

Indian Journal of Allergy, Asthma & Immunology

REVIEW ARTICLE

Year : 2012 | Volume : 26 | Issue : 2 | Page : 50-60

Respiratory allergic diseases and therapeutic intervention using allergen-specific immunotherapy

Shailendra N Gaur

Department of Pulmonary Medicine, V. P. Chest Institute, University of Delhi, Delhi, India

Correspondence Address:

Shailendra N Gaur

Department of Pulmonary Medicine, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi - 110 007
India

Abstract

Bronchial asthma and allergic rhinitis are the most common respiratory allergic disease mediated through type-1 immune reaction. The imbalance of TH1 and TH2 cells is responsible for increased immunoglobulin E (IgE) level and release of immune mediators, producing symptoms in the particular genetically predetermined target organ. Pharmacotherapy is effective in controlling the symptoms of allergic diseases, but withdrawal of medication leads to reappearance of symptoms in a short span of time. The therapeutic modality, namely allergen-specific immunotherapy, corrects the TH1 and TH2 imbalance and thus effects the natural course of allergic disease prescribed in addition to the pharmacotherapy. Immunotherapy has also been found to prevent development of newer allergies and progression of rhinitis to asthma. The subcutaneous immunotherapy with perennial subcutaneous injections and stepwise procedure is the standard and established method. Here, the injections are started with a very high dilution of antigen and then slowly increased to the optimal maintenance dose. However, precautions should be taken to take care of the related side-effects, including anaphylaxis. Patient education and use of this therapy only by trained physicians is mandatory, and it should be practiced at a center having facilities for the management of anaphylaxis. There are various trials in the literature to support improvement by immunotherapy. In Indian studies, favourable results were noted in symptom score, skin sensitivity, airway reactivity (PC20), specific immunoglobulin (Ig) E, Ig G1 and Ig G4. Researches are aimed on a safer and more acceptable route of administration of the allergen. The sublingual route for immunotherapy has been used from 1986 and, till today, has been found to be equally effective and safe. Considering the difference in geo-environmental variation in India from the western world, an Indian guideline for practice of allergen immunotherapy was published in 2009, where consideration was given to the Indian environment, pattern of seasons, presence of different/newer allergens, affordability and feasibility of certain tests in the Indian context.

How to cite this article:

Gaur SN. Respiratory allergic diseases and therapeutic intervention using allergen-specific immunotherapy. Indian J Allergy Asthma Immunol 2012;26:50-60

How to cite this URL:

Gaur SN. Respiratory allergic diseases and therapeutic intervention using allergen-specific immunotherapy. Indian J Allergy Asthma Immunol [serial online] 2012 [cited 2013 Sep 27];26:50-60

Available from: <http://www.ijaai.in/text.asp?2012/26/2/50/112548>

Full Text

Introduction

The immune system has been designed to protect the integrity of the host by maintaining the healthy "status quo" called homeostasis. The immune response is basically a mechanism used by vertebrates to eradicate infectious agents that succeed in penetrating the natural barriers, and it uses a complex array of protective mechanisms to control and eliminate invading organisms. But, inappropriate immune response to an antigen is harmful to the host and is termed as hypersensitivity or allergy. The term allergy was originally defined as altered reactivity to exogenous antigens, and is now used synonymously with atopy. Allergic reactions can be categorized as "immediate" or "delayed" based on the time taken for symptoms to appear after antigen exposure. There may be "humoral" or "cell mediated" immune response based on the type of components involved.

Allergic Diseases

Allergy represents a major health problem affecting more than 25% of the population worldwide. Allergic patients (type I) are characterized by the increased production of immunoglobulin E (IgE) antibodies against antigens (allergens) from different sources, e.g., pollen, mites, fungi, insects, animal dander and foods. The type I allergy (immediate) leads to allergic manifestations, e.g., allergic rhinitis, asthma, atopic dermatitis, urticaria and anaphylactic shock triggered by allergen-induced cross-linking of effector cell-bound IgE antibodies and the release of inflammatory mediators such as histamine and leukotrienes. [1],[2],[3] Allergic diseases affect an individual in terms of quality of life, work performance and productivity. [4] The prevalence of atopic diseases has increased in many industrialized countries during the past few decades. [5]

Allergic rhinitis

Allergic rhinitis (AR), commonly called "hay fever," is one of the most common atopic diseases affecting 10-25% of the population worldwide. [6] Prevalence of allergic rhinitis has been reported in 7.3% of the Indian children. [7] It is an IgE-mediated inflammation of the nasal mucosa characterized by inflammatory infiltrates containing eosinophils, T cells, mast cells and basophils that release several mediators like chemokines and cytokines on interaction with specific allergens. [8] AR is often associated with conjunctival symptoms. AR also triggers a systemic inflammation, which can augment inflammation in both the upper and the lower airways. Hence, asthma and rhinitis are common co-morbidities suggesting the concept of "one airway, one disease." [9] Epidemiologically, up to 40% of patients with AR have asthma, and up to 80% of patients with asthma experience nasal symptoms. [10]

Asthma

Asthma is one of the most common chronic diseases of the airways characterized by bronchial hyper-responsiveness and mucus hyper-secretion resulting in bronchoconstriction. According to the ISAAC study, the prevalence of asthma showed a wide range of 1.6-36.8%, with an eight-fold variation seen between the 10th and 90th percentiles (3.9-30.6%). [11] Recent reports from India showed a prevalence of asthma in the range of 2.3-16.6%, [12],[13],[14],[15] which is higher than that reported (<1%) earlier. [16] Approximately 50% or more cases of asthma are of an atopic origin.

The key symptoms of asthma are cough, shortness of breath, wheezing and chest tightness. Many cells and cellular elements play a role in asthma pathogenesis; in particular, mast cells, eosinophils, T-lymphocytes, macrophages, neutrophils and epithelial cells. In asthma, the predominant orchestrator of the chronic inflammation is the CD4 or T-helper lymphocyte, producing key regulatory cytokines such as interleukin (IL)-5 and IL-4. [17]

Atopic dermatitis (eczema)

The prevalence of atopic dermatitis in children is estimated to be 10-20%. [18] Studies show that the rate of atopic dermatitis first occurring in infants and persisting into adulthood ranges from 45% to 60%, and the risk of developing respiratory symptoms in later years is around 40-60% (pollen allergy 41.5%; perennial rhinitis 25%; asthma 25%).

Atopic dermatitis (IgE mediated) manifests red, inflamed rashes most often on the arms, legs, ankles or neck. It usually precedes the onset of allergic rhinitis and/or asthma. Acute exacerbations may be weepy and crusted, usually signifying superinfection with Staphylococci. Serum total IgE concentration may be markedly elevated with high levels of IgE against aero-allergens and/or food allergens. Following cutaneous challenge with allergen, there is an initial local response consisting of IL-4 and IL-5 (Th2-type cytokine response), followed by a mixed pattern (interferon [IFN]- γ , IL-4 and IL-5;

ThO). [19]

Urticaria

Hives, medically known as urticaria, are caused due to excessive mast cell activity in the skin on exposure with specific allergens. They are red, itchy, raised areas of skin that appear in varying shapes and sizes from a few millimeters to several inches in diameter. Hives can be round, or they can form rings or large patches. Hives are produced by histamine and other compounds released from mast cells. Histamine causes fluid to leak from the local blood vessels, leading to swelling in the skin. [20]

Allergy diagnosis

The diagnosis of allergy is based on a suggestive history, skin tests and/or specific IgE estimation to the relevant allergens and clinical corroboration with allergic symptoms. IgE plays a central role in allergic diseases; thus, in vivo and in vitro tests used in the diagnosis of allergic diseases are directed toward the detection of free or cell-bound IgE. In vivo tests, such as intradermal (ID)/skin prick and provocation tests, and in vitro tests, namely enzyme-linked immunosorbent assay (ELISA)/radioallergosorbent test, are followed for allergy diagnosis. [21],[22]

The common methods of skin testing are prick, ID and prick to prick. Skin prick test (SPT) is the most specific and safe test usually recommended for the diagnosis of immediate type of allergy. [23] The ID test is a more sensitive test, but may yield false-positive results. [24],[25] Another method for skin testing is the prick to prick method, mostly used for juicy fruits and other similar source of allergens. Skin reactions are graded in comparison with the positive control, i.e., histamine diphosphate, or negative control, i.e., phosphate buffer saline.

The histamine release assay is another in vitro test that detects histamine released from the patient's basophils in the presence of specific allergens. [26] The test employs an anti-histamine antibody in a sandwich ELISA to detect histamine released in response to allergen. The histamine released is expressed as a percentage of total histamine release. The cut-off point for significant release is determined in comparison with spontaneous release.

The provocation test (bronchial challenge) is a confirmatory test but is not employed in routine clinical practice because of the risk involved. However, a double-blind placebo-controlled food challenge is recommended to diagnose IgE-mediated allergies to various foods. [23],[27]

Therapeutic strategies

The key elements of allergy management are: (1) preventing the exposure of sensitized individuals to allergen and (2) treating these individuals with therapeutic agents appropriate to the disorder. Precisely, the management of allergic diseases includes allergen avoidance, medication (pharmacological treatment), immunotherapy and patient education. Allergen avoidance is the best treatment, but it may not be possible in case of many outdoor allergens. Pharmacotherapy provides symptomatic relief, and once the drugs are discontinued, the symptoms recur quickly. Immunotherapy is the only treatment modality that acts on effector cells and changes the course of disease.

Allergen-specific immunotherapy (SIT) forms an integrated part of the treatment of type I allergy diseases, [28] reducing the disease severity, improving the quality of life and diminishing the risk and cost of pharmacotherapy. Immunotherapy and pharmacotherapy should be integrated in the treatment for allergic diseases in order to gain the best results. SIT is a gradual immunizing process in which increasing doses of antigens responsible for causing allergic symptoms are administered to a patient to develop tolerance to the offending allergen on natural exposure. It reduces specific cutaneous, nasal, bronchial and/or ocular reactivity to allergen as assessed by in vivo and/or in vitro tests. There is an initial transient increase in serum-specific IgE, followed by gradual decrease over few months of treatment. Then, there is a class switch, where IgG1 shows an increase initially and the IgG4 level increases later as a result of successful immunotherapy. However, these changes may not always correlate with the symptoms.

Good candidates for immunotherapy are patients whose symptoms are not controlled adequately by medications and allergen avoidance measures. Analysis under a World Health Organization position paper revealed that SIT is an effective form of treatment for patients with allergic asthma. [29] Immunotherapy, when properly conducted, has the potential to reduce symptoms and the need for drug significantly. Moreover, it is possible to prevent progression into more severe disease using this therapy. [30] Immunotherapy can be considered effective if a substantial increase (10-100 folds) in allergen-specific IgG4 is induced.

Allergens for immunotherapy

Immunotherapy has been effective with pollen, fungi (molds), animal dander, dust mite, cockroach and hymenoptera sensitivity. [31] The selection of allergens for immunotherapy is based on clinical history, presence of specific IgE antibodies and allergen exposure. Phylogenetically related pollens contain allergens that are cross-reactive. Here, a selection of single pollen within the cross-reactive genus or subfamily may suffice the requirement for immunotherapy. Knowledge of cross-reactivity is important in the selection of allergens for immunotherapy, because limiting the number of allergens is necessary to attain optimal therapeutic dose.

SIT requires extracts with consistent allergenic activity and composition. Standardized extracts are therefore recommended with a defined potency for vaccines and are labeled with a common unit of measure. However, the commercial extracts vary widely in biologic activity and allergen constituents. [32] Natural products are also at risk of being contaminated with allergens from other sources, and the inherent proteolytic enzymes may degrade the proteins. [33] Allergen extracts for both diagnosis and treatment should be stored in a refrigerator between 2°C and 8°C (35.6°-46.4°F), because, at higher temperature, the extracts lose their potency. Concentrated aqueous extracts (1:10 w/v) in 50% glycerin are stable for about 3 years when stored at 4°C (39.2°F), but, without glycerin, they may lose half their original strength within 6 months. [34] A study on the stability of the *Periplaneta americana* extract shows that epsilon aminocaproic acid (EACA 0.05 M) and sucrose (0.25 M) in combination maintain the shelf-life of the extract for 1 year at 4°C. [35]

Types of immunotherapy

Immunotherapy has been classified according to the route of allergen administration.

Subcutaneous immunotherapy (SCIT): It involves repeated subcutaneous injections of increasing doses of allergen extracts until an optimal maintenance dose is reached [Table 1]. This dose is then injected subcutaneously on a regular basis (at intervals of approximately 20 days) for not less than 3 years. Treating patients with SCIT on the basis of positive allergy tests alone is not justified. Patients receiving immunotherapy need to have proven allergy by skin test or in vitro testing that should correlate with their clinical history. [Table 1]

Studies evaluating the long-term efficacy of subcutaneous immunotherapy with inhalant allergens have been undertaken in patients with rhinitis and asthma. In one report, the long-term advantages of this therapy have been shown over pharmacotherapy. [36] The relapse of symptoms after discontinuation of pharmacotherapy was faster (1 month) than immunotherapy (1 year). A pollen immunotherapy study of 3 years has demonstrated the favorable effects in clinical parameters as well as increased production of specific IgG4, TGF- β and IL-10 and decreased IgE binding affinities. [37] Another 10-year follow-up study investigating the time course changes in specific IgE and IgG4 in serum found that the IgE titer begins to decrease with many years of treatment, and increase in IgG4 plays a significant contribution in the clinical efficacy of immunotherapy. [38]

There are reports suggesting that allergen-SIT also prevent onset of new sensitization in allergic patients. [39] Administration of allergens in patients by the subcutaneous route might cause local and/or systemic reactions, such as urticaria, asthmatic attack and/or life-threatening anaphylactic shock. Therefore, attempts have been made to reduce therapy-induced side-effects. These include the adsorption of allergen extracts to adjuvant to delay the systemic release of allergenic material from the site of administration and the chemical modification of allergens to reduce their allergenicity and mutations to abrogate allergenic activity but retain immunogenicity.

Sublingual immunotherapy (SLIT): SIT by the subcutaneous route is effective for the treatment of allergic asthma and rhinitis, but it may be associated with severe or even fatal systemic reactions. [40],[41] Therefore, an alternative route of allergen administration has been proposed involving mucosal absorption. SLIT is safe because the oral mucosa lacks proinflammatory cells and is rich in Langerhans-like dendritic cells. [42],[43] Sublingual immunotherapy is raising a lot of interest as a non-invasive procedure and is demonstrated to be efficacious. But, it requires high doses of allergen, i.e., 50-100 fold more than that administered through the subcutaneous route. [44],[45],[46]

SLIT is reported to be effective as subcutaneous immunotherapy in reducing allergic symptoms and medication requirement. [47],[48],[49],[50] Here, the patients are instructed to hold the extract (drops) under the tongue for 2 min and then swallow the same. The dose is increased once every 3-4 weeks or earlier until a maintenance dose is reached, with minimal or no side-effects. Contact of the allergen with the oral mucosa is crucial for the success of SLIT. SLIT showed comparable efficacy with SCIT in allergic rhinitis patients, with less than 5% side-effects, and was safer even in children of less than 5 years of age. [50]

Oral immunotherapy: The extracts are given through the mouth in the form of enteric-coated capsules. Here, the allergen tolerance was reported to improve due to deviation of TH2/TH1 and regulation of IgE production. [51] Studies show a clear

clinical effectiveness supported by significant symptom improvement and decrease in dosage of drugs required to control the symptoms. [52],[53] However, with high doses, adverse reactions like intestinal bleeding were observed in this form of immunotherapy. [54],[55]

Intranasal immunotherapy: Of 18 studies available, 17 reported significant improvement in rhinitis by reduction of nasal sensitivity and local immunological responses. [56],[57] Both aqueous extracts and dry powders have been tried in nasal immunotherapy. Apparently, nasal therapy exerts its action only on the target organ and the efficacy of the treatment requires pre-seasonal administration. Here, mild adverse reactions such as rhinitis and itching in the nose have been reported.

Mechanism of Immunotherapy

Studies have shown modest reduction in allergen-specific IgE levels and induction of allergen-specific IgG subclasses after immunotherapy. [29],[58],[59],[60] Durham et al. suggested that the IgE allergen binds to CD23 on the surfaces of B cells leading to stimulation of allergen-specific T-cell clones. But, in patients getting immunotherapy, IgG antibodies could inhibit IgE-facilitated allergen binding by B cells and presentation to T cells. [61] Another surface receptor, CD32, is part of a large population of B cell co-receptors that act to modulate signaling. It has a low affinity for IgG antibodies and down-regulates IgE antibody production in the presence of IgG. CD32 (Fc II) receptor is involved in the regulation of the B cell response to antigen. [62] However, the importance of blocking antibodies (IgG) was questioned because induction of IgG is not always associated with clinical improvement after immunotherapy. [63] Recent studies carried out in defined molecular and cellular systems have rekindled interest in the concept of blocking antibodies. [64] Gradually, it was proven that immunotherapy altered the cytokine profile from a TH2 cytokine-dominated response (IL-4 and IL-5) to a TH1-type response with a local accumulation of cells producing IFN- γ . [65] Immunotherapy acts on effector cells inhibiting the release of inflammatory mediators. Early effects of immunotherapy are related to mast cell and basophil desensitization. Intermediate effects are associated with changes in allergen-specific T cells and late effects are on B cells and IgE as well as mast cells, basophils and eosinophils. [66]

Control of IgE synthesis is attributed to the actions of mutually antagonistic subsets of CD4⁺ T cells. Later, it was acknowledged that activated T cells and their products play a major role in the pathogenesis of allergic diseases, and allergen-specific T cells were considered as the major target for SIT. SIT is associated with a decrease in IL-4 and IL-5 production by CD4⁺ TH2 cells and a shift toward increased IFN- γ production by TH1 cells. Recent reports indicate that immunotherapy acts by modifying CD4⁺ T cell responses either by immune deviation, T cell anergy and/or both. [67] Studies in mice and in human subjects have suggested the presence of various types of T cells with regulatory activity (ie, T cells with inhibitory effects on immune responses), including TR cells (producing IL-10 and possibly TGF- β), CD4⁺ CD25⁺ T cells (possibly TGF- β) and TH3 cells (also TGF- β). [68] Induction of IL-10 might block B7/CD28 co-stimulation. [69] IL-10 has a number of documented anti-allergic properties that might be important to immunotherapy. [70]

Sublingual immunotherapy operates through a different route, wherein the allergen is captured by Langerhans-like dendritic cells in the oral mucosa and, subsequently, the dendritic cells mature and migrate to the proximal draining lymph nodes. The role of these lymph nodes lies in their preferential production of blocking IgG antibodies and the induction of T lymphocytes with suppressive function. One explanation for the success of SLIT is the profound difference between oral Langerhans cells and their skin counterparts. Oral Langerhans cells exhibit constitutive high expression of the Fc portion of IgE (Fc ϵ RI), major histocompatibility complex (MHC) class I and II, as well as co-stimulatory molecules (CD40, CD80/B7.1, CD86/B7.2), which suggest the specific function of these cells within the regional immune system of the oral mucosa. [71] A recent study [72] revealed that the lipopolysaccharide receptor CD14, present on both nasal and oral myeloid dendritic cells, was at higher density on oral dendritic cells. Allergen delivery through the sublingual-swallow route appears to be more efficient than either route alone, which suggests an inevitable absorption of allergen through the gastrointestinal tract that potentiates the induction of oral mucosal tolerance. [73]

Immunotherapy Trials in Asthma and Rhinitis

Trials supporting immunotherapy

Clinical efficacy of immunotherapy in rhinitis and asthma using potent standardized extracts in carefully selected patients has been well documented by many double-blind placebo-controlled studies. However, only a few studies have demonstrated a low degree of clinical efficacy. [74] A metaanalysis of clinical trials of allergen immunotherapy has shown benefits for the treatment of allergic conditions/asthma; however, the major risk associated is systemic reactions

(anaphylaxis). Immunotherapy is effective and well tolerated in children. Besides, it prevents the development of asthma in children having allergic rhinitis. Immunotherapy with hymenoptera venom allergens has been reported to give almost 100% benefits in patients. Several clinical trials have been performed the world over demonstrating beneficial effects of this therapy using clinico-immunologic parameters. [75],[76] The fundamental question regarding immunotherapy is whether it has the potential to provide long-term benefit after its discontinuation. Studies suggest that immunotherapy has a prolonged beneficial effect, lasting several years after the discontinuation of treatment. [77] To the best of our knowledge, more than 75 trials in asthma and more than 18 trials in allergic rhinitis has been reported in the literature. [78]

Immunotherapy trials with mixed grass-pollen allergoid used in a low-dose schedule (maximal dose: 2000 PNU) in 19 patients and in a high-dose schedule (maximal dose: 10,000 PNU) in 20 patients, with 18 patients in the placebo group, were conducted. Both the antigen immunotherapy groups presented a significant reduction in nasal and skin sensitivity and a significant increase in IgG. [79],[80] A highly significant correlation was recorded between nasal symptom and medication scores and the results of nasal challenges. [81] A double-blind, multicenter immunotherapy trial in children using a purified protein of *Cladosporium herbarum* showed a decrease in bronchial hyper-reactivity (BHR) and specific IgE and increase in specific IgG, with a marked increase in the ratio of specific IgG/specific IgE. [82],[83] A double-blind placebo-controlled rush immunotherapy with the *Alternaria* extract showed decreased symptom and medication scores, skin tests wheal and increased specific IgG significantly. [84] Immunotherapy trials with *Dermatophagoides pteronyssinus* and *D. farinae* showed improvement in asthma patients allergic to mite. [85],[86] A double-blind placebo-controlled immunotherapy trial of house dust mite demonstrated reduced bronchial sensitivity to allergen. [87] An immunotherapy trial with *D. pteronyssinus* showed improvement in asthma patients allergic to mite with increase in IgG. [88] A double-blind, multicenter immunotherapy trial in children using purified *D. pteronyssinus* resulted in a significant increase of specific IgG antibodies. [89] Successful immunotherapy with *D. pteronyssinus* in perennial childhood asthma was associated with a slight mean rise of serum IgG antibody to the mite antigen in contrast to the placebo group. [90] A double-blind, rush immunotherapy with standardized *D. pteronyssinus* showed improvement in bronchial provocation tests. [91] Further, a double-blind placebo-controlled trial with *D. pteronyssinus* demonstrated reduction in symptomatic medication than in the control group. [92] SIT with standardized *D. pteronyssinus* showed improvement in symptoms and increase in specific IgG. [93] A double-blind trial of immunotherapy in cat allergen-induced asthma showed a reduction in skin reactivity to the cat extract as well as a significant mean reduction in bronchial sensitivity to the same extract, with significant increases in specific IgG. [94],[95] Immunotherapy with the cat extract was well tolerated, significantly decreasing the skin and bronchial responses to the cat extract and specific IgE and significantly increasing the specific IgG antibodies. [96],[97] A double-blind trial of immunotherapy with cat allergen showed reduction in bronchial sensitivity toward cat, decrease in BHR and symptoms as well as increase in IgG. [98],[99] A double-blind immunotherapy trial with dog dander extract also showed decrease in conjunctival sensitivity and skin test wheal response and increase in IgG. [100],[101]

Trials not supporting immunotherapy

However, few studies on allergen immunotherapy did not find beneficial results. In one trial of ragweed allergic asthma patients (N = 25), there was no significant response in asthma or hay fever symptoms. [102] In another double-blind placebo-controlled trial with *D. pteronyssinus*, no improvement was observed in patients with asthma sensitive to house dust mite. [103]

The antigenic components, mainly proteins, are many in most of the antigens, except in venom. The response hence was very good with venom immunotherapy. The multiple proteins and the unwanted substances (fats and carbohydrate) in crude antigen (used at present) reduce the efficacy of immunotherapy. Efforts are being made to isolate the specific antigenic moiety in every antigen, but the cost and the procedure of procurement is tedious and is not at present commercially viable. The potency of the antigen mostly differs in every batch and is a cause of concern for the efficacy. However, there are natural variations in the characteristics of the antigen from place to place due to a difference in the soil characteristics and the environment. Development of new sensitivities has been noticed in few cases undergoing immunotherapy, for which no definite explanation is available. Standardization of antigen is highly essential for uniformity of potency and increasing the efficacy, but is a difficult task. The most important cause of reduced efficacy of immunotherapy in certain cases is due to its overuse and misuse.

Allergen immunotherapy in India

Immunotherapy is practiced in India since the last three decades. In an open study at the V.P. Chest Institute, Delhi, 50% of the seasonal allergic rhinitis cases showed considerable reduction in their symptom score and drug intake after 2 years of immunotherapy with mixed allergen vaccines. [104] Further, a placebo-controlled immunotherapy trial with a single allergen preparation of *Cocos nucifera* pollen showed significant clinical improvement and immunological changes as compared with the placebo group. [105] An open comparative trial taking immunotherapy versus inhaled budesonide showed faster improvement in symptoms of asthma with drug therapy. But, the decline in benefit after cessation of treatment was more

rapid in patients on corticosteroids than in patients on immunotherapy. [36] Immunotherapy in patients suffering from house dust allergy showed decrease in IgE symptoms and increase in specific IgG. [106] Allergen-SIT with mixed allergen extracts (vaccine) in nasobronchial allergy showed significant improvement in symptoms, lung function and reduced skin sensitivity in patients of asthma and rhinitis. [107] A single-blinded immunotherapy trial for 3 years with *Sorghum vulgare* (Jawar) pollen extract in 51 patients of asthma resulted in significant symptom improvement in 47% of the patients in the immunotherapy group as compared with 20% in the pharmacotherapy group. [108] A double-blind placebo-controlled immunotherapy trial with mosquitoes for 1 year in 40 patients of asthma and rhinitis showed significant symptom improvement, PC20, significantly high levels of IgG4 and reduced skin reactivity, IgE levels and decreased IgE/IgG4 ratio. [109] A double-blind control study of 1 year immunotherapy in 85 cases of asthma and rhinitis, including healthy controls, observed significant improvement in symptom score, skin sensitivity, airway reactivity (PC20), IgE/IgG4 and IgG1/IgG4 ratio. There was no significant difference in improvement by using single antigen or multiple antigen (up to 5) for immunotherapy. There was also a positive correlation with increase in IgG4 levels and clinical improvement in the study. This emphasizes that use of multiple antigens for immunotherapy is also justified. [110]

Future Immunotherapy Strategies

Reports from China on future therapeutic strategies suggest the beneficial effects of BCG immunization, [111] resulting in TH1 response and prevention of sensitization. Studies indicate an increased risk for sensitization on exposure to environmental allergens during infancy. [112] Thus, immunization with an antigen early in life (TH1 response) could prevent a subsequent TH2 response to the same antigen. Also, creating a pervasive TH1-dominated environment could influence the response occurring to the exposure of other allergenic proteins. Using various allergen-specific methods with adjuvants or DNA-based immunization at an early age to high-risk children is expected to give a long lasting protective TH1 response. [113] Here, the problem is to reliably identify children at high risk. With the developments in the human genome project, it has become possible to identify genes responsible for allergy/asthma that serve as markers for identifying children at high risk. These children can be benefited by proper immunotherapy at an early age before their immune responses get locked up in TH2 immunity.

Studies suggest that recombinant allergens show comparable IgE binding to their natural counterparts. [114],[115] Several studies with recombinant allergens have been performed to improve the efficacy and safety of immunotherapy. [116] Modified derivatives of major birch pollen allergen, Bet v1, have been tried successfully in two studies on immunotherapy. [117],[118] Recombinant allergens also offer the possibility of developing hypo-allergens with reduced IgE reactivity but intact immunogenicity.

Peptides are small fragments of protein that are too small to be recognized by IgE for cross-linking on mast cells. Synthetic peptides representing T cell epitopes, if presented without co-stimulation, can induce anergy to subsequent challenges with the peptide or full length protein. [119],[120],[121] Peptide allergen immunotherapy has been studied best with cat major allergen Fel d 1 [122] and with hymenoptera venom major allergen phospholipase A2. [123] Most recently, investigators have examined a collection of overlapping peptides of Fel d 1, each with 12 amino acids in length, which covers the whole protein. [124],[125] Because of the peptide overlap, they affect many T cells in a wide variety of different individuals.

The biology of CpG motifs had been reviewed by Krieg and Wagner, [126] followed by a review of toll-like receptor by Zuany-Amorin et al. [127] The potential use of CpG in the treatment of allergic rhinitis [128] and asthma has been suggested. [126],[127] Synthetic CpG motifs are either mixed with an allergen vaccine or covalently conjugated to an allergen. In the first trial of this concept, a conjugated product was developed by Dynavax Technologies Corporation that has an average ratio of six CpG residues per ragweed Amb a 1 molecule. Studies reveal that such conjugates promote allergen-specific IgG2a antibody production, a marker for TH1 responses in mice. [129] Studies in murine models have demonstrated that DNA immunization strategies prevent the development of antigen-specific IgE synthesis, airway hyper-responsiveness or food-induced anaphylaxis and induce inhibitory CD8 cells. [121],[122],[123]

The difference in environment, climate, soil characteristics and presence of different allergens present in India has prompted us to prepare a "Guidelines for practice of Allergy in India" to include most of the global recommendations, with few recommendations being modified to suit the Indian situations. The first such guidelines were prepared and published in 2009. [130] An editorial comment mentioning the scientific facts as known today further emphasizes the efficacy of allergen immunotherapy. [131] However, it is further emphasized that allergen immunotherapy should be prescribed under the direct supervision of a trained allergist/immunologist in the office having facilities with appropriate equipment, medications and personnel to deal with anaphylaxis. [130]

References

- 1 Liberman AC, Refojo D, Arzt E. Cytokine signaling/transcription factor cross-talk in T cell activation and Th1-Th2 differentiation. *Arch Immunol Ther Exp (Warsz)* 2003;51:351-65.
- 2 Dong C, Flavell RA. Th1 and Th2 cells. *Curr Opin Hematol* 2001;8:47-51.
- 3 Johnson K, Calame K. Transcription factors controlling the beginning and end of B-cell differentiation. *Curr Opin Genet Dev* 2003;13:522-8.
- 4 Bolin K, Lindgren B. Asthma and allergy: The significance of chronic conditions for individual health behaviour. *Allergy* 2002;57:115-22.
- 5 Wuthrich B. Epidemiology of the allergic diseases: Are they really on the increase? *Int Arch Allergy Appl Immunol* 1989;90:3-10.
- 6 International Consensus Report on Diagnosis and Management of Rhinitis. International Rhinitis Management Working Group. *Allergy* 1994;49 (19 Suppl):1-34.
- 7 Gaur SN, Rajpal S, Rohatgi A. Prevalence of bronchial asthma and allergic rhinitis among school children in Delhi. *Intern Med J Thai* 2004;20:8-13.
- 8 Naclerio RM. Allergic rhinitis. *N Engl J Med* 1991;325:860-9.
- 9 Grossman J. One airway, one disease. *Chest* 1997;111 (2 Suppl):11S-6.
- 10 Settipane RJ, Hagy GW, Settipane GA. Long-term risk factors for developing asthma and allergic rhinitis: A 23-year follow-up study of college students. *Allergy Proc* 1994;15:21-5.
- 11 ISAAC. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. *Lancet* 1998;351:1225-32.
- 12 Chhabra SK, Gupta CK, Chhabra P, Rajpal S. Prevalence of bronchial asthma in school children in Delhi. *J Asthma* 1998;35:291-6.
- 13 Gaur SN, Gupta K, Rajpal S, Singh AB, Rohatgi A. Prevalence of bronchial asthma and allergic rhinitis among urban and rural adult population of Delhi. *Indian J Allergy Asthma Immunol* 2006;20:90-7.
- 14 Aggarwal AN, Chaudhry K, Chhabra SK, D'Souza GA, Gupta D, Jindal SK, *et al.* Prevalence and risk factors for bronchial asthma in Indian adults: A multicentre study. *Indian J Chest Dis Allied Sci* 2006;48:13-22.
- 15 Jindal SK. Bronchial asthma: The Indian scene. *Curr Opin Pulm Med* 2007;13:8-12.
- 16 Viswanathan R, Prasad M, Thakur AK, Sinha SP, Prakash N, Mody RK. Epidemiology of asthma in an urban population; a random morbidity survey. *J Indian Med Assoc* 1966;46:480-3.
- 17 Hamid QA, Minshall EM. Molecular pathology of allergic disease: I: lower airway disease. *J Allergy Clin Immunol* 2000;105:20-36.
- 18 Kay AB. Overview of 'allergy and allergic diseases: With a view to the future'. *Br Med Bull* 2000;56:843-64.
- 19 Grewe M, Bruijnzeel-Koomen CA, Schopf E, Thepen T, Langeveld-Wildschut AG, Ruzicka T, *et al.* A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. *Immunol Today* 1998;19:359-61.
- 20 Kay AB. Allergy and allergic diseases: *N Engl J Med* 2001;344:109-13.
- 21 Dolen WK. Skin testing and immunoassays for allergen-specific IgE. *Clin Rev Allergy Immunol* 2001;21:229-39.
- 22 Voller A, Bartlett A, Bidwell DE. Enzyme immunoassays with special reference to ELISA techniques. *J Clin Pathol* 1978;31:507-20.
- 23 Kumari D, Kumar R, Sridhara S, Arora N, Gaur SN, Singh BP. Sensitization to blackgram in patients with bronchial asthma and rhinitis: Clinical evaluation and characterization of allergens. *Allergy* 2006;61:104-10.
- 24 Kausar MA, Viyayan VK, Bansal SK, Menon BK, Vermani M, Agarwal MK. Mosquitoes as source of inhalant allergens: Clinico-immunological and biochemical studies. *J Allergy Clin Immunol* 2007;120:1219-21.
- 25 Reid MJ, Lockey RF, Turkeltaub PC, Platts-Mills TA. Survey of fatalities from skin testing and immunotherapy 1985-1989. *J Allergy Clin Immunol* 1993;92:6-15.
- 26 Kleine Budde I, de Heer PG, van der Zee JS, Aalberse RC. The stripped basophil histamine release bioassay as a tool for the detection of allergen-specific IgE in serum. *Int Arch Allergy Immunol* 2001;126:277-85.
- 27 Kumar R, Srivastava P, Kumari D, Fakhr H, Sridhara S, Arora N, *et al.* Rice (*Oryza sativa*) allergy in rhinitis and asthma patients: A clinico-immunological study. *Immunobiology* 2007;212:141-7.
- 28 Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: Therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 1998;102:558-62.
- 29 Tonnel AB. Specific immunotherapy and therapeutic strategies in allergic diseases. What's new? *Bull Acad Natl Med* 2005;189:1475-87.
- 30 Moller C, Dreborg S, Ferdousi HA, Halken S, Høst A, Jacobsen L, *et al.* Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis. *J Allergy Clin Immunol* 2002;109:251-6.

- 31 Martin Munoz MF. Efficacy of immunotherapy in the treatment of asthma. *Allergol Immunopathol* 2004;32:133-41.
- 32 Ruiz Reyes H, Rodriguez Orozco AR. Allergic fungi: Importance of the standardization of fungal extracts and their application on clinical practice. *Rev Alerg Mex* 2006;53:144-9.
- 33 Van der Veen MJ, Mulder M, Witterman AM, Van Ree R, Aalberse RC, Jansen HM. False-positive skin prick test responses to commercially available dog dander extracts caused by contamination with house dust mite (*Dermatophagoides pteronyssinus*) allergens. *J Allergy Clin Immunol* 1996;98:1028-34.
- 34 Singh BP, Gangal SV. Defined allergen extracts; need for efficient diagnosis of allergy and immunotherapy. *Indian J Allergy Asthma Immunol* 2001;15:67-74.
- 35 Sudha VT, Srivastava D, Arora N, Gaur SN, Singh BP. Stability of protease rich *Periplaneta americana* allergen extracts during storage: Formulating preservatives to enhance shelf life. *J Clin Immunol* 2007;27:294-301.
- 36 Sheikh WA. Immunotherapy vs. inhaled budesonide in bronchial asthma: An open, parallel, comparative trial. *Clin Exp Allergy* 1997;27:1279-84.
- 37 Ye YM, Lee SK, Kim SH, Nahm DH, Suh CH, Park HS. Changes of serum cytokines after the long term immunotherapy with Japanese Hop pollen extracts. *J Korean Med Sci* 2006;21:805-10.
- 38 Ohashi Y, Nakai Y, Tanaka A, Kakinoki Y, Washio Y, Kato A, *et al.* Ten-year follow-up study of allergen-specific immunoglobulin E and immunoglobulin G4, soluble interleukin-2 receptor, interleukin-4, soluble intercellular adhesion molecule-1 and soluble vascular cell adhesion molecule-1 in serum of patients on immunotherapy for perennial allergic rhinitis. *Scand J Immunol* 1998;47:167-78.
- 39 Pajno GB, Barberio G, De Luca F, Morabito L, Parmiani S. Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy: A six-year follow-up study. *Clin Exp Allergy* 2001;31:1392-7.
- 40 Passalacqua G, Canonica GW. Sublingual immunotherapy: Update. *Curr Opin Allergy Clin Immunol* 2006;6:449-54.
- 41 Quirino T, Iemoli E, Siciliani E, Parmiani S, Milazzo F. Sublingual versus injective immunotherapy in grass pollen allergic patients: A double blind (double dummy) study. *Clin Exp Allergy* 1996;26:1228-31.
- 42 Scadding GK, Brostoff J. Low dose sublingual therapy in patients with allergic rhinitis due to house dust mite. *Clin Allergy* 1986;16:483-91.
- 43 Akdis CA, Barlan IB, Bahceciler N, Akdis M. Immunological mechanisms of sublingual immunotherapy. *Allergy* 2006;S81:11-4.
- 44 Grosclaude M, Bouillot P, Alt R, Leynadier F, Scheinmann P, Rufin P, *et al.* Safety of various dosage regimens during induction of sublingual immunotherapy: A preliminary study. *Int Arch Allergy Immunol* 2002;129:248-53.
- 45 Khinchi MS, Poulsen LK, Carat F, Andre C, Hansen AB, Malling HJ. Clinical efficacy of sublingual and subcutaneous birch pollen allergen-specific immunotherapy: A randomized, placebo-controlled, double-blind, double-dummy study. *Allergy* 2004;59:45-53.
- 46 Andre C, Perrin-Fayolle M, Grosclaude M, Couturier P, Basset D, Cornillon J, *et al.* A double-blind placebo-controlled evaluation of sublingual immunotherapy with a standardized ragweed extract in patients with seasonal rhinitis. Evidence for a dose-response relationship. *Int Arch Allergy Immunol* 2003;131:111-8.
- 47 Silvestri M, Spallarosa D, Battistini E, Sabatini F, Pecora S, Parmiani S, *et al.* Changes in inflammatory and clinical parameters and in bronchial hyperreactivity asthmatic children sensitized to house dust mites following sublingual immunotherapy. *J Investig Allergol Clin Immunol* 2002;12:52-9.
- 48 Mortemousque B, Bertel F, De Casamayor J, Verin P, Colin J. House dust mite sublingual swallow immunotherapy in perennial conjunctivitis: A double blind placebo controlled study. *Clin Exp Allergy* 2003;33:464-9.
- 49 Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: Systematic review and meta analysis. *Allergy* 2005;60:4-12.
- 50 Marogna M, Spadolini I, Massolo A, Canonica GW, Passalacqua G. Randomized controlled open study of sublingual immunotherapy for respiratory allergy in real-life: Clinical efficacy and more. *Allergy* 2004;59:1205-10.
- 51 Moller C, Dreborg S, Lanner A, Bjorksten B. Oral immunotherapy of children with rhinoconjunctivitis due to birch pollen allergy. *Allergy* 1986;41:271-7.
- 52 Giovane A, Bardare M, Passalacqua G. A three year double blind placebo controlled study with oral immunotherapy to pediatric patients. *Clin Exp Allergy* 1994;24:53-9.
- 53 Miller A, Lider O, Roberts AB, Sporn MB, Weiner HL. Suppressor T cells generated by oral tolerance to myelin basic protein suppress both *in vitro* and *in vivo* immune response by release of TGF- β following antigen specific triggering. *Proc Natl Acad Sci USA* 1992;89:421-5.
- 54 Mosbech H, Dreberg S, Madsenn F. High dose grass pollen tablets used for hyposensitization in hay fever patients: A 1 year double blind placebo controlled study. *Allergy* 1987;42:451-5.
- 55 Litwin A, Flanazan M, Eritis G. Oral immunotherapy with short ragweed in a novel encapsulated preparation: A double blind study. *J Allergy Clin Immunol* 1997;100:30-8.

- 56 Tari MG, Mancino M, Monti G. Immunotherapy by inhalation of allergen in powder in house dust allergic asthma: A double blind study. *J Invest Allergol Clin Immunol* 1992;2:59-67.
- 57 Schumacher MJ, Pain MC. Intranasal immunotherapy with polymerized grass pollen allergens. *Allergy* 1982;37:241-8.
- 58 Mailing HJ. Immunotherapy as an effective tool in allergy treatment. *Allergy* 1998;53:461-72.
- 59 Abramson M, Puy R, Weiner J. Immunotherapy in asthma: An updated systematic review. *Allergy* 1999;54:1022-41.
- 60 Durham SR, Till SJ. Immunologic changes associated with allergen immunotherapy. *J Allergy Clin Immunol* 1998;102:157-64.
- 61 Wachholz PA, Soni NK, Till SJ, Durham SR. Inhibition of allergen-IgE binding to B cells by IgG antibodies after grass pollen immunotherapy. *J Allergy and Clin Immunol* 2003;112:915-22.
- 62 Zhuang Q, Bisotto S, Fixman ED, Mazer B. Suppression of IL-4- and CD40-induced B-lymphocyte activation by intravenous immunoglobulin is not mediated through the inhibitory IgG receptor FcγRIIb. *J Allergy Clin Immunol* 2002;110:480-3.
- 63 Jeannin P, Delneste Y, Tillie-Leblond I, Wallaert B, Carlier A, Pestel J, *et al.* Abnormal IgG4 antibody response to aeroallergens in allergic patients. *Int Arch Allergy Immunol* 1994;104:191-8.
- 64 Ball T, Sperr WR, Valent P, Lidholm J, Spitzauer S, Ebner C, *et al.* Induction of antibody responses to new B-cell epitopes indicates vaccination character of allergen immunotherapy. *Eur J Immunol* 1999;29:2026-36.
- 65 Durham SR, Walker SM, Varga EM, Jacobson MR, O'Brien F, Noble W, *et al.* Long-term clinical efficacy of grass pollen immunotherapy. *N Engl J Med* 1999;341:468-75.
- 66 Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy. *J Allergy Clin Immunol* 2007;119:780-9.
- 67 McHugh SM, Deighton J, Stewart AG, Lachmann PJ, Ewan PW. Bee venom immunotherapy induces a shift in cytokine response from a TH-2 to a TH-1 dominant pattern: Comparison of rush and conventional immunotherapy. *Clin Exp Allergy* 1995;25:828-30.
- 68 Akdis M, Blaser K, Akdis CA. T regulatory cells in allergy: Novel concepts in the pathogenesis, prevention, and treatment of allergic diseases. *J Allergy Clin Immunol* 2005;116:961-8.
- 69 Akdis CA, Joss A, Akdis M, Faith A, Blaser K. A molecular basis for T cell suppression by IL-10: CD28-associated IL-10 receptor inhibits CD28 tyrosine phosphorylation and phosphatidylinositol 3-kinase binding. *FASEB J* 2000;14:1666-8.
- 70 Bellinghausen I, Knop J, Saloga J. The role of interleukin 10 in the regulation of allergic immune responses. *Int Arch Allergy Immunol* 2001;126:97-101.
- 71 Moingeon P, Batard T, Fadel R, Frati F, Sieber J, Van Overtvelt L. Immune mechanisms of allergen-specific sublingual immunotherapy. *Allergy* 2006;61:151-65.
- 72 Allam JP, Niederhagen B, Bücheler M, Appel T, Betten H, Bieber T, *et al.* Comparative analysis of nasal and oral mucosa dendritic cells. *Allergy* 2006;61:166-72.
- 73 Bagnasco M, Mariani G, Passalacqua G, Motta C, Bartolomei M, Falagiani P, *et al.* Absorption and distribution kinetics of the major *Parietaria judaica* allergen (Par j 1) administered by noninjectable routes in healthy human beings. *J Allergy Clin Immunol* 1997;100:122-9.
- 74 Fernandez-Caldas E, Iraola V, Boquete M, Nieto A, Casanovas M. Mite immunotherapy. *Curr Allergy Asthma Rep* 2006;6:413-9.
- 75 Dam Petersen K, Gyrd-Hansen D, Kjaergaard S, Dahl R. Clinical and patient based evaluation of immunotherapy for grass pollen and mite allergy. *Allergol Immunopathol (Madr)* 2005;33:264-9.
- 76 Casimir G, Cuvelier P, Allard S, Duchateau J. Life-threatening fish allergy successfully treated with immunotherapy. *Pediatr Allergy Immunol* 1997;8:103-5.
- 77 Des Roches A, Paradis L, Knani J, Hejjaoui A, Dhivert H, Chanez P, *et al.* Immunotherapy with a standardized Dermatophagoides pteronyssinus extract. V. Duration of the efficacy of immunotherapy after its cessation. *Allergy* 1996;51:430-3.
- 78 Abramson MJ, Puy RM, Weiner JM. Allergen immunotherapy for asthma. *Cochrane Database Syst Rev* 2003;4:CD001186.
- 79 Frankland AW, Augustin R. Prophylaxis of summer hay-fever and asthma: A controlled trial comparing crude grass-pollen extracts with the isolated main protein component. *Lancet* 1954;266:1055-70.
- 80 Citron KM, Frankland AW, Sinclair JD. Inhalation tests of bronchial hypersensitivity in pollen asthma. *Thorax* 1958;13:229-32.
- 81 Bousquet J, Maasch H, Martinot B, Hejjaoui A, Wahl R, Michel FB. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids: II, Comparison between parameters assessing the efficacy of immunotherapy. *J Allergy Clin Immunol* 1988;82:439-46.

- 82 Dreborg S, Agrell B, Foucard T, Kjellman NI, Koivikko A, Nilsson S. A double-blind, multicenter immunotherapy trial in children, using a purified and standardized *Cladosporium herbarum* preparation. I. Clinical results. *Allergy* 1986;41:131-40.
- 83 Karlsson R, Agrell B, Dreborg S, Foucard T, Kjellman NI, Koivikko A, *et al.* A double-blind, multicenter immunotherapy trial in children, using a purified and standardized *Cladosporium herbarum* preparation: II, *In vitro* results. *Allergy* 1986;41:141-50.
- 84 Horst M, Hejjaoui A, Horst V, Michel FB, Bousquet J. Double-blind, placebo-controlled with a standardized *Alternaria* extract. *J Allergy Clin Immunol* 1990;85:460-72.
- 85 Maunsell K, Wraith DG, Hughes AM. Hyposensitisation in mite asthma. *Lancet* 1971;1:967-8.
- 86 Smith AP. Hyposensitization with *Dermatophagoides pteronyssinus* antigen: Trial in asthma induced by house dust. *Br Med J* 1971;4:204-6.
- 87 Newton DA, Maberley DJ, Wilson R. House dust mite hyposensitization. *Br J Dis Chest* 1978;72:21-8.
- 88 D'Souza MF, Pepys J, Wells ID, Tai E, Palmer F, Overell BG, *et al.* Hyposensitization with *Dermatophagoides pteronyssinus* in house dust allergy: A controlled study of clinical and immunological effects. *Clin Allergy* 1973;3:177-93.
- 89 Pauli G, Bessot JC, Bigot H. Clinical and immunologic evaluation of tyrosine-adsorbed *Dermatophagoides pteronyssinus* extract: A double-blind placebo-controlled trial. *J Allergy Clin Immunol* 1984;74:524-35.
- 90 Turner MW, Yalcin I, Soothill JF. *In vitro* investigations in asthmatic children undergoing hyposensitization with tyrosine-adsorbed *Dermatophagoides pteronyssinus* antigen. *Clin Allergy* 1984;14:221-31.
- 91 Bousquet J, Calvayrac P, Guérin B, Hejjaoui A, Dhivert H, Hewitt B, *et al.* Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract: I, *In vivo* and *in vitro* parameters after a short course of treatment. *J Allergy Clin Immunol* 1985;76:734-44.
- 92 Price JF, Warner JO, Hey EN, Turner MW, Soothill JF. A controlled trial of hyposensitization with adsorbed tyrosine *Dermatophagoides pteronyssinus* antigen in childhood asthma: *In vivo* aspects. *Clin Allergy* 1984;14:209-19.
- 93 Bousquet J, Hejjaoui A, Clauzel AM. Specific immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. II. Prediction of efficacy of immunotherapy *J Allergy Clin Immunol* 1988;82:971-7.
- 94 Taylor WW, Ohman JL Jr, Lowell FC. Immunotherapy in cat-induced asthma. Double-blind trial with evaluation of bronchial responses to cat allergen and histamine. *J Allergy Clin Immunol* 1978;61:283-7.
- 95 Ohman JL Jr, Findlay SR, Leitermann KM. Immunotherapy in cat-induced asthma. Double-blind trial with evaluation of *in vivo* and *in vitro* responses. *J Allergy Clin Immunol* 1984;74:230-9.
- 96 Van Metre TE Jr, Marsh DG, Adkinson NF Jr, Kagey-Sobotka A, Khattignavong A, Norman PS Jr, *et al.* Immunotherapy for cat asthma. *J Allergy Clin Immunol* 1988;82:1055-68.
- 97 Van Metre TE Jr, Marsh DG, Adkinson NF Jr, Kagey-Sobotka A, Khattignavong A, Norman PS Jr, *et al.* Immunotherapy decreases skin sensitivity to cat extract. *J Allergy Clin Immunol* 1989;83:888-99.
- 98 Sundin B, Lilja G, Graff-Lonnevig V, Hedlin G, Heilborn H, Norrlind K, *et al.* Immunotherapy with partially purified and standardized animal dander extracts: I, Clinical results from a double-blind study on patients with animal dander asthma. *J Allergy Clin Immunol* 1986;77:478-87.
- 99 Hedlin G, Graff-Lonnevig V, Heilborn H, Lilja G, Norrlind K, Pegelow K, *et al.* Immunotherapy with cat- and dog-dander extracts: II: *In vivo* and *in vitro* immunologic effects observed in a 1-year double-blind placebo study. *J Allergy Clin Immunol* 1986;77:488-96.
- 100 Valovirta E, Koivikko A, Vanto T, Viander M, Ingeman L. Immunotherapy in allergy to dog: A double-blind clinical study. *Ann Allergy* 1984;53:85-8.
- 101 Valovirta E, Viander M, Koivikko A, Vanto T, Ingeman L. Immunotherapy in allergy to dog. Immunologic and clinical findings of a double-blind study. *Ann Allergy* 1986;57:173-9.
- 102 Bousquet J, Hejjaoui A, Soussana M, Michel FB. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids: IV, Comparison of the safety and efficacy of two dosages of a high-molecular-weight allergoid. *J Allergy Clin Immunol* 1990;85:490-7.
- 103 Gaddie J, Skinner C, Palmer KN. Hyposensitisation with house dust mite vaccine in bronchial asthma. *Br Med J* 1976;2:561-2.
- 104 Gaur SN, Gupta S. Clinical response of immunotherapy in cases of nasobronchial allergy. *Indian J Allergy Appl Immunol* 1996;10:65-8.
- 105 Karmakar PR, Das A, Chatterjee BP. Placebo-controlled immunotherapy with *Cocos nucifera* pollen extract. *Int Arch Allergy Immunol* 1994;103:194-201.
- 106 Chowdhury I, Chatterjee B. The immunological and clinical effects of immunotherapy in patients suffering from house dust allergy. *Ann Agric Environ Med* 1999;6:91-7.
- 107 Joshi SV, Tripathi DM, Dhar HL. Allergen specific immunotherapy in nasobronchial allergy. *Indian J Med Sci* 2003;12:527-34.

- 108 Pawar SS. Sensitivity to *Sorghum vulgare* (Jowar) pollens in allergic bronchial asthma and effect of allergen specific immunotherapy. Indian J Allergy Asthma Immunol 2002;16:41-5.
- 109 Srivastava D, Singh BP, Sudha VT, Arora N, Gaur SN. Immunotherapy with mosquito (*Culex quinquefasciatus*) extract: A double-blind, placebo-controlled study. Ann Allergy Asthma Immunol 2007;99:273-80.
- 110 Srivastava D, Singh BP, Arora N, Gaur SN. Clinico-immunologic study on immunotherapy with mixed and single insect allergens. J Clin Immunol 2009;29:665-73.
- 111 Guo YJ, Wu D, Wang KY, Sun SH. Adjuvant effects of *Bacillus Calmette-Guerin* DNA or CpG-oligonucleotide in the immune response to *Taenia solium* cysticercosis vaccine in porcine. Scand J Immunol 2007;66:619-27.
- 112 Stelmach I, Smejda K, Jerzynska J, Stelmach W, Majak P, Stelmach P, *et al.* Decreased markers of atopy in children with presumed early exposure to allergens, unhygienic conditions, and infections. Ann Allergy Asthma Immunol 2007;99:170-7.
- 113 Holt PG. A potential vaccine strategy for asthma and allied atopic diseases during early childhood. Lancet 1994;344:456-8.
- 114 Shankar J, Singh BP, Gaur SN, Arora N. Recombinant glutathione-S-transferase, a major allergen from *Alternaria alternata* for clinical use in allergy patients. Mol Immunol 2006;43:1927-32.
- 115 Tresch S, Holzmann D, Baumann S, Blaser K, Wüthrich B, Cramer R, *et al.* *In vitro* and *in vivo* allergenicity of recombinant Bet v 1 compared to the reactivity of natural birch pollen extract. Clin Exp Allergy 2003;33:1153-8.
- 116 Jutel M, Jaeger L, Suck R, Meyer H, Fiebig H, Cromwell O. Allergen-specific immunotherapy with recombinant grass pollen allergens. J Allergy Clin Immunol 2005;116:608-13.
- 117 Pauli G, Larsen TH, Rak S, Horak F, Pastorello E, Valenta R, *et al.* Efficacy of recombinant birch pollen vaccine for the treatment of birch-allergic rhinoconjunctivitis. J Allergy Clin Immunol 2008;122:951-60.
- 118 Niederberger V, Horak F, Vrtala S, Spitzauer S, Krauth MT, Valent P, *et al.* Vaccination with genetically engineered allergens prevents progression of allergic disease. Proc Natl Acad Sci U S A 2004;101:14677-82.
- 119 Haselden BM, Kay AB, Larche M. Peptide mediated immune response in specific immunotherapy. Int Arch Allergy Immunol 2000;122:229-37.
- 120 Von Garnier C, Astori M, Kettner A, Dufour N, Heusser C, Corradin G, *et al.* Allergen-derived long peptide immunotherapy down-regulates specific IgE response and protects from anaphylaxis. Eur J Immunol 2000;30:1638-45.
- 121 Larche M. Peptide immunotherapy for allergic diseases. Allergy 2007;62:325-31.
- 122 Pene J, Desroches A, Paradis L, Lebel B, Farce M, Nicodemus CF, *et al.* Immunotherapy with Fel d 1 peptides decreases IL-4 release by peripheral blood T cells of patients allergic to cats. J Allergy Clin Immunol 1998;102:571-8.
- 123 Muller U, Akdis CA, Fricker M, Akdis M, Blesken T, Bettens F, *et al.* Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. J Allergy Clin Immunol 1998;101:747-54.
- 124 Oldfield W, Larche M, Kay A. Effect of T cell peptides derived from Fel d1 on allergic reactions and cytokine production in patients sensitive to cats: A randomized controlled trial. Lancet 2002;360:47-53.
- 125 Maguire P, Nicodemus C, Aaronson D, Robinson D, Umetsu DT. The safety and efficacy of ALLERVAX CAT in cat allergic patients. Clin Immunol 1999;93:222-31.
- 126 Krieg AM, Wagner H. Causing a commotion in the blood: Immunotherapy progresses from bacteria to bacterial DNA. Immunol Today 2000;21:521-6.
- 127 Zuany-Amorim C, Hastewell J, Walker C. Toll-like receptors as potential therapeutic targets for multiple diseases. Nat Rev Drug Discov 2002;1:797-807.
- 128 Creticos PS, Schroeder JT, Hamilton RG, Balcer-Whaley SL, Khattignavong AP, Lindblad R, *et al.* Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. N Engl J Med 2006;355:1445-55.
- 129 Shirota H, Sano K, Kikuchi T, Tamura G, Shirato K. Regulation of murine airway eosinophilia and TH2 cells by antigen-conjugated CpG oligodeoxynucleotides as a novel antigen-specific immunomodulator. J Immunol 2000;164:5575-82.
- 130 Gaur SN, Singh BP, Vijayan VK, Agarwal MK. Guidelines for practice of allergen immunotherapy in India. Indian J Allergy Asthma Immunol 2009;23:1-21.
- 131 Gaur SN. Allergen immunotherapy: The scientific facts. Indian J Chest Dis Allied Sci 2011;53:205-6.

Table 1: Immunotherapy protocol

Antigen dilution	Volume of antigen	Dosing
1:5000 w/v	0.1 cc → 0.2 cc → → → 0.9 cc	Twice a week
1:500 w/v	0.1 cc → 0.2 cc → → → 0.9 cc	Twice a week
1:50 w/v	0.1 cc → 0.2 cc → → → 0.5 cc → 0.5 cc	Once a week
1:50 w/v	0.6 cc	Once in 2 weeks
1:50 w/v	0.7 cc	Once in 3 weeks
1:50 w/v	0.8 cc	Once in a month
1:50 w/v	0.9 cc	Once in a month
1:50 w/v	1.0 cc	Once in a month