

NOVEL IMMUNOTHERAPEUTIC APPROACHES FOR THE TREATMENT OF ALLERGIC DISEASES

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Advances in the knowledge of the cellular and molecular basis of immunity have led to an enhanced thorough understanding of the immunologic features that characterize an allergic response. A hallmark of an allergic response is a persistently elevated level of specific IgE antibodies to a variety of antigens, also referred to as *allergens*, to which the affected individual is regularly exposed by inhalation, ingestion, or contact with the skin.⁴⁸ Allergic sensitization involves processing of the antigen by an antigen-presenting cell (APC) and presentation, in association with a class II major histocompatibility complex (MHC) protein, to a T-cell receptor. Whereas dendritic cells (and macrophages) predominantly act as APCs in primary immunization, B cells may also participate in secondary immune responses to allergens. Activation of T cells requires the first signal from this trimolecular interaction and an additional costimulatory signal involving the ligation of B-7 on an APC (CD80/86) with CD28 or CTLA4 on a T cell.²⁷ When stimulated by antigens, helper T cells produce an array of specific cytokines, all of which have been designated as either T_H1 cytokines (interleukin [IL]-2, interferon [IFN]- γ , and tumor necrosis factor [TNF]- β) or T_H2 cytokines (IL-4, IL-5, IL-9, IL-10, and IL-13). The state of T-cell activation depends

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on several factors.^{27, 85} The first of these factors is the affinity of the interaction between the antigen and the T cell. The site of the antigen recognized by the T cell is termed the *epitope*. The affinity of epitope-T cell interaction is affected by the concentration of the antigen and the type of APC.⁸⁰ It also depends on the cytokine milieu of the T cell during antigen interaction. Thus, IFN- γ and IL-12 promote a T_H1-like response, whereas IL-4 promotes a T_H2-like response.¹² Additionally, host immune response genes may bias the overall immune responsiveness of an individual to favor a T_H1- or T_H2-like response. A T_H2-like cytokine profile is associated with the induction of IgE antibody (Ab) production in vitro and in vivo.⁷³ Specifically, IL-4 favors the development of T_H2-like cells from uncommitted T cells, and both IL-4 and IL-13 play a role in IgE antibody production.

Manifestation of an allergic reaction depends on the specific IgE levels and the amount of exposure at the time of the reaction. Although an allergic condition is a risk factor for asthma, about 20% to 30% of asthmatics do not show positive skin tests to allergens. In general terms, asthma is defined as an inflammatory disease where not only lymphocytes, but also other cells, such as mast cells, basophils, eosinophils, and epithelial cells, play a role. Studies to date suggest that T_H2-like cytokines, such as IL-4 and IL-5, also play an important role in nonatopic asthma.^{3, 85}

Developing immunotherapeutic approaches against allergic diseases, including asthma, may be pragmatically divided into two categories based on how soon they may make an inroad to the clinic, as shown in Figure 1. The first category includes therapies that are already in phase II/phase III clinical trials and are expected to be available in clinics within the next 1 to 3 years; such therapies include antihuman IgE antibodies and the soluble IL-4 receptor. The second category includes experimental approaches that are mainly at the stage of preclinical research in model systems and may proceed to clinical trials and the clinic within the next 5 to 10 years; these therapies include various allergen-specific and allergen-nonspecific approaches.

NONSPECIFIC THERAPEUTIC APPROACHES

Monoclonal Anti-IgE Antibody Therapy

Rationale

The development of an allergic state is a gradual process, and as a genetically predisposed individual undergoes the "allergic march," he or she may develop allergies to several antigens.¹³ Because IgE crosslinking on the mast cell membrane by allergens is an important first step in an allergic reaction, IgE has been an attractive therapeutic target. Although the idea of anti-IgE as a therapeutic agent was conceived of during the 1970s, the use of anti-IgE as a therapeutic strategy failed because the antibodies initially employed caused mast cell degranula-

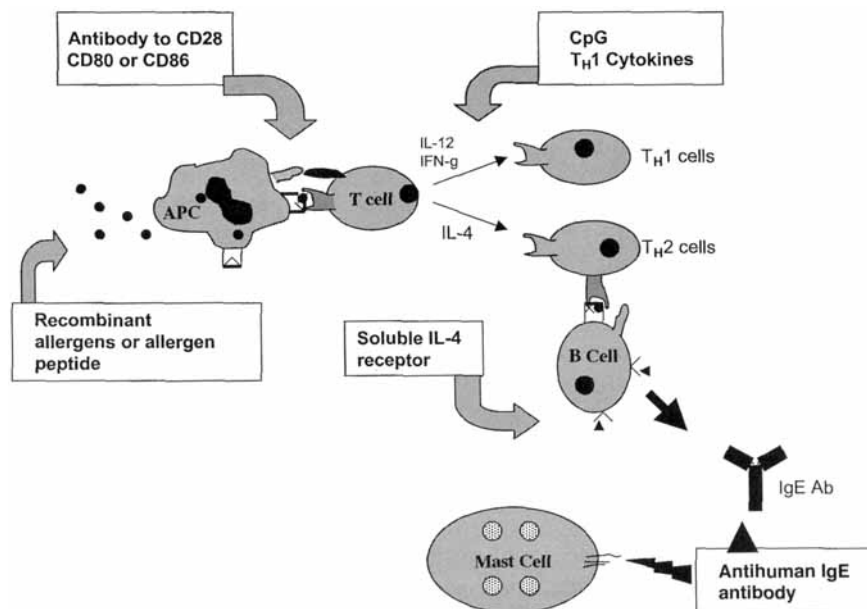


Figure 1. Mechanism underlying allergic diseases and potential interventions. In principle, each of the steps of the immune response against allergens can be targeted. Recombinant allergens or allergen peptides can be presented to T_H0 cells to make them differentiate into T_H1 cells and induce anergy to T_H2 cells. The use of antibodies against costimulatory molecules such as CD80/CD86 and its receptor, CD28, can inhibit costimulation and promote T_H1 differentiation. The use of soluble IL-4 receptors can block the binding of IL-4 on its receptors, affecting B-cell production of IgE and the development of T_H2 cells. Finally, humanized monoclonal anti-IgE antibodies can bind to free IgE and block the binding of free IgE to IgE-receptors on mast cells, preventing mast cell degranulation.

tion. With the advent of recombinant DNA technology, it has become possible to engineer monoclonal antibodies, which are capable of binding to circulating serum IgE antibodies but not to IgE antibodies bound to FcεRI or to FcεRII.⁶⁶ Thus, this strategy reduces unbound IgE levels in the serum. Several clinical studies have been conducted to evaluate the safety and effectiveness of using antihuman IgE antibodies to treat allergic asthma and allergic rhinitis. The results of these studies show that anti-IgE therapy reduces allergic symptoms, is well tolerated by patients, and does not have any severe adverse effects.²¹ The structural, functional, and immunomodulatory aspects of anti-human IgE antibodies and their potential in the treatment of allergic diseases are briefly reviewed later.

Design of Humanized Monoclonal Anti-IgE Antibodies

Previously, the use of nonhuman antibodies for clinical purposes indicated that these antibodies induced immune responses in patients, had a rapid clearance, and showed a weak recruitment of effector func-

tions. The fact that all of these factors reduced the therapeutic potential of such antibodies led to the concept of humanizing these antibodies.

The humanization of monoclonal antibodies refers to the *transplantation* of the nonhuman regions that bind the antigen, called complementary determining regions (CDRs), onto a human antibody framework using recombinant DNA protocols.^{36, 66} In addition to the CDR, certain amino acid residues in the variable framework region may be important not only for maintaining the CDR structure, but also for antigen binding. The selection of a human antibody framework is one of the most important considerations in the humanization process. There are two basic approaches: (1) selecting the human antibody most homologous to the murine antibody from a database of human antibody sequences, or (2) using a framework derived from consensus sequences of human variable light (VL) and variable heavy (VH) chain subgroups. Despite the advances in our knowledge of antigen-antibody interactions, it is difficult to determine the precise nonhuman CDR and variable framework sequences. Thus, humanization remains an empirical process.

The strategy to produce an antihuman IgE antibody involves three steps (Fig. 2). First, monoclonal antibodies are produced in mice using human myeloma-produced IgE antibodies as immunogen. Second, monoclonal antibodies are tested to identify which of them are capable of joining to free IgE but not to receptor-bound IgE. Thus, the antibodies are evaluated for their ability to inhibit IgE binding to the α chain of the high affinity Fc ϵ RI and the inability of these antihuman IgE:IgE complexes to degranulate mast cells and basophils. Third, the candidate monoclonal antibodies, which degranulate neither mast cells nor basophils, are humanized (i.e., the nonhuman sequences in these molecules are significantly reduced by genetic engineering techniques).

Two mouse monoclonal anti-IgE antibodies, TES-C21 and MAE11, have been humanized following the previously mentioned basic strategy^{36, 66}; however, only the humanized version of MAE11, called rhuMAb E25, has been evaluated in clinical studies. In humanizing MAE11, a framework derived from consensus sequences of human VL and VH chains was used, and the critical amino acids responsible for binding to IgE were engrafted onto a consensus human IgG₁ framework.⁶⁶

How Does the Anti-IgE Therapy Work?

In principle, antihuman IgE antibodies reduce free IgE levels and hinder the binding of IgE molecules with their high- and low-affinity receptors.⁷⁹ In addition, the physical interaction between IgE and anti-IgE leads to a number of immunomodulatory effects. Several studies have been conducted in vitro, as well as in vivo, using both murine and human systems to understand the mechanisms underlying these effects of antihuman IgE antibodies. The effectiveness of anti-IgE antibodies in asthma was examined in a murine model. Thus, using B-cell-deficient mice, the development of airway hyperresponsiveness was linked with

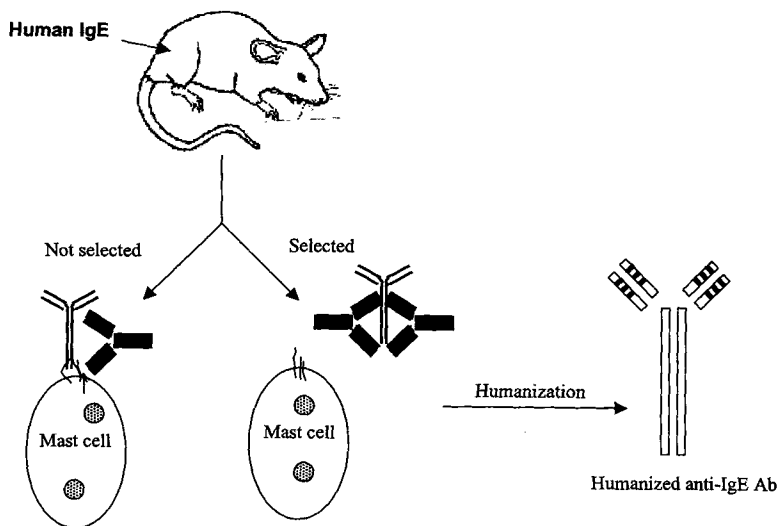


Figure 2. Humanization of mouse monoclonal antihuman-IgE antibodies. The process of generating antihuman IgE antibodies for treatment of allergic diseases involves three steps. (1) The monoclonal antihuman-IgE antibodies are produced in mice. (2) Such antibodies are subject to a selection process in which those that bind only to free IgE are selected. (3) The selected antibodies are humanized. This process involves: analysis of the complementary DNA (cDNA) encoding the variable region of the best (in terms of affinity and ability to bind to free IgE but not mast cells) antihuman-IgE antibody; making the appropriate changes on the cDNA sequence of human consensus variable regions of κ and IgG1 chains using site-directed mutagenesis based on the sequence analysis; and synthesis of the humanized mouse monoclonal antihuman-IgE antibody by recombinant DNA technology.

the presence of IgE.²⁶ Moreover, the administration of an anti-IgE monoclonal antibody to sensitized mice neutralized serum IgE and inhibited the recruitment of eosinophils into the lungs as well as the production of T_H2 cytokines, such as IL-4 and IL-5.¹⁵

Studies have shown that most of these anti-IgE antibodies do not bind to receptor-bound IgE, with the exception of anti-IgE BSW17, a murine antibody that binds to IgE attached to mast cells or basophils but does not induce degranulation.^{67, 74} Biochemical studies using sedimentation analysis and size exclusion chromatography have shown that recombinant humanized monoclonal anti-IgE E25 (rhuMAb E25) is able to form different complexes with IgE, depending on the molar ratio of IgE and anti-IgE.⁴¹ Thus, the molar ratio determines whether E25-IgE forms either a predominant heterohexamer or a heterotrimer structure. In addition, these complexes are very stable and the kinetic studies have revealed that IgE binds to rhuMAb E25 with a very high affinity and with a very low K_d (0.06 nM).⁵¹ An important finding is that the relation between the levels of free IgE and receptor-bound IgE are maintained in the system at a certain ratio. The binding of anti-IgE antibodies to free

IgE results in a decrease of IgE levels in circulation; consequently, cell-bound IgE is freed from the receptors in order to maintain the serum ratio.⁷⁴ Thus, anti-IgE antibodies cause a reduction of the mast cell- and basophil-bound IgE. Treatment with rhuMAb E25 for a 3-month period resulted in a decrease of free IgE levels and a concomitant down-regulation of the median density of FcεRI from 220,000 receptors to 8300 receptors per basophil.⁴⁴ This FcεRI reduction is accompanied by a decline of histamine release response to *in vitro* allergen exposure. In addition, in a murine model, an anti-IgE antibody inhibits FcεRII (CD23) expression, up-regulation on murine B cells, and IgE synthesis.²⁵ CD23 is known to efficiently participate in the capture and presentation of antigens associated with IgE and may favor the development of T_H2 responses.^{23, 25} Thus, the reduced levels of CD23 may partially explain IgE synthesis inhibition.

Clinical Studies

Two monoclonal anti-IgE antibodies, rhuMAb E25 and CGP51901, have been used in clinical trials to demonstrate safety, tolerance, and efficacy. These clinical trials have included subjects with asthma or allergic rhinitis. The anti-IgE antibody CGP51901, which is a chimeric version of the mouse monoclonal anti-IgE TES-C21, has been evaluated in patients with seasonal allergic rhinitis.^{14, 68} These studies have shown a decrease in serum-free IgE levels but an increase in total IgE levels. The slow clearance of IgE-anti-IgE complexes explains the increase, with complexed IgE having a half-life of 11 to 13 days. The reduction in serum-free IgE levels was dose dependent upon anti-IgE doses and was reversible. It was necessary to sustain 85% or greater reduction in serum-free IgE levels to achieve improved clinical symptoms. An eighty-five percent reduction in IgE required a serum CGP51901 concentration of 5,000 ng/mL. The treatment was safe, with no serum sickness and one case of urticaria among 153 evaluated patients.⁶⁸

rhuMAb E25 has been evaluated in both allergic rhinitis and asthma. It decreased serum-free IgE in a dose-dependent fashion in patients with seasonal allergic rhinitis¹⁰; however, in this study, it was not possible to evaluate its clinical efficacy because only 11 subjects had undetectable IgE levels. To achieve undetectable IgE concentration, the dose of rhuMAb E25 was 0.005 mg/kg/wk for each international unit per milliliter of baseline IgE. There were no adverse effects related to this treatment. rhuMAb E25 has also been studied in asthma.^{7, 20, 24} Asthma symptoms, rescue 2-agonist use, or forced expiratory volume in one second (FEV₁) did not improve; however, there was improvement in the allergen early- and late-asthmatic response, methacholine reactivity, and some inflammatory parameters in induced sputum.^{7, 20} The evaluated patients had mild asthma, which may have limited the potential for improvement.

The administration of rhuMAb E25 significantly increased the allergen doses required to cause a 15% fall in FEV₁, with a correlation between the decrease in serum-free IgE and the protection against inhaled allergen.⁷ The same study also showed that a higher dose of

methacholine was necessary to reduce FEV₁ by 20% after administration of rhuMAb E25. Another study showed rhuMAb E25 reduced maximal bronchoconstriction by 60% during the late asthmatic response following allergen challenge. Reduction of the early response after allergen bronchial challenge was less affected.²⁸ The late asthmatic response following allergen challenge correlates with airway hyperresponsiveness, airway inflammation, and improvement of asthma symptoms, suggesting rhuMAb E25 may have a clinical benefit.^{8, 17} Additional studies support this possibility. rhuMAb E25 administration reduced the number of asthmatic exacerbations and facilitated a 50% dose reduction of inhaled and oral steroids.^{22, 52} Minimal toxicity was observed with one case of urticaria in all of these reports. Thus, anti-IgE is a potentially safe and effective therapy for asthma.

Soluble Interleukin-4 Receptor Therapy

Interleukin-4 was discovered during the 1980s as a murine 20-kDa T-cell-derived B-cell growth factor-1 (also referred to as B-cell stimulatory factor-1 [BSF-1]).⁶¹ As shown in Figure 1, B-cell production of allergen-specific IgE antibodies is regulated by helper T cells producing cytokines, such as IL-4 and IL-13. IL-4 is one of the most important cytokines in a variety of allergic diseases, including asthma. IL-4 is produced primarily by CD4 T_H2 cells but also by CD8 T cells, eosinophils, mast cells, and basophils. IL-4 activity in different cell types is shown in Table 1. The role of IL-4 in human asthma has been established in several studies. The proof of the importance of IL-4 includes the following: (1) characterization of T-cell clones producing IL-4 in allergic and nonallergic individuals^{73, 84}; (2) *in situ* localization of IL-4-producing cells in bronchial biopsies⁸⁵; (3) expression of IL-4 receptor mRNA and protein in epithelium, subepithelium, and endothelial cell layers in bronchial biopsies of atopic asthmatics compared with controls³⁷; and (4) analysis of mutations of the IL-4 receptor (IL-4R) and their association with asthma.⁵⁴

Basis for Modulation of Asthma with Interleukin-4 Receptor

Interleukin-4 binds to the cell surface IL-4R heterodimer, which consists of an independent high-affinity α -chain and γ -chain shared with IL-2, IL-7, IL-9, and IL-15.³¹ The IL-4R α -chain also is similar to the IL-13R- α chain, which allows the binding of both IL-4 and IL-13.⁴² The existence of both a membrane-bound and a soluble form of IL-4R in the mouse was reported in 1989.⁵⁷ Soluble IL-4 is primarily a product of proteolysis of the IL-4 α -chain before release (shedding).³⁴ A soluble form of human IL-4R α , resulting from alternate splicing of the human IL-4R α gene, has also been described.³⁸ Secreted forms of the α -chain of the IL-4 receptor lack the transmembrane and cytoplasmic domains but still interact with IL-4. The importance of IL-4 in the regulation of the allergic response, coupled with the identification of the soluble receptor,

Table 1. EFFECTS OF IL-4 ON VARIOUS CELL POPULATIONS

Cell Type	Biological Effect	Clinical Effect
T cell	Differentiation of naïve T cells to T _H 2 cells that produce IL-4, IL-5, IL-9, and IL-13	Propagation of T _H 2-immune responsiveness
B cell	Proliferation of T _H 2 cells IgE isotype switch Up-regulation of low-affinity IgE receptor and class II MHC	Elevated IgE production and positive skin test Enhanced IgE production
Mast cell	Proliferation of B cells Up-regulates high affinity IgE receptor, ICAM-1 expression, and production of IL-5, IL-8, IL-13, MIP1- α , and GM-CSF	Increased histamine release
Basophils	Up-regulates high-affinity IgE receptor	Increased histamine release
Mononuclear phagocytes	Up-regulation of low-affinity IgE receptor and class II MHC	Enhanced IgE production
Fibroblasts	Mediator release Up-regulate chemokine production	Fibrosis and airway remodeling
Epithelial cells	Up-regulate mucin gene expression Facilitate differentiation into goblet cells	Mucus hypersecretion
Endothelial cells	Up-regulate VCAM-1	Eosinophil recruitment to airways

GM-CSF = granulocyte-macrophage colony-stimulating factor; ICAM-1 = intercellular adhesion molecule-1

suggested the evaluation of the soluble IL-4 receptor (sIL-4R) as a therapeutic agent.

Soluble IL-4R inhibited the IL-4-induced proliferation of B cells, the expression of low affinity IgER and MHC class II, and the secretion of both IgE and IgG1 antibodies in mice.⁴⁶ In a murine model of allergen sensitization, murine-soluble IL-4R inhibited both polyclonal and, particularly, antigen-specific IgE and IgG1 production following restimulation with these allergens.⁷⁰ Kinetic experiments indicated that the newly induced IgE production by IL-4 was inhibited by murine sIL-4R.⁷⁰ In summary, murine sIL-4R inhibits immunoglobulin class switching, IgE production, allergen-induced airway reactivity, VCAM-1 expression, allergen-induced eosinophilia in bronchoalveolar lavage, and allergen-induced pulmonary infiltration and airway occlusion by inflammatory cells. The safety of sIL-4 receptor therapy is suggested by transgenic mice that produce a 100-fold greater sIL-4R concentration than nontransgenic littermates. Mice with increased sIL-4 receptor compared with normals have similar numbers of B and T lymphocytes, lymphocyte surface marker expression, and antigen-specific antibody responses (Table 2).⁴⁷

Clinical Studies

Soluble IL-4R blocked CD8 T-cell-mediated IgE production in allergic patients, suggesting that sIL-4 may be useful for treating allergic diseases.⁵⁰ Studies on the effects of recombinant humanized sIL-4R (rhusIL-4R) on IL-4/staphylococcal enterotoxin B-stimulated peripheral blood mononuclear cells from patients with eczema suggested that rhusIL-4R may be an immunomodulatory drug for atopic eczema.⁷⁸

A study of humans with asthma is encouraging. rhusIL-4R (trade name, Nuvance, Immunex, Seattle, WA), was nebulized to 62 patients with moderate asthma at doses of 0.75, 1.5, or 3 mg. The study was double-blind and placebo controlled; corticosteroid therapy was discontinued at entry. The results showed that sIL-4R was well tolerated, and the 3-mg sIL-4R treated group demonstrated less labile FEV₁ and improved asthma symptom scores compared with the placebo group.⁴ In an open-label, randomized, dose-ranging study, the safety and tolerability of sIL-4R was evaluated in 16 adult patients with mild atopic asthma (FEV₁ > 70% of predicted). A single nebulized dose (50–100 µg) was generally tolerated and pulmonary function improved.⁶ In a phase I/II randomized, placebo-controlled trial,⁵ 25 patients with moderate, inhaled corticosteroid-dependent asthma were randomly assigned to receive a single nebulized dose of IL-4R or placebo after discontinuing the inhaled corticosteroid. The results indicate that sIL-4R is effective with once-weekly inhalation. The treatment is generally well tolerated and prevents a decline in FEV₁, improves asthma symptom scores, and reduces β_2 -agonist rescue following discontinuation of inhaled corticosteroids. Pulmonary inflammation, as assessed by exhaled nitric oxide, was significantly lower in the sIL-4R treated group compared with the placebo group. The treated group had nonstatistically decreased levels of VCAM-1, intercellular adhesion molecule-1 (ICAM-1), and eosino-

Table 2. EFFECTS OF sIL-4R ON MURINE MODELS OF INFLAMMATION

Model (Organ)	Features	Effect of sIL-4R
Local inflammation (footpad)	Injection of T _H 2 cells produce local tissue inflammation	Complete inhibition of inflammatory response
Polyclonal total IgE (systemic)	Anti-IgD stimulates IL-4-dependent polyclonal B activation and IgE production	IgE production inhibited
Cutaneous hypersensitivity (skin)	Ova sensitization leads to specific IgE production and ICH	Inhaled sIL-4R inhibits allergen specific IgE/IgG1, total IgE, suppressed ICH, and normalized BHR
Allograft model (cardiac)	Anti-CD3 mAb induces VCAM-1 expression	Inhibition of VCAM-1 and mECA-32 expression, and cellular infiltration

BHR = bronchial hyperresponsiveness

phil peroxidase (EPO) and increased CD23 expression compared with the placebo group. These results support a potential role of sIL-4R as a therapy for asthma and other allergic diseases. Additional studies are needed.

Other Potential Therapeutic Approaches

In addition to the new treatment approaches described, several other potential approaches are being explored. Most of these are aimed at modulating the allergic immune response and inflammation in murine models using either soluble receptors or antibodies that alter cytokines, immunocyte coreceptors, IgE receptor binding, and cellular adhesion.

Costimulatory Molecules

The activation of T cells requires the first signal from the antigen in context of the HLA molecule and an additional costimulatory signal involving the ligation of B-7 on APC (CD80/86) with CD28 or CTLA4 on T cells.³⁷ Without costimulation, T cells become anergic. Thus, the costimulatory molecules have become targets for developing drugs that will modulate an allergic immune response. The chimeric CTLA4-Ig molecule, which consists of the extracellular domain of CTLA4 and the hinge, CH2, and CH3 regions of IgG, blocks the costimulatory signal that T cells receive through the interaction of CD28 with the counter receptors CD80 or CD86 expressed on the APC membrane. CTLA4-Ig is able to down-regulate or suppress the mucosal immune response in allergic rhinitis.⁷⁵ CTLA4-Ig inhibits airway eosinophilia and hyper-responsiveness by enhancing T_H1 -like cell activity.⁶² IgE formation is enhanced by the T_H2 cytokine IL-4. Therefore, it is expected that down-regulating T_H2 activity will decrease IgE formation, as has been demonstrated in CTLA4-Ig-treated mice.⁶² The combination of antibodies to CD80/CD86, the ligand for CTLA-4, blocks the development of allergic airway inflammation, whereas a partial reduction occurs using either of these antibodies alone.⁴⁹ However, the only anti-CD86 antibody inhibits the production of IgE.⁴⁹ Therefore, the CD28-CD80/CD86 complex is a potential target for developing new strategies of allergy treatment.

IgE Binding Receptors

The IgE antibody binds to FcεRI and CD23 on mast cells and basophils and also binds to CD23 on these cells, in addition to monocytes and eosinophils. Both of these receptors are potential therapeutic targets. Antibodies attached to CD23 may be able to facilitate antigen presentation to T cells, but IgE regulation has not been affected. Transgenic mice that overexpress CD23 on T and B cells exhibit decreased IgE production,⁶⁵ which suggests enhancing CD23 expression on B cells before activation can be a strategy to inhibit IgE production. A decrease in CD23 levels with a metalloprotease resulted in an increase in IgE.¹¹

The administration of anti-CD23 antibodies inhibits eosinophil airway recruitment and reduces airway hyperresponsiveness.^{15, 16} This observation suggests anti-CD23 antibodies may be used to block the interaction between IgE and CD23, reducing eosinophil recruitment and inflammation. More studies are necessary before IgE receptors can be viewed as important therapeutic targets for allergic disease.

ALLERGEN-BASED IMMUNOTHERAPEUTIC APPROACHES

Specific allergen injection immunotherapy is highly effective in selected patients with IgE-mediated disease, including allergic rhinitis and venom anaphylaxis. Specific immunotherapy leads to the modulation of allergen-specific T-cell responses from T_H2 -like to T_H1 -like and the reduction of inflammatory cells and mediators in the target nasal mucosa and the airways⁶⁰; however, classical immunotherapy is limited by the lack of standardized extracts for many allergens and the batch-to-batch variation of allergen concentrations. The availability of recombinant allergens makes it possible to treat allergic patients with identical, consistent vaccines.¹⁹ Other advantages of recombinant allergens include: (1) immunization with vaccines tailored to the major allergens to which the patient is allergic; (2) administration of allergens in optimal doses; (3) more accurate dosing; and (4) the possibility of modifying IgE-binding structure allergen epitopes, thereby increasing the safety of allergen immunotherapy. Recombinant allergens are not yet used in clinical medicine. These allergens, like other recombinant proteins, must undergo rigorous clinical trials before they are available for clinical use. This section discusses their potential uses in clinical practice.

Developing Prophylactic Vaccines Against Allergic Diseases

In a murine model, vaccination with a recombinant allergen before sensitization resulted in a state of immune deviation—a shift from a T_H2 , IgE response to a T_H1 , IgG2a response.⁵⁵ Because the immune system of neonates and adult mice is similar in terms of their ability to develop immune deviation, and because allergic sensitization occurs predominantly in the first 2 years of human life,^{2, 28} the preventive approach to immunotherapy using recombinant allergens holds promise and needs further investigation.³² A controlled study of traditional allergen vaccine immunotherapy to prevent asthma in atopic children demonstrated that more children in the nontreated group developed asthma than in the immunotherapy group. Moreover, it has been shown that immunotherapy in children sensitive to a single aeroallergen prevented new sensitization to other allergens.¹⁸

Recombinant allergens and their corresponding cDNAs could be used to prevent allergen-specific IgE responses. Allergen genes expressed

in the appropriate hosts may be used as live vaccines, genes introduced via living bacteria, or viruses, or plasmid vaccines. Two models are noteworthy. First is oral immunization with *Salmonella typhimurium*, which expresses a Bet v 1—cDNA (the cDNA coding for the major birch tree allergen) promoted IgG2a instead of the default IgE antibody response in mice⁸¹; however, an IgG2a response specific for Bet v 1 could be detected in only 10% of the immunized mice. Second, a live vaccine to treat allergic diseases using recombinant bacille Calmette-Guerin-expressing allergens has been proposed.³⁹ These experimental approaches appear promising and may lead to effective prophylactic allergy vaccines. About one quarter of the population is genetically predisposed to develop allergic disease. With advances in the identification of genes, it may be possible to develop methods for predicting atopic predisposition, which may then allow for the vaccination of predisposed individuals against the dominant allergens in their environments.

Alternatively, plasmids expressing allergens could also be used for therapeutic vaccination. The immunization of mice with an allergen-cDNA cloned in a plasmid vehicle resulted in an allergen-specific IgG2a and T_H1-like response, with no detectable IgE response. This was in contrast to immunizing with an allergen, which induced an IgE antibody response.^{30, 69} Furthermore, a T_H1-like response induced by gene immunization reversed the ongoing allergen-specific T_H2-like response. These studies suggest that immunization with allergen-cDNAs may provide a novel type of immunotherapy for allergic diseases; however, the application of DNA vaccines as a therapy appears less appealing than as a prophylactic because of the possibility of inducing anti-DNA antibodies and autoimmunity.

Allergen-Specific Immunotherapy

Studies with a murine model have suggested that recombinant allergens switch immune responses from a T_H2-like profile to the preferred T_H1-like response. The recombinant allergens may be used for immunotherapy as aqueous, enteric-coated, or liposome-packed vaccines for immunotherapy.^{1, 82} Furthermore, recombinant allergens may be polymerized as recombinant allergoids using formaldehyde.⁴³ The problem with the available polymerized allergen vaccines is that they are not standardized, making it uncertain whether some allergens have been denatured and whether all allergens have been uniformly polymerized. Consequently, considerable batch-to-batch variation exists. Polymerized recombinant allergens, which are antigenic but nonallergenic, may improve allergen-specific immunotherapy. Recombinant allergens also may be conjugated with n-formyl-methionyl-leucyl-phenylalanine and then used to treat allergic patients.⁸³ These chemically modified recombinant allergen vaccines should provide a safer and more effective vaccine with fewer injections than are needed with current allergen vaccines.

Furthermore, subsequent to the identification of epitopes, an aller-

gen cDNA can be altered specifically to reduce the IgE binding ability of the corresponding recombinant allergen without compromising its T-cell stimulation capacity. Thus, genetically modified allergens not only appear to be safe, but also may prove to be effective agents for patient-tailored immunotherapy.

Allergen-Specific Therapy with T_H1-Stimulating Adjuvants

Immune deviation from a T_H2-like to T_H1-like response may be achieved by designing vaccines with allergens or recombinant allergens in conjunction with adjuvants. The adjuvants may either be cytokines or other synthetic compounds, such as immunostimulatory DNA sequences containing a cytosine-phosphoguanosine (CpG) motif that induces T_H1-like cytokine response.^{33, 35, 64} The adjuvant may be injected with natural allergens or may be genetically linked with allergen cDNA.

Among the cytokines, IFN- α and IL-12, both of which induce a strong T_H1-like cytokine profile, have the potential to convert allergen-specific T_H2-like responses to T_H1-like responses.⁵⁶ IL-12 has been suggested as an adjuvant for vaccination against diseases in which the T_H2 profile predominates. IFN-tau, a type I IFN that lacks the toxicity associated with type I IFNs, inhibited IgE production in a murine model allergy and in an IgE-producing human myeloma cell line.⁵⁸ Administration of a recombinant allergen (ovalbumin) with the IL-12 (subunit p40) fusion protein vaccine down-regulated ovalbumin-specific IgE responses *in vivo*³⁵; however, IL-12 may cause side effects, and its effectiveness requires IFN- α production by the cells in question. IL-12's ability to convert an established T_H2 to a T_H1 response remains uncertain. Therefore, IL-12 as an adjuvant for allergen vaccines is unproven.

Peptide-Based Therapies

Because T cells play a dominant role in IgE synthesis, T-cell peptides may be useful as immunotherapeutic vaccines. T-cell peptide vaccines induce anergy to allergen-specific T cells or interfere with the formation of trimolecular complexes involving the interaction of major histocompatibility complex-peptide duplexes with the appropriate Th-cell receptor.^{72, 76} Peptide therapeutic vaccines have several advantages (e.g., their defined chemical nature, simplicity of preparation, and prolonged shelf life). They also appear to be safe because T-cell peptides may not bind to IgE⁷¹; however, human immune responses to allergens are more complex than murine immune responses. Various theoretical constraints are predictable: (1) some major allergens, particularly pollen allergens, contain several T-cell epitopes that are recognized by allergic individuals; (2) certain major allergens appear to have various isoforms containing cross-reacting and non-cross-reacting epitopes, which increase the repertoire of allergenic epitopes⁷⁷; (3) allergic individuals differ with respect

to their recognition of these epitopes; (4) B-cell and T-cell epitopes may be present on the same peptide, which increases the risk of systemic reactions; and (5) excessive doses of some peptides may induce autoimmune reactions. Thus, in individuals allergic to certain complex aeroallergens, treatment with peptides may not be effective or appropriate.

In experimental animals, synthetic Fel d 1 (cat major allergen) or Der p 1 (house dust mite major allergen) T-cell peptides induced peripheral T-cell tolerance.^{9, 29} The activation of allergen-specific T cells and IgE antibody synthesis was inhibited by the *in vivo* administration of peptides by intranasal, oral, and subcutaneous routes. Tolerance to the dominant peptides was surprisingly achieved with the minor (cryptic) epitopes of Der p 1.²⁹ In ragweed-allergic patients, treatment with the peptidic fragments of the Amb a 1 allergen, produced by proteinase digestion, reduced the clinical symptom scores in treated patients^{40, 53}; however, conclusions from these studies are limited because few patients were treated.

In Hymenoptera-sensitive patients, treatment with a cocktail of phospholipase A₂-derived peptides had no adverse effects.⁵⁹ Five patients were treated for 70 days with a total dose of 397.1 µg each of three phospholipase A₂ peptides, 12 to 20 amino acids in length, and then challenged with native phospholipase A₂. The treatment was effective in three of five patients, as judged by the reduction in symptom scores, lymphocyte proliferation studies, and a shift in the cytokine profile from an IL-4-dominant to an IFN-dominant cytokine response. No decrease in specific IgE occurred in treated patients. The largest clinical study with allergen peptides was conducted using two peptides of the cat allergen Fel d 1, referred to as ALLERVAX-CAT (ImmuLogic, Boston, MA) (a total of four injections of 750 µg each at 2-week intervals).⁴⁵ In a multicenter, randomized, double-blind, placebo-controlled study of 133 cat-allergic patients chronically exposed to cats or who had failed previous conventional cat immunotherapy, ALLERVAX-CAT, given as a 750 µg dose, improved pulmonary function in patients with reduced baseline FEV₁.⁴⁵ Side effects of treatment were reported in the respiratory system organ, with 77 patients reporting at least one event in this body system. Such symptoms included chest tightness, dyspnea, coughing, throat irritation, wheezing, and asthma aggravation. The most common dermatologic reaction presented was pruritus. The majority of adverse events were considered to be of mild or moderate severity. Although severe adverse reactions were experienced, there was no statistical difference in the frequency of the appearance of such severe adverse reactions between the treated and placebo group.⁴⁵ Therefore, although peptide therapy in severely cat-allergic patients was associated with some adverse effects, the therapy was found to be effective.

CONCLUSION

The molecular cloning of allergens has advanced our knowledge of their primary structures and has enabled these allergens to be produced in virtually unlimited quantities for the potential diagnosis and treat-

ment of allergic diseases. Furthermore, with the availability of recombinant allergens, new forms of allergen immunotherapy may use these recombinant allergens or even plasmid DNAs that encode allergens. It will be necessary to demonstrate the effectiveness and safety of these vaccines in humans before they can be used clinically. Allergic conditions are complex; therefore, it is naive to think that one form of allergen-specific immunotherapy will be effective to treat all allergic conditions. Nonetheless, all new technologies should be explored to optimize the diagnosis and treatment of allergic diseases.

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