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IMMUNOTHERAPY FOR ALLERGIES AND ASTHMA: PRESENT AND FUTURE

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Abstract

Allergen immunotherapy (IT) is a proven approach for treating allergic rhinitis and allergic asthma that has been practiced since 1911 and has undergone significant development in the last two decades. As currently practiced, IT involves subcutaneous or sublingual administration of allergens, both methods of which have been extensively investigated. In addition to allergen IT, a number of additional nonspecific IT approaches are being used or are in phase II/phase III clinical trials, which may be available in clinics within the next one to three years. Such therapies include anti-IgE antibodies and the soluble IL-4 receptor. Other experimental IT approaches are at the preclinical research stage and may proceed to clinical trials and the clinic within the next five to ten years. This review discusses the pros and cons of recent developments in both currently practiced and experimental IT approaches.

Keywords

allergy; asthma; allergen immunotherapy; subcutaneous; sublingual

Introduction

Advances in basic immunology research have enhanced our understanding of the cellular and molecular basis of the allergic response. A prominent characteristic of the allergic response is a persistently elevated level of IgE antibodies against specific antigens (allergens) to which the affected individual is regularly exposed by inhalation, ingestion, or contact with the skin [1]. 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. While 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

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signal from this trimolecular interaction and an additional costimulatory signal resulting from the binding of B-7 (CD80/86) on an APC to CD28 or CTLA4 on a T cell [2]. When stimulated by antigens, helper T (T_H) cells produce specific cytokines that have been designated as either T_H 1 cytokines (interleukin [IL]-2, interferon [IFN]-gamma, and tumor necrosis factor [TNF]-beta) or T_H 2 cytokines (IL-4, IL-5, IL-9, IL-10, and IL-13). The state of T-cell activation depends on several factors [2,3]. The first of these factors is the strength 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 a T cell for a specific epitope depends on the concentration of the antigen, the type of APC [4] and the cytokine milieu of the T cell during antigen interaction. Thus, IFN-gamma and IL-12 promote a T_H 1-like response, whereas IL-4 promotes a T_H 2-like response [5]. Additionally, host immune system genes may bias the overall immune responsiveness of an individual to favor a T_H 1- or T_H 2-like phenotype. A T_H 2-like cytokine profile is associated with the induction of IgE antibody (Ab) production in vitro and in vivo [6]. Specifically, IL-4 favors the development of T_H 2-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, 20% to 30% of asthmatics do not show positive skin tests to allergens. In general terms, asthma is an inflammatory disease in which not only lymphocytes but mast cells, basophils, eosinophils, and epithelial cells play a role. Studies to date suggest that T_H 2-like cytokines, such as IL-4 and IL-5, also play an important role in nonatopic asthma [7,8].

SPECIFIC ALLERGEN IMMUNOTHERAPY

The term 'allergy' was first introduced by an Australian pediatrician, Clemens Freiherr von Pirquet, who believed that it was a pathological state of altered immune reactivity. It was not until 1911, however, that Leonard Noon and John Freeman proposed the concept of allergen immunotherapy while working at St. Mary's hospital in London. They hypothesized that toxins from grass pollen somehow accounted for symptoms seen in patients suffering from hay fever. By inoculating the patients with gradually increasing doses of the toxin itself to stimulate the immune system against the toxin, the symptoms could be reduced or even abolished. Current allergen-specific IT involves administering increasing doses of the causative allergen in order to reduce the clinical signs and symptoms associated with exposure to the allergen and thereby produce tolerance. The allergens are administered by two different routes: parenteral or subcutaneous (SCIT) and sublingual (SLIT), which was introduced relatively recently. The current state of the art for each of these immunotherapeutic approaches is reviewed below.

Subcutaneous Immunotherapy (SCIT)

As currently practiced, SCIT has proven effective in allergic rhinitis and asthma and is FDA approved and reimbursable. SCIT has been extensively studied in double-blind trials to determine effective doses, establish duration, define the mechanisms and to investigate the persistence of efficacy after treatment ends. However, the need for multiple visits to the clinic for shots is inconvenient, only a few allergens have been standardized for SCIT and the potential for systemic anaphylactic reactions is a serious limitation.

Although allergen immunotherapy has been around for about a century, little is known about the absorption and fate of subcutaneously administered allergen. The pharmacokinetics of subcutaneous immunotherapy has been studied using leukocytes labeled with ^{99m}Tc -HMPAO on allergic patients injected intravenously in contralateral arms. Local inflammatory activity was noted in the first hour and the increase was time-dependent. The immune system responded quickly as the activity was traced in the lymphoid tissue of the upper mediastinum and anterior region of the neck. Thoracic and bowel focalization was

also noted for the subcutaneous route [8]. Factors important in SCIT include the dose of allergen being administered [9], the quality of the allergen extract [10], and the duration over which it is given. The effects of duration of allergen dosing appear to depend on individual factors according to a review of studies performed between 1976 and 2006 in which the rate of relapse ranged from 0-50% [11].

Many studies evaluating the clinical efficacy of SCIT have used symptom scores and decreased use of medication to assess efficacy. One such meta-analysis in patients with allergic rhinitis included double-blind placebo-controlled trials of 2,871 participants receiving 18 injections on average. Symptom score showed statistically significant reduction in the treatment group (standard mean deviation -0.73 (95% CI -0.97 to -0.50, $p < 0.00001$). Similar results were found in the medication score data showing an overall reduction in medication requirement in the immunotherapy group (standard mean deviation of -0.57 (95% CI -0.82 to -0.33, $p < 0.00001$) [12]. A comparison of meta-analyses of SCIT with those of SLIT is shown in Table 1.

The immune mechanism of SCIT has been extensively studied. Specific immunotherapy alters allergen-specific T cell responses from T_H 2-like to T_H 1-like and reduces inflammatory cells and mediators in the target nasal mucosa and the airways [13]. For example, immunotherapy with house dust mite extract induces antigen-specific suppressive activity by $CD4 + CD25 + T$ cells in allergic subjects, which causes the regulatory/suppressor T cells to secrete IL-10 and TGF- β . This in turn increases IgG4 and IgA antibody production and suppression of IgE antibodies by B cells, thus mimicking a healthy immune response to environmental allergens.

Interestingly, 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 [14].

Currently, only 2-3 million people of 55 million with allergic diseases are on SCIT. The main reasons for this are that the target populations dislike injections or cannot afford the time and expense of frequent visits to a doctor's office. There is also a concern about the safety of SCIT and it cannot be used for young children with allergy or asthma. In an effort to decrease the frequency of allergen administrations, intralymphatic allergen administration was attempted in a randomized (but not blinded) controlled trial [15]. The responses of a SCIT group ($n = 54$) receiving 54 injections of 100,000 SQUs of grass vaccine were compared with those of a group ($n = 58$) who received 3 injections of 1000 SQUs of the same vaccine into superficial inguinal lymph nodes under ultrasound guidance. The latter was safer and showed equivalent reductions in IgE and improvements in symptom score compared to SCIT. While the results of this study are promising, it was not a blinded study and the technology of ultrasound-guided intralymphatic injection may not be available in all clinics.

Sublingual Immunotherapy

Sublingual immunotherapy (SLIT) consists of placement of an aqueous allergen extract (50% glycerin and 0.4% phenol) in single allergen tablets or capsules under the tongue. It has been extensively practiced in Europe [16], and tested in clinical trials in the United States but is not yet FDA approved. Presently, SLIT is considered to have about one-tenth the risk of SCIT and has been shown to prevent new sensitization and progression from rhinitis to asthma. In addition, SLIT provides effective protection that persists for a long

time after treatment is stopped. The disadvantages are that SLIT is less effective than SCIT and has not been tested for multiple allergen mixes.

The pharmacokinetics of local immunotherapy by SLIT has been studied in allergic volunteers using radiolabeled allergen (Par J 10) given in the form of soluble tablets. Plasma radioactivity was measured at different intervals using early and late sequential scintigraphic acquisition. No absorption through the oral mucosa was observed, and the plasma radioactivity, which peaks at two hours, was increased only after swallowing. No allergen was detected in the blood stream [17]. Another study in which the biodistribution of ^{125}I -radiolabelled Der p2 was assessed in allergic volunteers confirmed these findings. Plasma radioactivity was only noted after the tablets were swallowed and the effect peaked at 1-2 hours [18]. It was noted that in order for the immunotherapy to be successful, the allergen had to be swallowed after the tablet had dissolved sublingually. This confirms that the contact with oral mucosa is an important factor although no allergen was absorbed through the oral mucosa. It was also found that duodenal enzymes were necessary for hydrolyzing the allergen [19]. Treatment with SLIT is initiated with a build-up phase lasting 4-6 weeks starting with the lowest concentration. The dosage is then gradually increased up to a maintenance dose. The recommended duration of the treatment is three to four years for optimal results [20]. SLIT can be administered co-seasonally, pre-seasonally or continuously [21].

The majority of SLIT studies have shown clinical efficacy in the form of improvement in symptom scores or medication scores, or both for allergic rhinitis and allergic asthma. A meta-analysis of five randomized double-blind, placebo-controlled studies on patients with allergic asthma showed significantly improved symptom scores and decreased use of rescue medication [22].

High-dose SLIT has also been studied with seasonal allergens given as co-seasonal or perennial treatment and was effective and well tolerated. An individual patient data (IPD) meta-analysis on a total of 1052 patients that included three open, prospective observational studies on high-dose SLIT in patients with allergic rhinitis with and without asthma showed consistent improvement in symptom score, reduction in medication use or both. The fact that 88% of the patients in the study decided to continue SLIT after the study was completed reflects well on the effectiveness of the medication [23]. A comparison of meta-analyses of SLIT with SCIT is presented in Table 1.

Mechanism of SLIT

Both humoral and cellular responses are altered following SLIT and SLIT-induced are functional and have allergen neutralizing activity. There is good evidence for dose and time-dependent increases in IgG1, IgG4 and IgG2 antibodies following grass pollen SLIT [24]. At the T cell level, SLIT induces suppression of allergen-stimulated T cell proliferation and cytokine production. Similar to SCIT, there is also induction of systemic T regulatory cells [25]. In addition, there is evidence for local sublingual mucosal Treg cells, which may inhibit local inflammation [26].

Safety and Adverse Effects

There is abundant data in the literature documenting adverse outcomes from allergen immunotherapy. Although very effective in treating allergic airway diseases, IT comes with its own price of local and systemic reactions among which the most severe is anaphylaxis. Multiple studies have been done assessing adverse reactions to SCIT. A prospective, multicenter, placebo-controlled trial was conducted including 768 patients using depigmented allergen extract in Madrid, Spain. Patients received four injections of

increasing doses at weekly intervals followed by monthly shots. A total of 5923 shots were given, from which 14 local reactions and 27 systemic reactions were reported [27]. Anaphylaxis is the most devastating adverse outcome of SCIT immunotherapy and in a prospective study of patients treated in large-in-hospital allergy clinics, 131 anaphylactic reactions occurred among 453 patients receiving 21,022 shots over 10,040 visits [28]. Since adverse effects (AEs) are related to both the dose of the allergen and the duration over which it is given a short, a high-dose cluster titration study was performed in adults with allergic rhinitis, with or without mild to moderate allergic asthma. In this open-label, prospective trial pollen-allergic patients (n = 110) received a three-week titration cluster and house dust mite (HDM)-allergic patients (n = 47) received a two-week titration cluster. One third of patients did not experience any AEs, while 248 local reactions were noted in the pollen group and 113 in the HDM group. The treatment was well tolerated and no life threatening systemic anaphylactic reactions were observed [29].

SLIT, on the other hand, has been characterized by a high degree of safety as its most notable feature. Mild, short lasting and self resolving local side effects include oral cavity pruritis, throat irritation, rhinitis, itchy eyes, nausea, and GI complaints [30]. Systemic side effects include asthma exacerbation and rhinoconjunctivitis [31]. A meta-analysis done in 2009 to assess the safety of SLIT at the beginning of pollen season and during the pollen season found it to be comparable to conventional pre-seasonal SLIT (Table 1). This study included 34 children with allergic rhinitis and 36 adults with allergic asthma. Out of 54 AEs reported, most were short lasting and self-resolving local effects, while systemic effects were mild, and no anaphylactic reactions were reported [32]. Although anaphylaxis was not an adverse event of any of the studies done in the past, individual case reports have been cited. The first one was reported in 2006 and involved generalized pruritis, dyspnea, and dizziness on the second day of therapy [33]. Occurrences of anaphylaxis on the first dose [34] as well as after maintenance doses [35] have also been reported. Although this type of severe AE is not commonly seen in SLIT, it still must be kept in mind when offering this therapy to patients.

Experimental IT Approaches

Introduction of Recombinant Allergens for IT

SCIT is limited by the lack of a standardized extract for many allergens and the batch-to-batch variation in allergen concentration. The availability of recombinant allergens makes it possible to treat allergic patients with identical, consistent vaccines [36]. 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 the structure of IgE-binding allergen epitopes, thereby increasing the safety of allergen immunotherapy. Recombinant allergens are not yet used in medicine since like other recombinant proteins, they must undergo rigorous clinical trials before they are available for clinical use.

In a murine model, vaccination with a recombinant allergen before sensitization resulted in a state of immune deviation--a shift from a T_H 2, IgE response to a T_H 1, IgG2a response [37]. 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 two years of human life, [38,39] using recombinant allergens for immunotherapy holds promise and needs further investigation [40].

The recombinant allergens for immunotherapy may be used as aqueous, enteric-coated, or liposome-packaged vaccines [41,42] or polymerized as recombinant allergoids using formaldehyde [43]. Polymerized recombinant allergens are antigenic but nonallergenic and

therefore may improve allergen-specific immunotherapy. The problem with the available polymerized allergen vaccines is that they are not standardized and considerable batch-to-batch variation exists, making it uncertain whether all allergens have been uniformly polymerized or if some have been denatured. Recombinant allergens may also be conjugated with n-formyl-methionyl-leucyl-phenylalanine before being used to treat allergic patients [44]. 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, once the epitopes have been identified, an allergen cDNA can be altered specifically to reduce the IgE binding ability of the corresponding recombinant allergen without compromising its capacity to stimulate T cells. Genetically modified allergens not only appear to be safe, but also may prove to be effective agents for patient-tailored immunotherapy.

While recombinant allergens and their corresponding cDNAs can be used to prevent allergen-specific IgE responses, the allergen genes expressed in an appropriate host may be used as live vaccines. Genes can be introduced via live bacteria, or viruses, or using plasmid vectors. Two models are noteworthy. First is oral immunization with *Salmonella typhimurium* expressing the cDNA coding for the major birch tree allergen, Bet v 1. This live vaccine promoted IgG2a instead of the default IgE antibody response in mice [45]; however, the Bet v 1-specific IgG2a response 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 [46]. 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 associated with asthma, 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 can 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_H 1-like response, with no detectable IgE. This was in contrast to immunization with allergen extract, which induced an IgE antibody response [47,48]. Furthermore, the gene immunization induced a T_H 1-like response by reversed the ongoing allergen-specific T_H 2-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 prophylactic may be more feasible because of the possibility of inducing anti-DNA antibodies and autoimmunity.

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 in allergen-specific T cells or interfere with the formation of the trimolecular complexes involving the interaction of major histocompatibility complex-peptide duplexes with the appropriate T-cell receptor [49,50]. Peptide vaccines have several advantages: a defined chemical structure, simplicity of preparation, and prolonged shelf life. They also appear to be safe because, at least in mice, T-cell peptides do not bind to IgE [51]; 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 epitopes [52]; (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 d1 (cat major allergen) or Der p1 (house dust mite major allergen) T-cell peptides induced peripheral T-cell tolerance [53,54]. The activation of allergen-specific T cells and IgE antibody synthesis was inhibited by in vivo administration of peptides by intranasal, oral, and subcutaneous routes. Surprisingly, tolerance to the dominant peptides was achieved with the minor (cryptic) epitopes of Der p1 [54]. In ragweed-allergic patients, treatment with the peptidic fragments of the Amb a1 allergen produced by proteinase digestion reduced the clinical symptom scores in treated patients [55,56]; 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 [57]. Five patients were treated for 70 days with a total dose of 397.1 g 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 and lymphocyte proliferation 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 a total of four injections of 750 µg each of two peptides from the cat allergen Fel d1 (ALLERVAX-CAT; ImmuLogic, Boston, MA) at 2-week intervals [58]. In a multicenter, randomized, double-blind, placebo-controlled study, 133 cat-allergic patients chronically exposed to cats or who had failed previous conventional cat IT, were given ALLERVAX-CAT as a 750 µg dose, and patients with reduced baseline FEV1 showed improved pulmonary function [58]. Side effects of the treatment were considered to be of mild to moderate severity and were reported by 77 patients who had at least one respiratory system event including chest tightness, dyspnea, coughing, throat irritation, wheezing, and asthma aggravation. The most common dermatologic reaction presented was pruritus. Although severe AEs did occur, there was no statistical difference in the frequency of their appearance between the treated and placebo group [58]. Therefore, although peptide therapy in severely cat-allergic patients was associated with some adverse effects, the therapy was found to be effective.

Allergen-Specific Therapy with TH1-Stimulating Adjuvants

Immune deviation from a T_H 2-like to T_H 1-like response may be achieved by designing vaccines with allergens or recombinant allergens in conjunction with adjuvants. The adjuvants may be cytokines or synthetic compounds, such as immunostimulatory DNA sequences containing a cytosinephosphoguanosine (CpG) motif that induces a T_H 1-like cytokine response [59-61]. The adjuvant may be injected with natural allergens or genetically linked with allergen cDNA.

Among the cytokines, IFN-α and IL-12 both induce a strong T_H 1-like cytokine profile and therefore have the potential to convert allergen-specific T_H 2-like responses to T_H 1-like responses [62]. IL-12 has also been suggested as an adjuvant for vaccination against diseases in which the T_H 2 profile predominates. IFN-τ, a type I IFN that lacks the toxicity associated with type I IFNs, inhibited IgE production in a murine allergy model and in an IgE-producing human myeloma cell line [63]. Administration of a recombinant allergen (ovalbumin) vaccine with the IL-12 (subunit p40) fusion protein down-regulated ovalbumin-specific IgE responses in vivo [60]; however, IL-12 may cause side effects, and its effectiveness requires IFN-α production by the target cells. Because IL-12's ability to convert an established T_H 2 to a T_H 1 response remains uncertain, the use of IL-12 as an adjuvant for allergen vaccines is unproven.

Also of note are the studies involving Toll-like receptor (TLR) antagonists, which are based on the observation that LPS, the ligand of TLR-4, inhibits Th2 cytokine production in nasal explants of children [64]. MPL, a chemically modified derivative of LPS, was still a TLR-4 agonist and decreased combined symptom and medication scores compared to placebo in clinical trials [65]. A ragweed-TLR-9 agonist vaccine for IT was also tested in clinical trials in allergic rhinitis patients. While the results for the primary endpoint were not statistically significant, the secondary endpoints were significantly reduced [66]. A similar study with this TLR-9 ligand in asthmatics showed a clear reduction of FEV1 and sputum eosinophils after allergen challenge [67]. These studies need to be repeated.

Another potential form of IT involves the Fcγ1-linker-major cat allergen, Fel d1, fusion protein (Gamma Fel D), which was effective in inhibiting allergic responses in mice in vivo and human cells in vitro [68]. Gamma Fel D inhibits cat allergen-induced degranulation of human basophils and cord blood mast cells in culture. Since results of mouse studies may not be translatable to humans, the proof-of-concept needs to be obtained in humans and the potential adverse effects need to be examined. Also, IT with mite allergen Der p1 on virus-like particles was reported to be safe and highly immunogenic in healthy adults [69]. The IgG response to dust mite allergens, particularly IgG1 and IgG3, but not IgG2 and IgG4, was significantly induced by these particles. However, this study was limited by lack of comparisons between Der p1 on virus and Der p1 without virus, lack of cellular studies and appropriate clinical endpoints relevant to human allergy and asthma.

NONSPECIFIC THERAPEUTIC APPROACHES

Monoclonal Anti-IgE Antibody Therapy

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 [70]. Because IgE cross-linking 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 during the 1970s, the use of anti-IgE as a therapeutic strategy failed because the antibodies initially employed caused mast cell degranulation. With the advances in recombinant DNA technology, it has become possible to engineer monoclonal antibodies that reduce unbound IgE levels in the serum by binding to circulating serum IgE antibodies but not to IgE antibodies bound to Fc-epsilon RI or to Fc-epsilon RII [71].

Two mouse monoclonal anti-IgE antibodies, TES-C21 and MAE11, have been humanized [71,72] and MAE11 (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 IgE were engrafted onto a consensus human IgG1 framework [69]. 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 cause any severe AEs [73]. The two monoclonal anti-IgE antibodies, rhuMAb E25 and CGP51901, have been shown in a number of clinical trials including subjects with asthma or allergic rhinitis to be safe and effective in generating tolerance. 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 [74,75]. These studies have shown a decrease in free serum IgE 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 free serum IgE levels was dependent upon anti-IgE doses and was reversible. It was necessary to sustain 85% or greater reduction in free serum IgE levels to achieve improved clinical

symptoms, and an 85% reduction in IgE required a serum CGP51901 concentration of 5,000 ng/mL. The treatment was safe, with no serum sickness and only one case of urticaria among 153 evaluated patients [75].

The anti-IgE antibody rhuMAb E25 has been evaluated in both allergic rhinitis and asthma. It decreased free serum IgE in a dose-dependent fashion in patients with seasonal allergic rhinitis [76]; 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 concentrations, the dose of rhuMAb E25 was 0.005 mg/kg/wk for each international unit per milliliter of baseline IgE. There were no AEs related to this treatment. The rhuMAb E25 antibody has also been studied in asthma [77-79], but asthma symptoms, rescue medication 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 [77,78]. 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% decrease in FEV₁, with a correlation between the decrease in free serum IgE and the protection against inhaled allergen [77]. 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 bronchial allergen challenge was less affected [39]. 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 [80,81]. Additional studies support this possibility with rhuMAb E25 administration reducing the number of asthmatic exacerbations and facilitating a 50% dose reduction in use of inhaled and oral steroids [82,83].

Among all of these reports, only minimal toxicity was observed with one case of urticaria. Recently, however, more side effects have been noted including local injection site irritation, upper respiratory infection, headache and urticaria [84]. Among the total of 118 cases of anaphylaxis found by the FDA AE reporting system, 19 had anaphylaxis within the first hour, 32 had reaction after receiving the first dose while 14 reacted after the second dose and 77 patients required hospitalization. These patients were treated with epinephrine or corticosteroids, and had anti-IgE withheld or discontinued [85]. The vaccine was given subcutaneously every two or four weeks and the dose was determined by body weight and total pretreatment serum IgE levels. Absorption is slow from the inoculation site and the mean half-life of the drug is 26 days [86].

While the evidence shows that anti-IgE is a safe and effective immunotherapeutic treatment for asthma, it is noteworthy that anti-IgE antibodies also aid with allergen immunotherapy and reduce the risk of adverse reactions. Casale et al. [87] reported that anti-IgE reduces acute allergic reactions with SCIT rush immunotherapy, which reduces the frequency of hospital visits inherent with classical SCIT.

Soluble Interleukin-4 Receptor Therapy

IL-4 is one of the most important cytokines in a variety of allergic diseases, including asthma [88]. IL-4 is produced primarily by CD4 T_H 2 cells but also by CD8 T cells, eosinophils, mast cells, and basophils. 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 [6,89]; (2) *in situ* localization of IL-4-producing cells in bronchial biopsies [3]; (3)

expression of IL-4 receptor mRNA and protein in epithelium, subepithelium, and endothelial cell layers in bronchial biopsies of atopic asthmatics compared with controls [90]; and (4) analysis of mutations of the IL-4 receptor (IL-4R) and their association with asthma [91].

Basis for Modulation of Asthma with the Interleukin-4 Receptor

Interleukin-4 binds to the cell surface IL-4R heterodimer, which consists of an independent high-affinity alpha-chain and a gamma-chain shared with IL-2, IL-7, IL-9, and IL-15 [92]. The IL-4R-alpha chain is similar to the IL-13R-alpha chain, which allows the binding of both IL-4 and IL-13 [93]. The existence of both a membrane-bound and a soluble form of IL-4R in the mouse was reported in 1989 [84]. Soluble IL-4 is primarily a product of proteolysis of the IL-4R-alpha chain before release (shedding) [95]. A soluble form of human IL-4R-alpha, resulting from alternate splicing of the human IL-4R-alpha gene, has also been described [96]. Secreted forms of the IL-4R-alpha chain 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, suggested that the soluble IL-4 receptor (sIL-4R) might be effective 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 [97]. In a murine model of allergen sensitization, soluble murine IL-4R inhibited polyclonal and particularly antigen-specific IgE and IgG1 production following re-stimulation with these allergens [98]. In addition to inhibiting immunoglobulin class switching and IgE production, murine sIL-4R reduces 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-4R therapy is demonstrated by transgenic mice that produce a 100-fold greater sIL-4R concentration than non-transgenic littermates. Mice with increased sIL-4 receptor compared with controls have similar numbers of B and T lymphocytes, lymphocyte surface marker expression, and antigen-specific antibody responses [99].

In 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 [100]. 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 [101]. In another study of human asthmatics, rhusIL-4R, was nebulized and given to 62 patients with moderate asthma at doses of 0.75, 1.5, or 3 mg. The study was double-blinded and placebo-controlled, and corticosteroid therapy was discontinued at entry. The results showed that sIL-4R was well tolerated, and the group treated with 3 mg sIL-4R demonstrated less labile FEV₁ and improved asthma symptom scores compared to the placebo group [102].

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 well tolerated and pulmonary function improved [103]. In a phase I/II randomized, placebo-controlled trial [17], 25 patients with moderate, inhaled corticosteroid-dependent asthma were randomly assigned to receive a single nebulized dose of IL-4R or placebo after discontinuing inhaled corticosteroids. 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 beta₂-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 to the placebo group. The treated group had non-statistically decreased levels of VCAM-1, intercellular adhesion molecule-1 (ICAM-1), and eosinophil

peroxidase (EPO) and increased CD23 expression compared to the placebo group. These results support the potential use of sIL-4R as a therapy for asthma and other allergic diseases, but additional studies are needed.

Other Potential Therapeutic Approaches

In addition to the new treatments described above, 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 first requires a signal from the antigen in the context of the HLA molecule and then an additional costimulatory signal involving the binding of B-7 on APCs (CD80/86) to CD28 or CTLA4 on T cells [90]. 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 [104]. CTLA4-Ig inhibits airway eosinophilia and hyperresponsiveness by enhancing T_H 1-like cell activity [105]. IgE formation is enhanced by the T_H 2 cytokine IL-4. Therefore, it is expected that down-regulating T_H 2 activity will decrease IgE formation, as has been demonstrated in CTLA4-Ig-treated mice [105]. The combination of antibodies to CD80/CD86 with the ligand for CTLA-4, blocks the development of allergic airway inflammation, whereas only a partial reduction occurs using either of these antibodies alone [106]. However, only the anti-CD86 antibody inhibits the production of IgE [106]. 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 the Fc-epsilonRI and CD23 on mast cells and basophils and to CD23 on monocytes and eosinophils. Both receptor and ligand are potential therapeutic targets. Antibodies attached to CD23 may be able to facilitate antigen presentation to T cells, but IgE regulation was not affected. Transgenic mice that overexpress CD23 on T and B cells exhibit decreased IgE production [107], which suggests that enhancing CD23 expression on B cells before activation can effectively inhibit IgE production. Conversely, a decrease in CD23 levels with a metalloprotease resulted in an increase in IgE [108]. The administration of anti-CD23 antibodies inhibits eosinophil airway recruitment and reduces airway hyperresponsiveness [109,110]. This observation suggests that 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.

Conclusions

The current forms of allergen IT, SCIT and SLIT, are effective for allergic rhinitis and asthma, and extensive studies have shown that SCIT is more effective than SLIT. On the other hand, SLIT, which is mostly used in Europe and is presently in clinical trials in the US, is safer than SCIT. Both forms of IT will continue to evolve, for example, by incorporating an increased number of different standardized allergens and by utilizing recombinant allergens. New forms of immunotherapy with plasmid DNAs that encode allergens or cytokine adjuvants are also being developed and preclinical pilot studies have

shown them to be effective. Also, several immunomodulatory treatments such as anti-IgE and anti-IL-5 antibodies, and soluble IL-4 receptor are being tested in clinical trials. Allergic conditions are complex and it would be naive to think that one form of allergen IT will suffice for all patients. It is likely that a combination of immunotherapeutic agents with patient-tailored dosages and treatment durations and continued monitoring of immunoparameters will be necessary. In this new decade we should begin to see the fruits of combining basic and translational approaches with interdisciplinary research to develop better ways to treat allergic diseases.

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Figure 1. Mechanism underlying allergic diseases (adapted from Steve Holgate, JACI 2006). In principle, allergens induce an immunologic response via APC-T cell interactions leading to the production of specific cytokines, which in turn help B cells produce IgE antibodies. Each of these steps can potentially be targeted for therapeutic intervention.

Table 1Meta-analysis comparison of immunotherapy approaches¹

Parameters	SCIT	SLIT
Number of double-blind, placebo controlled trails	51	22
Total number of subjects	2,871	979
Mean reduction in symptoms	73%	42%
Mean reduction in medications	57%	43%
Geographic distribution	world-wide	Practiced in Europe and experimental in USA
Advantages	time-honored, proven efficacy	Ease of administration, safety
Disadvantages	potential systemic side effects	Local oral side effects

¹References: Calderon MA et al., Cochrane Review 2007; Wilson DR et al., Allergy 2005, 60:4-12; Nieto A et al., JACI, 2009, 124:157-61.

Table 2

Mechanisms of immunotherapy

Ranking	Mechanism	Quality of evidence
1	¹ Th2 downregulation	² High
2	Treg cell induction	High
3	Th1 upregulation	High
4	IL-10	Moderate
5	Eosinophil/basophil suppression	Moderate
6	IgG4	Moderate
7	IFN-gamma	Low
8	IL-12	Low
9	Histamine receptor modulation	Low
10	IgA	Very Low

¹ Abbreviations: Th2, type 2 helper T lymphocytes; Th1, type 1 helper T lymphocytes; IL, Interleukin; Treg, regulatory T cell.

² Grading quality of evidence: **High**: Further research is very unlikely to change our confidence in the estimate of effect; **Moderate**: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate; **Low**: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate; **Very Low**: Any estimate of effect is very uncertain (www.gradeworkinggroup.org).