



Advances in subcutaneous immunotherapy

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Purpose of review

Current limitations in subcutaneous immunotherapy (SCIT) include the length of time required to achieve tolerance as well as the potential for systemic side-effects. Advances in allergen immunotherapy include targeted therapies to B-cell and T-cell pathways that can lead to more rapid desensitization and potentially the prevention of allergic disease.

Recent findings

Novel molecularly engineered compounds and delivery vehicles allow for rapid and efficient desensitization. Combination with immune modifiers, treatment with epitope-based compounds, and hypoallergenic recombinant vaccines have the potential to improve immunogenicity while limiting systemic side-effects.

Summary

Advances in SCIT create new therapeutic opportunities for patients to improve safety, efficacy, and compliance. Concepts using epitope-based immunotherapy and carrier-fusion peptides have the potential to induce tolerance quickly via selective B-cell and T-cell pathways.

Keywords

adjuvant, allergic rhinitis, epitope, immunotherapy, recombinant technology

INTRODUCTION

Allergen immunotherapy (AIT) has entered a new era with improved understanding of the pathophysiology of atopy aligned with advances in molecular technology. Although AIT has been in practice for over a century, rapid advances in the development of immune-modulating agents has occurred only recently. Conventionally, AIT has involved the regular and frequent administration of allergen via a subcutaneous route over several years in order to achieve tolerance. In general, subcutaneous immunotherapy (SCIT) is considered to be well tolerated, but adverse reactions and the possibility of anaphylaxis still exist. In a 3-year survey between 2007 and 2009, which included approximately 8 million injection visits per year, the reported rate of systemic reactions to SCIT was approximately 0.1% of injections [1]. Considering the time commitment and potential for serious reactions, it is not surprising that both utilization and compliance with therapy are suboptimal [2]. Therapeutic or even prophylactic treatments of the future, however, may involve the administration of antibodies, peptides, nucleotides, and other immune effectors that may allow for short courses of immunotherapy without the adverse effects associated with current regimens [3[¶]]. Efforts have also been made to standardize and modify

allergens via recombinant technology, which can improve immunogenicity while limiting systemic reactions. Novel approaches to improve the efficacy of SCIT include the use of combination treatment with immune response modifiers, adjuvants, recombinant allergens, and peptides targeting B-cell and T-cell epitopes to treat allergic rhinitis.

COMBINED TREATMENT WITH IMMUNE RESPONSE MODIFIERS

Concomitant treatment with a monoclonal anti-IgE antibody in patients undergoing rush protocols or moderate persistent asthma has shown efficacy and improved tolerance. In a double-blind, placebo-controlled trial in ragweed-allergic patients undergoing rush immunotherapy, patients who were pretreated with omalizumab showed significant improvement in symptom severity and a five-fold

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Curr Opin Otolaryngol Head Neck Surg 2014, 22:216–220

DOI:10.1097/MOO.0000000000000053

KEY POINTS

- New developments in SCIT include therapy with immune response modifiers, adjuvants, recombinant allergens, and peptides that can selectively target B-cell and T-cell pathways in allergic rhinitis.
- Mechanisms to achieve improved efficacy and safety of allergen immunotherapy include bypassing the IgE pathway via epitope-based therapies and treatment with recombinant carrier-bound proteins to create 'hypoallergenic' vaccines.
- The next generation of allergen immunotherapy includes immune modifiers that selectively target B-cell and T-cell pathways, which can potentially lead to quicker achievement of tolerance and even prevention of allergic disease.

decrease in the risk of anaphylaxis compared with patients receiving immunotherapy alone [4]. Omalizumab or placebo was given subcutaneously for 9 weeks prior to a 12-week course of immunotherapy, and patients receiving omalizumab demonstrated a 10-fold reduction in serum free IgE. The authors concluded that pretreatment with omalizumab could enhance the safety of rush protocols and perhaps allow for more rapid and efficacious treatment of patients with allergic rhinitis.

Omalizumab has been utilized for the treatment of uncontrolled persistent allergic asthma and has shown to improve asthma control. A similar trial evaluating 248 patients with at least moderate persistent allergic asthma inadequately controlled with inhaled corticosteroids showed that pretreatment with omalizumab prior to immunotherapy with perennial allergens (cat, dog, and house mite) resulted in fewer systemic reactions including respiratory-related complications and more patients were able to reach the maintenance dose ($P=0.004$) [5].

Other strategies include targeting the IgE receptor directly with chimeric proteins. Fusion proteins to the IgE receptors, Fc epsilon and gamma, have shown promise in human cell culture and animal models. Initial studies demonstrated inhibition of downstream signaling and ultimately a decrease in mast cell and basophil degranulation [6,7].

ADJUVANTS AND ALLERGENS

Recent clinical trials have evaluated the use of adjuvants in enhancing the innate immune response in the treatment of allergic rhinitis and asthma. Toll-like receptor (TLR) agonists have the potential to downregulate the Th-2 response and induce Th-1 and T-regulatory type responses.

Several clinical trials have evaluated the efficacy of adjuvants composed of oligonucleotides resembling bacterial DNA (CpG-ODN) in the inhibition of the atopic response. CpG-ODN consists of unmethylated cytosine–guanine dinucleotide motifs which bind to TLR9. Treatment with these adjuvants has shown to reduce airway inflammation and IgE levels [8].

In a phase II clinical trial, 25 patients with ragweed allergy were treated with a vaccine consisting of Amb a 1 conjugated to CpG-ODN, which was administered once weekly over 6 weeks. Patients demonstrated improved symptom scores sustained over the subsequent ragweed season 2 years after the vaccination. The primary endpoint for the study, nasal vascular permeability, however, was not found to be significantly affected [9]. Although there was suppression of seasonal increase in Amb a 1-specific IgE, the production of specific IgG was transient. The authors concluded that the treatment appeared to offer long-term clinical efficacy without any report of adverse systemic reactions. Furthermore, in contrast to conventional SCIT, successful treatment was thought to be independent of the production of allergen-specific IgG.

Novel methods of delivery of these adjuvants include the use of virus-like particles (VLPs), which are self-assembling, noninfectious structures. In a phase I/IIa clinical trial, 21 patients with a history of house dust mite (HDM) allergy with associated asthma were treated over a 10-week period with standard HDM extract mixed with an adjuvant, CpG-ODN contained in a VLP consisting of a bacteriophage coat protein, Q β [10]. There was a significant improvement in rhinitis and asthma symptoms lasting for 38 weeks after treatment. Allergen-specific IgG increased and skin reactivity to HDM was reduced.

A randomized phase I trial of 24 healthy volunteers treated with a synthetic 16-amino acid sequence of the allergen Der p 1 coupled with a VLP was found to be effective in inducing Der p 1-specific IgG and IgM production [11]. The study found that mainly IgG1 and IgG3 were produced but the authors postulated that therapeutic benefit could be seen even without the production of IgG4, which is usually seen after long-term antigenic stimulation. The response was seen 4 weeks after a single injection of the agent, with no difference seen between the subcutaneous and intramuscular routes. The authors concluded that treatment with peptide antigens coupled to VLPs could show promise as an adjuvant-free means of inducing a potentially therapeutic antibody response.

Conversely, in order to determine whether the adjuvant without associated allergen could result in

clinical and immunologic tolerance, Klimek *et al.* [12] conducted a large phase IIb study ($n=299$) investigating CpG-ODN packaged in a VLP in patients with HDM allergy over a 6-week period with weekly injections. Low-dose and high-dose groups were compared with placebo. Symptom and medication scores were significantly improved, and a 10-fold increase in conjunctival provocation testing was seen in the high-dose treatment group. There was no difference in the low-dose group. The authors proposed that stimulation of TLR9 might play a central role in decreasing the Th2 response, while inducing a Th1 response.

Another TLR agonist, monophosphoryl lipid A (MPL) derived from a species of *Salmonella* has been used as an adjuvant for SCIT. MPL coupled with antigen binds to TLR4 and can result in sustained antigen-specific IgG production. In a randomized, double-blind placebo-controlled study, patients allergic to grass pollen received either placebo ($n=514$) or four injections of a mixture of modified pollen allergens (allergoids) from 13 grass species adsorbed onto MPL ($n=514$) in a preseasonal SCIT course of weekly injections. Patients in the treatment group had significant reduction in the combined symptom and medication score compared to placebo by 13.6% [13]. Ninety-five percent of patients in this study completed the course of treatment, which the authors attributed to the shorter course of therapy in comparison to conventional immunotherapy.

EPITOPE-BASED IMMUNOTHERAPY

Several concepts in targeting both B-cell and T-cell mechanisms in allergic rhinitis include the use of peptide immunotherapy, fusion of major allergens using recombinant technology, and modification of native allergen. T-cell-based epitope immunotherapy seeks to bypass the IgE pathway and several clinical trials have been performed showing efficacy. Vaccines based on nonallergenic B-cell epitopes induce protective IgG, which can serve as a blocking antibody and a signal for deactivation of mast cells.

PEPTIDE IMMUNOTHERAPY

Peptide immunotherapy is designed to induce tolerance via binding major histocompatibility complex class II antigen presenting cells and interacting with T regulatory cells without cross-linking IgE. This may be accomplished via IgG-blocking antibodies or tolerance induction in T cells [14]. Larché [15] demonstrated that delivery of T-cell epitope fragments intradermally induces tolerance

in allergen-specific T cells with downregulation of the antigen response. The fragments are too short to cross-link IgE but can result in tolerance, which is thought to arise from systemic presentation of peptides to naïve T cells by dendritic cells and epithelial cells.

In a phase IIa clinical trial, Worm *et al.* [16] administered a set of seven synthetic peptides containing major T-cell epitopes from the cat allergen, Fel d 1, via intradermal ($n=10$) or subcutaneous ($n=12$) routes. In comparison to cat allergen extract, administration of peptide immunotherapy did not induce histamine release in serum basophils and was at least 1000-fold less allergenic than the native allergen, although cytokine response appeared to be similar. In a follow-up to this study, a randomized, double-blind placebo-controlled trial was performed to determine the efficacy and persistence of therapeutic benefit. After treatment with four administrations of peptide immunotherapy over 12 weeks, a significant improvement in symptoms of cat allergen-induced rhinoconjunctivitis was seen at 1 year in the treatment group in comparison with placebo [17].

Peptide immunotherapy has also been investigated for bee venom allergy consisting of a mixture of three T-cell epitope peptides of the major allergen, Api m 1. Five patients with bee venom allergy were treated over a 2-month period and then challenged with a bee sting 1 week later in a monitored setting. Three patients had no systemic reaction to the challenge and the remaining two patients had mild systemic reactions, including angioedema of the lips and eyelid [18]. The response to Api m 1 was also reduced in the peripheral blood mononuclear cells, with isotype switching in favor of IgG4.

FUSION PROTEINS

Fusion proteins using genetic engineering have also been utilized to treat bee venom allergy, incorporating the major bee venom allergens, Api m 1 and Api m 2. The technique preserves T-cell epitopes while deleting the B-cell epitopes, with the goal to eliminate IgE cross-linking and decrease the risk of anaphylaxis. Administration of the fusion protein resulted in reduced IgE binding, basophil degranulation, and skin reactivity, while retaining the ability to stimulate T-cell responses. In a murine model, the authors also demonstrated that vaccination with the fusion protein suppressed IgE and IgG antibodies against the native allergen. In contrast to peptide therapies, the authors argued that fusion proteins offer an advantage by offering the entire complement of T-cell epitopes necessary because of the wide diversity of T-cell receptors [19].

RECOMBINANT ALLERGEN DERIVATIVES AND FUSION CARRIER PEPTIDES

Recombinant technology by cloning allergen proteins seeks to develop 'hypoallergenic' molecules that can induce favorable T-cell responses without IgE binding [14]. Hybrid molecules derived from the major dust mite allergens, Der p 1 and Der p 2, demonstrated significant reduction in IgE reactivity *in vitro* and *in vivo* by skin prick testing in HDM-allergic patients. Higher T-cell proliferative responses were seen with the recombinant allergen in comparison with the native allergen extract. Furthermore, immunization in the murine model produced allergen-specific IgG, inhibiting the IgE response [20].

Genetically engineered allergens have also been demonstrated in birch allergy, based on recombinant Bet v 1. Initial studies showed favorable responses, including reduced allergen-specific IgE and improvement in symptoms [21,22]. Hypoallergenic Bet v 1 derivative resulted in induction of IgG antibodies with significantly decreased binding to the IgE receptor, CD 23, which serves to enhance allergen-specific T-cell responses [23].

Targeting of B-cell epitopes to induce production of allergen-specific IgG has been developed based on the 'hapten-carrier' concept, in which allergen peptides are coupled to carrier proteins. These complexes can potentially reduce IgE-mediated and T-cell-mediated side-effects [24].

In a study of recombinant hypoallergenic allergen derivatives by Linhart *et al.* [25^{***}], vaccination with carrier-bound allergen-derived peptides lacking allergen-specific T-cell epitopes was able to induce allergen-specific IgG antibodies and suppress allergen-specific T-cell responses and allergic lung inflammation. This concept has also been demonstrated for birch pollen allergy, in which peptides derived from the IgE-reactive areas of Bet v 1 were fused to a hepatitis B surface protein [26^{*}]. This fusion peptide was found to reduce T-cell activation *in vitro* as well as induce allergen-specific IgG in a rabbit model.

Chen *et al.* [27] synthesized two carrier-bound Der p 2 peptides that induced IgG antibodies and inhibited IgE binding to allergen and basophil activation in in-vitro studies on allergic patients' serum and *in vivo* in both murine and rabbit models. In a later study, they demonstrated recombinant rDer p 2/1 mosaic proteins showed almost no detectable IgE reactivity or allergenic activity in IgE-binding assays from the serum of HDM-allergic patients and demonstrated reduced in-vivo allergenicity compared with the native allergen [28].

CONCLUSION

Advances in AIT have created a new frontier for reconceptualizing treatment for allergic rhinitis. Strategies for inducing tolerance include bypassing IgE via T-cell epitope immunotherapy as well as combination therapy with immune modifiers that inhibit the Th2 pathway further downstream. Novel techniques in recombinant technology and genetic engineering have resulted in targeted immunologic therapies that can potentially make immunotherapy more efficient, safe, and well tolerated.

Acknowledgements

None.

Conflicts of interest

The authors have no conflicts of interest to disclose.

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- of special interest
- of outstanding interest

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