

Immunomodulation

The Future Cure for Allergic Diseases

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ABSTRACT: Allergies are the result of aberrant immune reactivity against common innocuous environmental proteins (allergens). A pivotal component of allergic pathogenesis is the generation of allergen-specific Th cells with an effector phenotype. These Th cells activate a complex immune cascade that triggers the release of potent mediators and enhances the mobilization of several inflammatory cells types, which in turn elicit the acute allergic reactions and promote the development of chronic inflammation. The current therapies for allergic diseases focus primarily on pharmacological control of symptoms and suppression of inflammation. This approach is beneficial, but not curative, since the underlying immune pathology is not inhibited. In an attempt to develop more effective therapeutic strategies, the scientific interest has been directed toward methods down-modulating the immune mechanisms that initiate and maintain the allergic cascade. Today, the only widely used disease-modifying form of allergy treatment is the specific immunotherapy with allergen extracts. More recently the use of anti-IgE has been approved for patients with allergic asthma. Other immunomodulatory methods being currently explored are the administration of microbial adjuvants that inhibit Th2 reactivity and the design of molecules that interrupt the activity of key allergic cytokines, chemokines, or other Th2 effector mediators.

KEYWORDS: allergy; asthma; immunomodulation; T cells; IgE

Allergies affect approximately the 20% of the population in industrialized countries and cause a broad spectrum of problems ranging from mild inconvenience to severe respiratory distress and life-threatening anaphylactic shock. Over the past 40 years the prevalence and severity of allergic diseases has increased dramatically. For asthma in particular, the overall annual mortality and morbidity have almost doubled during the last decade. Today, despite the

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development of new “antiallergic” drugs, a permanent cure for most of the allergic diseases cannot be achieved. The problem with the current pharmacological regimes (antihistamines, antileukotrienes, and corticosteroids) is that they target primarily the control of symptoms and/or suppression of inflammation, without affecting significantly the allergic pathogenesis. Thus, these regimes cannot offer long-lasting effects and the patients’ relief depends on the continuous use of medication. To overcome these shortcomings the academic and industrial research efforts have been directed toward the design of novel therapeutic approaches altering the dysregulated immune mechanisms that are responsible for the development of acute allergic reactions and chronic inflammation. To this end, the improvement of our knowledge of the pathophysiology of allergic diseases has provided a new arsenal of exciting opportunities for therapeutic intervention that promise to bring a permanent solution to the allergy problem.

ALLERGY, A DYSREGULATED IMMUNITY TO INNOCUOUS ENVIRONMENTAL PROTEINS

Allergic diseases arise as a result of aberrant immune responsiveness against innocuous environmental proteins (allergens) present in pollen, house dust, mites, fungal spores, animal danders, food, insect venom, etc. We are all exposed to allergens, but in healthy individuals the natural immune response to allergens is characterized by lack of effector reactivity and maintenance of a status of immune tolerance. In contrast, in allergic individuals the immune mechanisms that preserve the development of tolerance to allergens are disrupted and a window allowing the induction and perpetuation of active immune responses is generated. The pathologic immunity to allergens is orchestrated by the induction of allergen-specific CD4⁺ T cells with an effector phenotype.¹⁻³ The presence of these cells is an essential requirement not only for allergen sensitization, but also for the full expression of the inflammatory characteristics of allergic diseases. Activated allergen-specific Th cells are present in high frequency in the peripheral blood of allergic patients. Moreover, there is severe infiltration of the organ targets, such as the nose, lungs, skin, and gut.^{1,3} Depletion of T cells with a specific neutralizing Ab inhibits the development of disease in animal models of allergic sensitization.^{4,5} Accordingly, asthma cannot be generated in RAG-deficient mice, whilst reconstitution of CD4⁺ T cells restores the potential for induction of airway inflammation and airway hyperresponsiveness (AHR).^{5,6} The allergen-specific CD4⁺ T cells involved in the initiation of allergic reactivity have a distinctive cytokine profile (Th2 cells), characterized by the enhanced production of IL-4, IL-5, IL-9, and IL-13.^{1,7} The presence of high levels of Th2 cytokines in the immune environment sets in motion a wide range of events that lead to the development and polarization of the features of allergic pathophysiology.^{1,2,8} For example,

IL-4 and IL-13 drive the IgE isotype switching on B cells, IL-5 promotes the maturation and recruitment of eosinophils, and IL-9 triggers the activation of mast cell and basophils.^{8,9} Moreover, some Th2 cytokines directly contribute to the generation of specific disease symptoms, such as the AHR and mucus hypersecretion in asthma.^{3,10}

The differentiation of naïve Th cells toward the Th2 phenotype and the production of Th2 cytokines is regulated by a complex network of signaling molecules and transcription factors. Presence of bystander IL-4 in the local microenvironment is essential for the initiation of Th2 programming.¹¹ Triggering of the IL-4R stimulates the activation of several intracellular molecules that transduce the signal to the nucleus and promote the transcription of the Th2 cytokine genes.^{11,12} Of these molecules, activation of STAT6 is of paramount importance for effective IL-4 signal transduction and Th2 differentiation.^{12,13} Knockout of the *Stat6* gene leads to deficient Th2 cell development and failure to produce IL-4.¹³ The key signaling event downstream of STAT6 activation is the upregulation of the two principal Th2 transcription factors: GATA-3 and c-maf.^{13,14} During Th cell differentiation GATA-3 promotes selectively the acquisition of Th2 phenotype, not only by enhancing the Th2 cytokine gene transcription, but also by controlling the remodeling of chromatin structure and the opening of the IL-4 locus.¹⁵ GATA-3 is suppressed during Th1 cell development.¹⁵ On the contrary, overexpression of GATA-3 leads to increased Th2 cytokine synthesis.¹⁵ In addition to GAT-3 and c-maf, members of the NF-AT family of transcription factors (NF-ATc and NF-ATp) are also involved in the molecular regulation of Th2 responses.¹⁴

The most catalytic immune event promoting the generation of allergic symptoms following the initial shift to Th2 responsiveness is the increased synthesis of allergen-specific IgE. The presence of high IgE levels is deterministic for the development of acute allergic reactions, but also contributes significantly to the development of chronic allergic inflammation.¹⁶ In clinical practice allergic sensitization is defined by the presence of elevated serum levels of allergen-specific IgE antibodies. IgE sustains allergic reactivity primarily through its tendency to bind to high-affinity receptors (FcεRI) on the surface of tissue mast cells and circulating basophils. Cross-linking of cell-bound IgE with the specific allergens causes degranulation of the cells and release of several proinflammatory mediators (e.g., histamine, leukotrienes, prostagladins, PAF) and cytokines that cause pathophysiological changes within minutes, such as smooth muscle contraction, mucus production, and vascular leakage, which are responsible for the acute allergic reactions.¹⁶ In addition to the immediate symptoms, the release of allergic mediators triggers another more prolonged inflammatory process associated with the attraction of various immune cells at the sites of allergenic exposure, including Th2 lymphocytes, eosinophils, basophils, and monocytes.¹⁶ The recruited cells secrete several inflammatory factors that exacerbate the symptoms of the early phase and injure the local tissues. Upon continuous exposure to allergens this immunopathologic process

becomes resistant to resolution and leads to refractory inflammatory changes that can cause serious tissue damage and dysfunction of the organ-targets.

The role of allergens in driving and maintaining chronic allergic inflammation is well established. However, with age and recurrent challenge the inflammatory process in diseases such as asthma and atopic dermatitis may become IgE-independent and be perpetuated by a variety of other nonallergenic stimuli.¹⁷ Infections play a particularly important role, since they not only transiently exacerbate the allergic symptoms, but they also shape the disease progression by enhancing the inflammation and aggravating the tissue damage.¹⁷ Nonspecific irritants, intrinsic proteins cross-reacting with allergen-specific T cells, autocrine stimulation of inflammatory cells, and epithelial defects are also among the factors that appear to be implicated in the persistence of inflammation.^{17,18} The complexity of the immune mechanisms involved in the chronic forms of allergic disorders is often reflected in the type of activated T cells accumulated in the inflamed tissues. Thus, in addition to Th2 cells, Th1 cells or Th cells secreting a mixture of Th1- and Th2-type cytokines (e.g., secretion of IL-5 and IFN- γ , with little IL-4) can be detected, while CD8⁺ T cells can also be prominent.^{18,19}

The recruitment of effector cells at the sites of allergen exposure is controlled by the chemokines and adhesion molecules expressed in the local immune environment. In susceptible individuals the early signals elicited after the allergen entry and stimulation of an immune response lead to rapid release of chemokines in the local tissues (namely eotaxin, RANTES, MCP-3, MCP-5 and MDC) that act as chemoattractants.^{9,20} The secreted chemokines upregulate the expression of integrins in the blood vessels, which facilitates transmigration of leukocytes into the tissues.²¹ Once in the tissues the leukocytes follow the chemokine gradient and move to the sites of immune reactivity.²¹ Since each chemokine attracts only inflammatory cells that express the corresponding receptor, the chemokine profile induced in response to a particular immune stimulus dictates the type of inflammation that develops.^{20,22} Recent data suggest that the expression of chemokines and chemokine receptors is under stringent developmental and organ-specific control.²³ In particular, it has been demonstrated that the various types of leukocytes differ in their capacity to express certain chemokine receptors and therefore to respond to local signals.²³ Th1 and Th2 cells express diverse sets of chemokine receptors and because of that they are differentially recruited to the sites of inflammation.^{23,24} Thus, the secretion of CCL11, CCL22, and CCL17 in the allergen-inflamed tissues leads to preferential recruitment of the Th2 cells that selectively express the matching receptors CCR3, CCR4, and CCR8.²⁴ The importance of chemokines in the development of allergic inflammation is tremendous and this is highlighted by studies showing that experimental allergic diseases cannot be induced in animals with specific chemokine deficiencies.^{9,22} Of all the known chemokine-chemokine receptor systems, the eotaxin system seems to play the most critical role in allergic inflammation.²⁵ Animals deficient in the

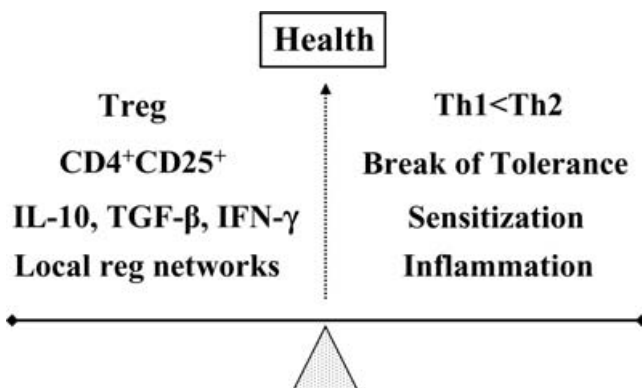


FIGURE 1. Tolerance to allergens. The natural immunity to allergens is characterized by the maintenance of a status of tolerance. Several mechanisms are involved, including T cell deletion, T cell anergy, Treg cell induction, and development of local regulatory networks. In allergic individuals the mechanisms preserving the immune tolerance to allergens are disrupted and effector Th2 reactivity leading to the development of disease is generated.

expression of eotaxin receptors (CCR3 and CCR2) have been found unable to develop lung and skin eosinophilia or disease features such as AHR in response to allergen challenge.²⁵

In contrast to what happens in allergic individuals, in healthy subjects the maintenance of tolerance to allergens is the result of multiple systemic and local immune mechanisms that prevent or quickly eliminate the development of inappropriate reactivity (FIG. 1).^{26,27} Studies in animals examining the natural immune responses to allergen exposure via the respiratory or gastrointestinal tract indicate that there is immediate activation of several mechanisms of immune tolerance including T cell deletion, T cell anergy, and T cell suppression.^{28,29} The early activation of these mechanisms prevents the induction of allergen-specific T cell reactivity and protects from the development of allergic inflammation.^{29,30} Exposure of healthy people to allergens is not associated with the rise of robust effector Th cell responses. Occasionally, few allergen-specific Th1 cells may be detected in the peripheral blood of local tissues, but the dominant cell subset that is present consistently and expands after allergen challenge is the Treg cells.²⁶ The allergen-specific Treg cells are characterized by the production of high amounts of IL-10 and display strong immunosuppressive potential.³¹ Data from experiments using Treg cells isolated from the peripheral blood of healthy individuals confirm that these cells suppress very effectively the proliferation and cytokine production of allergen-specific Th2 cells in an IL-10-dependent fashion.^{31,32} Enhancement of the number and function of these cells has also been noticed in individuals

who have overcome their allergies.³² On the contrary, in atopic individuals only few allergen-specific Treg cells with reduced immunosuppressive capacity are present in the periphery.³² It has been proposed that after exposure to allergens the ratio of allergen-specific effector Th2 cells and immunosuppressor Treg cells determines whether a healthy or allergic immune response will be generated. The naturally induced CD4⁺CD25⁺ Treg cells may also participate in the development of tolerance to allergens and protection from allergic diseases; however more investigations are needed to define their exact contribution.³² Apart from the different types of peripheral T cells involved in tolerance induction, there are also indications that in the various organs additional local immunoregulatory mechanisms exist and provide an extra layer of protection against the development of aberrant immune reactivity. For example, it has been shown that the local epithelium, organ-specific cells, such as the alveolar macrophages in the lungs, and local immunosuppressive cytokines, such as TGF- β , favor the ablation of inflammatory responses and the maintenance of immune homeostasis.³³

IMMUNOMODULATION: THE TARGET FOR EFFECTIVE THERAPEUTIC INTERVENTION

For the design of novel strategies for allergy prevention and therapy, several targets have emerged from the elucidation of the cellular and molecular mechanisms regulating the immunity to allergens. Theoretically, the ideal approach for a radical allergy therapy should include the eradication of reasons and mechanisms that sustain the development of effector reactivity to allergens and the redirection toward the induction of tolerogenic responses (FIG. 2). The

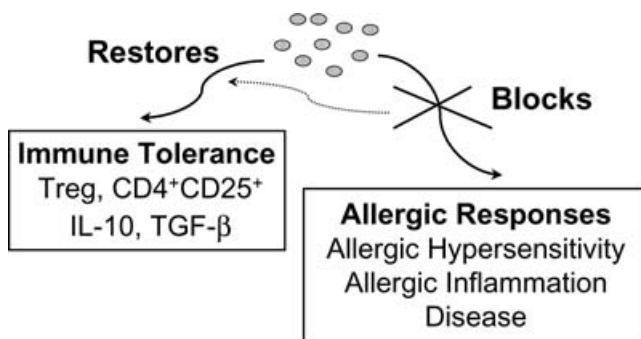


FIGURE 2. Ideal immunointervention. An ideal curative therapy for allergic diseases should include the suppression of the existing effector allergen-specific responses and the enhancement of mechanisms promoting the induction of tolerance to allergens.

TABLE 1. Immunomodulatory methods

Used in clinical practice	Under development
Allergen-specific immunotherapy	Th cell inhibition
Anti-IgE	Use of microbial adjuvants
	Inhibition of allergic cytokines
	Inhibition of allergic cell migration

break in immune tolerance to allergens is accompanied in most types of allergic diseases by generation of allergen-specific Th2 responses. Therefore, elimination of the existing pool of allergen-specific Th2 cells, deviation of the new responses away from the Th2 phenotype, and enhancement of the tolerance process are important goals for successful disease manipulation. Modulation of the phenotype of Th cell responses could be achieved with allergen-specific, as well as nonspecific methods. The various immunotherapy regimes using allergen extracts belong to the first category, while the methods aiming to alter the Th cell functions by blocking major molecules involved in Th cell differentiation (e.g., costimulatory molecules, cytokines, or transcription factors) represent examples of the second approach. An alternative strategy to suppress or reduce allergic reactivity is through inhibition of the key events of the allergic cascade. Blockade of IgE activity, inhibition of allergic second messengers, or suppression of the inflammatory cell migration are among the obvious targets. Downregulation of these immune events may not alter profoundly the nature of aberrant immune responsiveness to allergens, but it provides significant qualitative and quantitative restriction of allergic reactivity that prevents the full development and persistence of allergic features (TABLE 1).

IMMUNOMODULATORY THERAPIES USED
IN CLINICAL PRACTICE

Allergen-Specific Immunotherapy

The goal of allergen immunotherapy is to eliminate allergic symptoms and achieve clinical desensitization through the exposure of patients to progressively increasing doses of extracts, made up of the allergens to which they are allergic. Subcutaneous allergen immunotherapy (SCIT) has been used in clinical practice for nearly 100 years and is the only widely used method of immunomodulatory treatment. It is highly effective in selected patients with IgE-mediated allergies and sensitivity to a limited number of allergens (e.g., patients with seasonal allergic rhinitis, asthma, or insect venom hypersensitivity). SCIT inhibits efficiently the early- and late-phase allergic responses and confers protection for several years after its discontinuation.³⁴ There is

solid scientific evidence that SCIT exerts its therapeutic effects by altering the nature of immune responsiveness to allergens.^{34,35} Successful SCIT induces an increase in the allergen-specific IgG (especially the IgG4 subtype), which results in diminished IgE-mediated immune activity.³⁶ This happens because the IgG antibodies compete with IgE for allergen binding to the surface of basophils and mast cells.³⁶ Furthermore, SCIT promotes the suppression of allergen-specific Th2 responses and enhances the generation of tolerant T cells.^{35,37} In particular, it has been found that rapidly after the initiation of traditional, as well as rush protocols of SCIT there is significant rise of the IL-10-producing Treg cells in the peripheral blood and local tissues.³⁷ Some researchers believe that SCIT may also favor a shift of the allergen-induced Th cell responses toward the Th1 profile.³⁵

Despite its efficacy SCIT has several drawbacks. The fact that it is allergen-specific restricts its use to patients with known and limited allergen-sensitivities. The compliance of the patients is also a common problem, since in order to achieve sustainable therapeutic results a long and expensive commitment is required (most SCIT protocols last for at least 3 years). More importantly, SCIT is not entirely free of side effects and there is the rare but existing risk of severe anaphylactic reaction, even when standardized extracts are used. To avoid these defects alternative forms of allergen-immunotherapy have been developed aiming to reproduce the immunomodulatory effects of SCIT, without bearing the potential risk of strong IgE-mediated reactions. Among them, sublingual and oral forms of allergen immunotherapy have been tested in clinical practice. Meta-analysis of the performed studies suggests that sublingual immunotherapy is probably safer than SCIT and equally effective for the treatment of allergic rhinitis and asthma.³⁸ However, much higher doses of sublingually administered allergens may be required to obtain beneficial results.³⁸ In an attempt to decrease the allergenicity (IgE reactivity) of allergen extracts and reduce the number of doses required to achieve clinical benefit (e.g., by prolonging the absorption), physical and chemical modification of the allergens have been employed. Thus, allergen absorption to alum hydroxide, calcium phosphate, tyrosine and liposomes, or chemical modification with polyethylenoglycol (PEG) or polymerization (allergoids) with formaldehyde, glutaraldehyde, or alginic salt have been tested with variable results.^{34,39} An alternative option that has been recently discovered is the use of recombinant technology to produce large quantities of allergen derivatives genetically engineered to present reduced IgE binding capacity and therefore reduced allergenicity, but good ability to stimulate T cell responses.⁴⁰ An additional advantage of the recombinant allergens is that they are unable to generate new IgE antibodies, something that happens often when crude allergen extracts are used.⁴⁰ The efficacy of immunotherapy with recombinant allergens remains to be evaluated in the future. On the basis of the same rationale, immunotherapy using allergen-derived peptides that fail to bind to IgE, but are

potent T cell stimulators has been proposed.⁴¹ Preliminary trials assessing the subcutaneous administration of peptides derived from the cat allergen Feld 1 have shown modest reduction of the symptoms in cat-allergic patients.⁴¹

Anti-IgE

Since IgE is a key mediator of allergic pathogenesis, efforts to develop safe agents that block its activity have been going on for years. Recently a humanized recombinant monoclonal antibody (omalizumab) that is directed against IgE and inhibits its binding to FcεI receptor has reached the bedside.⁴² Omalizumab cannot provoke undesirable IgE cross-linking because its design is such that it does not allow the interaction with IgE molecules already bound to the surface of mast cells or basophils.⁴² Omalizumab was initially tested in patients with allergic asthma. The studies performed so far conclude that it is well-tolerated and effective in cases of both mild and severe allergic asthma.^{42,43} In particular, they show that treatment with Omalizumab leads to diminished asthma symptoms and amelioration of lung function.⁴³ In parallel, Omalizumab reduces the frequency of asthma exacerbations and the need for rescue and other anti-inflammatory medication.⁴³ The improvement observed after therapy with Omalizumab seems to be associated with decline of the free IgE levels, downregulation of the FcεIR expression, and decrease of the early and late allergic reactivity following aeroallergen exposure.⁴⁴ Omalizumab has been recently launched in clinical practice; therefore further evaluations are required to exclude the potential development of long-term adverse effects, to better define the categories of patients that benefit most, and to evaluate the right use of anti-IgE in combination with other antiallergic therapies. Another question that remains to be answered is the cost-effectiveness of anti-IgE therapy compared to the current antiasthmatic drug regimes. Furthermore, the role of anti-IgE in the treatment of other allergic diseases, such as atopic eczema, allergic rhinitis, and food allergy needs to be investigated thoroughly.

IMMUNOMODULATORY METHODS UNDER EVALUATION

Inhibition of Th Cell Activation

The recognition of the central role of Th cells in allergic pathophysiology has propelled significant effort toward the development of anti-T cell therapeutic strategies. To this end a trial assessing the efficacy of a chimeric anti-CD4 monoclonal antibody to inhibit allergic Th cell responses and therefore to control the disease progress has been conducted in asthmatic patients.³ Preliminary results suggest that a single intravenous administration of anti-CD4

leads to considerable decrease of CD4⁺ T cell counts, alleviation of asthma symptoms, and increase in peak flow, but not in FEV1, measurements.⁴⁵ To avoid the side effects of generalized T cell suppression, other more elegant approaches targeting the qualitative restriction of Th2 responsiveness have been pursued. A lot of interest focused on the design of agents blocking the T cell costimulatory pathways that influence the acquisition of Th2 phenotype.⁴⁶ It has been proposed that selective interaction of CD28 with the CD86 molecule on the surface of antigen-presenting cells may preferentially promote Th2 cell differentiation.⁴⁷ Administration of antibodies blocking the binding of CD28 to CD80/CD86 in murine models of allergic airways disease has been shown to diminish the levels of Th2 cytokines, the degree of lung inflammation, and the development of AHR.⁴⁸ ICOS is another costimulatory molecule critically implicated in the regulation of Th2 responses to aeroallergens and thus an interesting candidate for therapeutic manipulation.⁴⁹ Some reports suggest that blocking the binding of ICOS to its ligand attenuates the development of experimental allergic diseases.^{46,49} However, the issue remains controversial. The costimulatory activators OX40, CD30, or 4-1BB and the negative costimulator CTLA-4 are considered potential targets for allergy therapy.⁴⁶

Immune Deviation Using Microbial Products

The observation that Th1 cells antagonize the proliferation and functions of Th2 cells led to the idea that preferential induction of Th1 immunity to allergens could have a protective effect in allergic patients. In this context, various experimental methods aiming to shift the immune responsiveness to allergens from a Th2- to a Th1-type have been examined. Exposure to microbes is the most efficient stimulus to activate Th1 immunity. For this purpose the use of attenuated microbes or other microbial components, alone or as adjuvants to allergen immunotherapy, has attracted considerable interest.⁵⁰ Experiments with heat-killed *Listeria* or attenuated mycobacterial strains (BCG vaccine or *Mycobacterium vaccae*) have demonstrated that these agents can protect against the development of allergic disease in mice, if used at the right time.⁵¹⁻⁵³ However, clinical studies have to be carried out to evaluate their real potential in allergy prevention and therapy. More data for the beneficial effect of microbial products have been accumulated from studies using the 3-deacylated monophosphoryl lipid A (MLP), an LPS-derived adjuvant that has been successfully used in viral vaccines.⁵⁰ Early trials with a combination of MLP with grass pollen allergen extracts have shown alleviation of allergic rhinitis symptoms and reduction of the need for supporting medication.⁵⁰ The Th2-modifying microbial adjuvants are potent promoters of Th1 responses through induction of IL-12 and IFN synthesis by antigen-presenting cells.^{35,50} In parallel, they boost the activation of Treg cells secreting IL-10

and/or TGF- β .^{35,50} An alternative approach to safely stimulate the generation of Th1/Treg cells is the use of DNA immunostimulatory sequences.⁵⁴ These sequences contain repeated dinucleotide cytosine-guanine (CpG) motifs and are highly preserved in microbial DNA, but are absent in mammals. The CpG motifs are universally recognized by the innate immune system of vertebrates through toll-like receptors and trigger the activation of protective immune responses.⁵⁴ Sole administration of synthetic oligodeoxynucleotides containing unmethylated CpG dinucleotides (ISS-ODN) have been found effective in preventing and reversing allergen-induced Th2 inflammation in murine models of asthma.^{55,56} The protective effect of ISS-ODNs appears to be even higher when they are used directly conjugated to allergens.⁵⁵ An ISS-ODN-ragweed allergen (Amb1) conjugate has been proven to be a very effective suppressor of allergen-specific Th2 responses in mice as well as in humans.⁵⁶ Results from clinical trials in ragweed-sensitive adults have demonstrated that treatment with the ISS-ODN conjugate reduces the nasal eosinophilia and local expression of Th2-type cytokines.⁵⁷ Furthermore, there is significant lessening of rhinitis symptoms. The ISS-ODN conjugate appears to be 100-fold less allergenic than the conventional ragweed immunotherapy.⁵⁷ The successful results from the early phases of clinical trials have given rise to considerable enthusiasm about the development of DNA vaccines for allergy prevention, whereas an important caveat that remains to be delineated is the potential long-term stimulation of Th1 pathology that may lead eventually to severe deterioration of the inflammatory disease.

Inhibition of Allergy-Inducing Cytokine Networks

Cytokines play a deterministic role in the generation of allergic T and B cell responses, as well as in the maintenance of allergic inflammation. Therefore, it has been proposed that blocking key cytokines, such as IL-4, IL-13, and/or IL-5, may alter radically not only the phenotype of allergen-specific immune responses, but also the effector features of the disease. To examine this hypothesis several antiallergic cytokine agents have been developed, including humanized blocking antibodies against cytokines, antibodies against cytokine receptors, and soluble cytokine receptors.^{11,58,59} Initial studies evaluating the effect of inhibiting the IL-4 activity with a humanized anti-IL-4 monoclonal antibody (pascolizumab) or a soluble recombinant IL-4 receptor had encouraging results. Administration of these agents in mice inhibited profoundly the development of allergen-mediated AHR and inflammation.⁶⁰ Unfortunately, the efficacy of the anti-IL-4 treatment has not been confirmed in large-scale clinical trials in patients with moderate to severe asthma.⁶⁰ Administration of anti-IL-4 in patients led to downregulation of several immune parameters, including IL-4 activity, Th2 cell activation, or IgE synthesis. However, these changes were not accompanied by a significant therapeutic benefit.⁶⁰ Mixed

conclusions were also reached in the trials assessing the treatment of asthmatics with an anti-IL-5 monoclonal antibody (mepolizumab).⁶¹ Thus, although reduction in the number of circulating eosinophils and prevention of their recruitment to the lungs were observed, marked improvement in lung function and airway reactivity was not achieved.⁶¹ In contrast, therapy with anti-IL-5 had excellent results in other diseases primarily driven by eosinophils, such as the hypereosinophilic syndrome.⁶¹ Agents blocking the pathways triggered by IL-13 are still under development (i.e., IL-13Ra2- IgGFc fusion protein) and their efficacy remains to be determined.⁶² The failure to reach the therapeutic goals with the anti-IL-4 and anti-IL-5 agents has halted the initial optimism about the potential of anticytokine strategies. Nevertheless, this cannot preclude the possibility that the efforts to interrupt the cytokine pathways by using small molecules that block critical signal transduction events (e.g., suppressors of STAT6 or GATA3 activity) may not be more successful.⁶³ Another issue that needs to be examined is the use of multiple anticytokine agents in combination, as it is known that there is significant redundancy in the allergic cytokine network. Alternatively, the combination of anticytokine therapy with other disease-modifying approaches can also be evaluated.

Inhibition of Inflammatory Cell Transmigration

A new concept to treat allergic inflammation is by inhibiting the inappropriate recruitment of leukocytes at the sites of allergen exposure. This could be achieved by interfering selectively with the system of chemokines and adhesion molecules that regulate the trafficking of Th2 cells, eosinophils, basophils, and mast cells. For example, blocking the adhesion molecule LFA-1 or the binding to its ligand ICAM-1 has been shown effective in abrogating the early- and late-phase reactivity to allergens and the development of inflammation in animals.⁶⁴ Data from preliminary trials using an anti-LFA-1/CD11a monoclonal antibody (efalizumab) in allergic patients support this finding and show that following treatment the inflammatory infiltration at the sites of allergen challenge is attenuated.⁶⁴ Further studies are required to determine the therapeutic potential of this approach. Other suitable targets for suppressing the migration of allergic effector cells include the VLA-4, which serves as a receptor for VCAM-1, the eotaxin receptors, as well as several other chemokine–chemokine receptor interactions (e.g., CCL17/CCL22-CCR4, CCL1-CCR8, CXCL12-CXCR4) that appear to facilitate preferentially the development of Th2 inflammation.^{20,25}

CONCLUSION

The immunomodulatory strategies provide a promising alternative for the treatment of allergic patients in the future. However, many questions remain to

be answered before we have novel immunomodulatory tools in clinical practice. Apart from issues relevant to the efficacy, safety, and longevity of the novel forms of treatment, another point that needs to be clarified is the optimum timing for intervention in order to maximize the therapeutic benefit. It is probable that early intervention may be more successful in altering the disease pathogenesis and in preventing the progression beyond the stage of mild allergy. For this purpose, the development of prophylactic forms of immunotherapy targeting high-risk atopic children may have several merits. Further studies are also required to define the best therapeutic options for the treatment of the chronic forms of allergic inflammation where several allergen-independent mechanisms are involved (e.g., asthma, atopic dermatitis). In these cases, the design of comprehensive multilayer strategies targeting all the coexisting dysfunctional immune pathways may yield better results.

REFERENCES

1. ROMAGNANI, S. 2000. The role of lymphocytes in allergic disease. *J. Allergy Clin. Immunol.* **105**: 399–408.
2. KAY, A.B. 2001. Allergy and allergic diseases. First of two parts. *N. Engl. J. Med.* **344**: 30–37.
3. LARCHE, M., D.S. ROBINSON & A.B. KAY. 2003. The role of T lymphocytes in the pathogenesis of asthma. *J. Allergy Clin. Immunol.* **111**: 450–463.
4. LAMBERT, L.E., J.S. BERLING & E.M. KUDLACZ. 1996. Characterization of the antigen-presenting cell and T cell requirements for induction of pulmonary eosinophilia in a murine model of asthma. *Clin. Immunol. Immunopathol.* **81**: 307–311.
5. DE SANCTIS, G.T. *et al.* 1997. T-lymphocytes regulate genetically determined airway hyperresponsiveness in mice. *Nat. Med.* **3**: 460–462.
6. CORRY, D.B. *et al.* 1998. Requirements for allergen-induced airway hyperreactivity in T and B cell-deficient mice. *Mol. Med.* **4**: 344–355.
7. UMETSU, D.T. & R.H. DEKRUYFF. 1997. TH1 and TH2 CD4⁺ cells in human allergic diseases. *J. Allergy Clin. Immunol.* **100**: 1–6.
8. BROIDE, D.H. 2001. Molecular and cellular mechanisms of allergic disease. *J. Allergy Clin. Immunol.* **108**: S65–S71.
9. ROMAGNANI, S. 2002. Cytokines and chemoattractants in allergic inflammation. *Mol. Immunol.* **38**: 881–885.
10. HERRICK, C.A. & K. BOTTOMLY. 2003. To respond or not to respond: T cells in allergic asthma. *Nat. Rev. Immunol.* **3**: 405–412.
11. LI-WEBER, M. & P.H. KRAMMER. 2003. Regulation of IL4 gene expression by T cells and therapeutic perspectives. *Nat. Rev. Immunol.* **3**: 534–543.
12. PERNIS, A.B. & P.B. ROTHMAN. 2002. JAK-STAT signaling in asthma. *J. Clin. Invest.* **109**: 1279–1283.
13. TAKEDA, K., T. KISHIMOTO & S. AKIRA. 1997. STAT6: its role in interleukin 4-mediated biological functions. *J. Mol. Med.* **75**: 317–326.
14. MOWEN, K.A. & L.H. GLIMCHER. 2004. Signaling pathways in Th2 development. *Immunol. Rev.* **202**: 203–222.

15. ZHOU, M. & W. OUYANG. 2003. The function role of GATA-3 in Th1 and Th2 differentiation. *Immunol Res.* **28**: 25–37.
16. PLATTS-MILLS, T.A. 2001. The role of immunoglobulin E in allergy and asthma. *Am. J. Respir. Crit. Care Med.* **164**: S1–S5.
17. EL BIAZE, M. *et al.* 2003. T cell activation, from atopy to asthma: more a paradox than a paradigm. *Allergy* **58**: 844–853.
18. HOLGATE, S.T. 2002. Airway inflammation and remodeling in asthma: current concepts. *Mol. Biotechnol.* **22**: 179–189.
19. GIROLOMONI, G. *et al.* 2001. T-cell subpopulations in the development of atopic and contact allergy. *Curr. Opin. Immunol.* **13**: 733–737.
20. BISSET, L.R. & P. SCHMID-GRENDELMEIER. 2005. Chemokines and their receptors in the pathogenesis of allergic asthma: progress and perspective. *Curr. Opin. Pulm. Med.* **11**: 35–42.
21. ONO, S.J. *et al.* 2003. Chemokines: roles in leukocyte development, trafficking, and effector function. *J. Allergy. Clin. Immunol.* **111**: 1185–1199.
22. LUKACS, N.W. 2001. Role of chemokines in the pathogenesis of asthma. *Nat. Rev. Immunol.* **1**: 108–116.
23. SALLUSTO, F. & A. LANZAVECCHIA. 2000. Understanding dendritic cell and T-lymphocyte traffic through the analysis of chemokine receptor expression. *Immunol. Rev.* **177**: 134–140.
24. COSMI, L. *et al.* 2001. Chemoattractant receptors expressed on type 2 T cells and their role in disease. *Int. Arch. Allergy Immunol.* **125**: 273–279.
25. AMERIO, P. *et al.* 2003. Eotaxins and CCR3 receptor in inflammatory and allergic skin diseases: therapeutical implications. *Curr. Drug Targets Inflamm. Allergy* **2**: 81–94.
26. AKBARI, O. *et al.* 2003. Mucosal tolerance and immunity: regulating the development of allergic disease and asthma. *Int. Arch. Allergy Immunol.* **130**: 108–118.
27. KUIPERS, H. & B.N. LAMBRECHT. 2004. The interplay of dendritic cells, Th2 cells and regulatory T cells in asthma. *Curr. Opin. Immunol.* **16**: 702–708.
28. VAN PARIJS, L., V.L. PEREZ & A.K. ABBAS. 1998. Mechanisms of peripheral T cell tolerance. *Novartis Found. Symp.* **215**: 5–14.
29. TSITOURA, D.C. *et al.* 1999. Intranasal exposure to protein antigen induces immunological tolerance mediated by functionally disabled CD4⁺ T cells. *J. Immunol.* **163**: 2592–2600.
30. TSITOURA, D.C. *et al.* 2000. Mechanisms preventing allergen-induced airways hyperreactivity: role of tolerance and immune deviation. *J. Allergy Clin. Immunol.* **106**: 239–246.
31. AKDIS, M., K. BLASER & C.A. AKDIS. 2005. T regulatory cells in allergy: novel concepts in the pathogenesis, prevention, and treatment of allergic diseases. *J. Allergy Clin. Immunol.* **116**: 961–968.
32. ROBINSON, D.S., M. LARCHE & S.R. DURHAM. 2004. Tregs and allergic disease. *J. Clin. Invest.* **114**: 1389–1397.
33. BLUMENTHAL, R.L. *et al.* 2001. Human alveolar macrophages induce functional inactivation in antigen-specific CD4 T cells. *J. Allergy Clin. Immunol.* **107**: 258–264.
34. NELSON, H.S. 2004. Advances in upper airway diseases and allergen immunotherapy. *J. Allergy Clin. Immunol.* **113**: 635–642.
35. TILL, S.J. *et al.* 2004. Mechanisms of immunotherapy. *J. Allergy Clin. Immunol.* **113**: 1025–1034.

36. WACHHOLZ, P.A. & S.R. DURHAM. 2004. Mechanisms of immunotherapy: IgG revisited. *Curr. Opin. Allergy Clin. Immunol.* **4**: 313–318.
37. AKDIS, C.A., K. BLASER & M. AKDIS. 2006. Mechanisms of allergen-specific immunotherapy. *Chem. Immunol Allergy*. **91**: 195–203.
38. PASSALACQUA, G., C. LOMBARDI & G.W. CANONICA. 2004. Sublingual immunotherapy: an update. *Curr. Opin. Allergy Clin. Immunol.* **4**: 31–36.
39. WHEELER, A.W. & S.R. WORONIECKI. 2004. Allergy vaccines—new approaches to an old concept. *Expert Opin. Biol. Ther.* **4**: 1473–1481.
40. LINHART, B. & R. VALENTA. 2005. Molecular design of allergy vaccines. *Curr. Opin. Immunol.* **17**: 646–655.
41. LARCHE, M. & D.C. WRAITH. 2005. Peptide-based therapeutic vaccines for allergic and autoimmune diseases. *Nat. Med.* **11**: S69–S76.
42. BROWNELL, J. & T.B. CASALE. 2004. Anti-IgE therapy. *Immunol. Allergy Clin. North Am.* **24**: 551–568.
43. BUHL, R. 2005. Anti-IgE antibodies for the treatment of asthma. *Curr. Opin. Pulm. Med.* **11**: 27–34.
44. INFUHR, D. *et al.* 2005. Molecular and cellular targets of anti-IgE antibodies. *Allergy*. **60**: 977–985.
45. KON, O.M. *et al.* 2001. The effects of an anti-CD4 monoclonal antibody, keliximab, on peripheral blood CD4⁺ T-cells in asthma. *Eur. Respir J.* **18**: 45–52.
46. KROCZEK, R. & E. HAMELMANN. 2005. T-cell costimulatory molecules: optimal targets for the treatment of allergic airway disease with monoclonal antibodies. *J. Allergy Clin. Immunol.* **116**: 906–909.
47. LARCHE, M. *et al.* 1998. Costimulation through CD86 is involved in airway antigen-presenting cell and T cell responses to allergen in atopic asthmatics. *J. Immunol.* **161**: 6375–6382.
48. CHEN, Y.Q. & H.Z. SHI. 2006. CD28/CTLA-4-CD80/CD86 and ICOS-B7RP-1 costimulatory pathway in bronchial asthma. *Allergy*. **61**: 15–26.
49. GONZALO, J.A. *et al.* 2001. ICOS is critical for T helper cell-mediated lung mucosal inflammatory responses. *Nat. Immunol.* **2**: 597–604.
50. FRANCIS, J.N. & S.R. DURHAM. 2004. Adjuvants for allergen immunotherapy: experimental results and clinical perspectives. *Curr. Opin. Allergy Clin. Immunol.* **4**: 543–548.
51. HANSEN, G. *et al.* 2000. Vaccination with heat-killed *Listeria* as adjuvant reverses established allergen-induced airway hyperreactivity and inflammation: role of CD8⁺ T cells and IL-18. *J. Immunol.* **164**: 223–230.
52. LI, X.M. *et al.* 2003. Engineered recombinant peanut protein and heat-killed *Listeria monocytogenes* coadministration protects against peanut-induced anaphylaxis in a murine model. *J. Immunol.* **170**: 3289–3295.
53. ZUANY-AMORIM, C. *et al.* 2002. Long-term protective and antigen-specific effect of heat-killed *Mycobacterium vaccae* in a murine model of allergic pulmonary inflammation. *J. Immunol.* **169**: 1492–1499.
54. CHU, R.S. *et al.* 1997. CpG oligodeoxynucleotides act as adjuvants that switch on T helper 1 (Th1) immunity. *J. Exp. Med.* **186**: 1623–1631.
55. HORNER, A.A. & E. RAZ. 2002. Immunostimulatory sequence oligodeoxynucleotide-based vaccination and immunomodulation: two unique but complementary strategies for the treatment of allergic diseases. *J. Allergy Clin. Immunol.* **110**: 706–712.
56. HUSSAIN, I. & J.N. KLINE. 2003. CpG oligodeoxynucleotides: a novel therapeutic approach for atopic disorders. *Curr. Drug Targets Inflamm. Allergy*. **2**: 199–205.

57. BROIDE, D.H. 2005. Immunostimulatory sequences of DNA and conjugates in the treatment of allergic rhinitis. *Curr. Allergy Asthma Rep.* **5**: 182–185.
58. FOSTER, P.S. *et al.* 2002. Interleukins-4, -5, and -13: emerging therapeutic targets in allergic disease. *Pharmacol. Ther.* **94**: 253–264.
59. CHUNG, K.F. 2003. Individual cytokines contributing to asthma pathophysiology: valid targets for asthma therapy? *Curr. Opin. Investig. Drugs.* **4**: 1320–1326.
60. STEINKE, J.W. 2004. Anti-interleukin-4 therapy. *Immunol Allergy Clin. North Am.* **24**: 599–614.
61. KAY, A.B. & A.D. KLION. 2004. Anti-interleukin-5 therapy for asthma and hyper-eosinophilic syndrome. *Immunol. Allergy Clin. North Am.* **24**: 645–666.
62. YANG, G. *et al.* 2004. Anti-IL-13 monoclonal antibody inhibits airway hyperresponsiveness, inflammation and airway remodeling. *Cytokine* **28**: 224–232.
63. NAKAMURA, Y. & M. HOSHINO. 2005. TH2 cytokines and associated transcription factors as therapeutic targets in asthma. *Curr. Drug Targets Inflamm Allergy.* **4**: 267–270.
64. GAUVREAU, G.M. *et al.* 2003. The effects of an anti-CD11a mAb, efalizumab, on allergen-induced airway responses and airway inflammation in subjects with atopic asthma. *J. Allergy Clin. Immunol.* **112**: 331–338.