate allergen-specific CD4 T cells in the absence of costimulation and appear to induce functional inactivation or anergy. It appears that peptide immunotherapy differs from con-

ventional immunotherapy in this respect, where allergy rather than immune deviation is primarily induced by peptide therapy. Initial trials in humans with cat allergy utilized only two antigen peptides containing immunodominant epitopes of the major cat allergen *Fel d1* and demonstrated the efficacy of these peptides in reducing allergic symptoms on allergen challenge in patients with cat and ragweed allergy. However, late-occurring reactions (1–6 h after injection) have been observed in some subjects, presumably due to activation of allergen-specific T cells (67, 68). In some subjects, IgE against the injected peptides developed, occasionally associated with increased immediate reactions. Additional studies are required to determine whether the suppressive effects are persistent over several years times, whether a broader number of smaller but overlapping peptides covering the whole antigen may be more effective in a greater number of patients, and if reduction of allergen-specific IgE occurs following peptide immunotherapy.

Oral Immunotherapy

Sublingual swallow immunotherapy, performed by oral administration of allergen, has been intensively studied recently in patients as a form of immunotherapy. Such therapy has been shown to be effective in reducing symptoms of allergic rhinitis and asthma and may have less risk of serious immediate reactions, such as anaphylaxis and bronchospasm (69, 70). It also offers the benefit of not involving injections, which require administration under supervision by medical personnel. The underlying mechanisms of action of sublingual swallow immunotherapy, which requires relatively high dosages of allergen to overcome potential digestion in the gastrointestinal tract, are poorly understood. These mechanisms may be quite distinct from that of conventional immunotherapy; for example, high doses of oral antigen induce deletion or apoptosis in antigen-specific T cells, whereas low doses of oral antigen induce the development of regulatory cells, often associated with the production of IL-10 and TGF- β 1 (71). Therefore, oral administration of allergen may be very effective in diminishing allergic immune responses and asthma symptoms in a very safe and effective manner.

DNA Vaccination

A large number of recent studies have demonstrated the effectiveness of genetic vaccination approaches in models of infectious disease (72–74), cancer (75), and autoimmune disease (76, 77). Because Th1-biased responses are thought to be effective in protecting against allergic disease and asthma, DNA vaccination has been applied to allergic disease and asthma to

downmodulate the Th2-biased immune responses. In murine models of allergic disease and asthma, the recipient is vaccinated with allergen in the form of naked plasmid cDNA, which stimulates allergen-specific immune responses with a Th1 bias. CpG motifs present on the vector backbone enhance immunogenicity (78) and effectively induce IL-12, IL-18, IFN- γ production, and antigen-specific CD8 cells (79). Studies in murine models have demonstrated that DNA immunization strategies prevent the development of antigen-specific IgE synthesis, airway hyperresponsiveness, or food-induced anaphylaxis and induce inhibitory CD8 cells (80–82). Preliminary studies in our laboratory suggest that fusing IL-18 cDNA with allergen cDNA in a single vector may enhance the effectiveness of the DNA vaccination and results in a potent vaccine construct that is much more capable of reversing established airway hyperreactivity than a simple allergen cDNA plasmid.

Fusion Proteins with Enhanced Immunogenicity

The fusion of cytokine and antigen has been shown to be an effective vaccine strategy in several other systems, for example, with idiotypic antigens of B cell lymphoma conjugated to GM-CSF, which enhanced the induction of protective immunity against a subsequent lethal tumor challenge (75). The fusion construct focuses the effects of the cytokine on antigen-specific cells, which reduces the absolute amount of cytokine required, thereby reducing potential adverse effects of the cytokine.

This approach was used with the cytokine IL-12, which was fused to the prototypic allergen OVA (83). IL-12, like IL-18, potently induces IFN- γ production and enhances Th1-biased immune responses. This approach reduces the significant antigen-nonspecific effects of IL-12 when it is administered *in vivo*. For example, *in vivo* administration of IL-12 has been associated in clinical trials with substantial morbidity and mortality (75), has been ineffective in suppressing Th2 recall responses (84, 85), and when administered in high doses to mice, paradoxically enhances IgE synthesis (86). In contrast, administration of the IL-12, when fused to OVA protein, reduced antigen-nonspecific IL-12 effects and maximized induction of IFN- γ production in an antigen-specific fashion. Moreover, the OVA–IL-12 fusion protein increased the immunogenicity of the OVA and greatly improved the capacity of the allergen to induce Th1-biased responses and reverse established allergic responses (83). The antigen specificity of this response was dependent upon covalent linkage of antigen and IL-12, since immunization of mice with allergen and free recombinant IL-12 enhanced T cell production of IFN- γ , but the IFN- γ production was not allergen-specific. Thus modification of allergen by covalent linkage to cytokines, such as IL-12, may be an effective methodology for

enhancing the immunogenicity of proteins in inducing Th1-biased responses and for treating patients with allergic disease.

ISS as Adjuvant for Allergen Immunotherapy

Because immunostimulatory sequences (also known as unmethylated CpG motifs) in DNA have been found to greatly enhance Th1 responses (80), there has been great interest in using ISS as an adjuvant in allergen immunotherapy. ISS, when administered prior to or with allergen, has been shown to prevent airway hypersensitivity in murine models of asthma (87, 88). ISS motifs activate pattern recognition receptors and MyD88 pathways involved in innate immunity, expressed on antigen presenting cells (dendritic cells, macrophages, and B cells) and NK cells (89) and appear to be effective in reducing Th2 responses even in IFN- γ and IL-12 knockout mice. Moreover, conjugates composed of ISS covalently linked to allergen have been shown to be 100-fold more efficient in reducing airway eosinophilia and airway hyperreactivity than unconjugated mixtures (90). Thus, allergen-ISS constructs or the coadministration of ISS and allergen may be effective in immunotherapy for asthma and atopic disorders, and clinical trials with these agents are currently under way.

Other Adjuvants for Allergen Immunotherapy

While many modified approaches to allergen immunotherapy induce immune deviation toward a Th1 form of immunity, as discussed above, immune modulation with the induction of other regulatory cell types may be important for the elimination of allergen-induced inflammation. For example, the adjuvant heat-killed *Listeria monocytogenes* (HKL), which is potent in inducing IFN- γ , IL-12, IL-18, as well as IL-10 production, appeared to be extremely effective in converting Th2-dominated immune responses into protective responses. Moreover, the effect of HKL occurred in an allergen-specific manner and was effective in reversing established airway hyperreactivity (38, 91). HKL as adjuvant was much more effective than *Mycobacteria* as adjuvant (38), even though respiratory infection with *Mycobacteria* has been shown to suppress allergen-induced airway eosinophilia (92). The inhibitory effect of HKL on airway hyperreactivity depended on the presence of IL-12, IL-10, TGF- β , and CD8⁺ T cells, was associated with an increase in IL-18 mRNA expression, and required close physical association between HKL and the antigen. Thus, these studies demonstrate that HKL as an adjuvant very effectively promotes complex regulatory protective immune responses in the respiratory tract, which downmodulates ongoing Th2-dominated responses and indicates that

HKL as an adjuvant for immunotherapy may be clinically effective in the treatment of allergic asthma. Further study of such regulatory responses induced with HKL or with other adjuvants, such as cholera toxin, subunit B, which appears to induce tolerance (93, 94), are likely to lead to very effective therapies for allergic diseases and asthma.

SUMMARY

The most effective immunotherapies for allergic diseases will be those that replicate the regulatory processes that occur in nonallergic individuals and which allow individuals to tolerate exposure to allergens without symptoms. At the present time the mechanisms which underlie this process in nonallergic people are still poorly understood, but are likely to involve regulatory/suppressor cells, including Th1 CD4, CD8 cells, and regulatory T cells secreting IL-10 or TGF- β (Th3 or Tr1 cells). In addition, mechanisms such as anergy or deletion may also be involved, as is thought to occur during gastrointestinal exposure to antigens. Although antigen-specific IFN- γ producing cells may play an important role in mediating the beneficial effects of conventional immunotherapy, the simple conversion of Th2-dominated allergic inflammatory responses into classical Th1 responses may result in enhanced rather than decreased inflammation and exacerbation of allergic disease. Conventional allergen immunotherapy is currently available for treating allergic disease, and it appears to attenuate the underlying detrimental allergic inflammatory responses and has the potential for altering the natural course of the disease. Future therapies will be much more efficacious and will be based on our developing knowledge of the mechanism of action of tolerance and immune deviation. Such allergen-specific therapies, however, will be long lasting and not require chronic administration, since exposure to allergen should reinforce the protective immune response. Moreover, these therapies will convert allergic individuals into nonallergic individuals and produce a cure for these problems.

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