REVIEW ARTICLE
Iran J Allergy Asthma Immunol
March 2011; 10(1): 1-9.

A Review of Allergy and Allergen Specific Immunotherapy

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Received: 28 October 2010; Received in revised form: 13 December 2010; Accepted: 20 December 2010

ABSTRACT

Since 20th century, when allergy was defined, an ongoing attempt for discovering the mechanisms underlying it and its treatment began. Defining allergens as well as cells such as regulatory T-cells and characterizing the antibodies involved in the pathogenesis (including blocking antibodies) have helped very much towards a better understanding of the immunologic process.

However, Allergen specific immunotherapy (SIT), as a specific curative treatment for allergy also dates back to the beginning of the previous century and has progressed considerably during these years. SIT similar to natural immunomodulation, directs the immune response towards tolerance.

New strategies in this field, such as using recombinant allergens, T- and B-cell-epitope-containing peptides, and DNA vaccination have shown promising results. Sublingual immunotherapy, although not yet FDA-approved, as an alternative strategy in SIT has demonstrated efficacy as well as safety.

Furthermore, allergen extracts, their standardization and their modification have also been the focus of much research. Undoubtedly, specific immunotherapy is proven to be an efficacious method to treat allergy, so its cost-effectiveness should be estimated in developing countries in order to include it in the country's health priorities. Informing physicians about the new anti-vaccination movement is also crucial.

Key words: Allergens; Allergen Immunotherapy; Allergy; Blocking Antibodies; T Cells

INTRODUCTION

The term allergy was first used by von Pirquet in the year 1906 to describe harmful immune responses for the host. The word comes from the Greek word “alol”, meaning, “change in the original state”.

The first report on specific immunotherapy goes back to the beginning of the 20th century (1911), when Noon injected an extract of grass pollen into a person whose allergic symptoms coincided with the pollination of grass.[1] At that time, it was believed that pollens contain undefined toxins. Ten years later, allergen-specific sensitivity was passively transferred by a serum factor[1] and it took several years until IgE and

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then IgG blocking antibodies were discovered. Today, by expansion of allergy epidemic all around the world, immunotherapy is widely used and the mechanisms underlying allergy and immunotherapy are extensively investigated. New developments in this field and the emergence of Regulatory T-cells (Treg cells) and T helper 17 cells (T_H17 cells) however have enlightened the concepts in allergy and hypersensitivity and filled the gap in the understanding of inflammatory processes.[2] Furthermore, the implementation of good clinical practice (GCP) in the conduct of clinical trials on medicinal products for human use introduced by the Directive 2001/20/EC[3] improved the evidence-based medicine and the researches in the field of immunotherapy.

What Is an Allergen?

Allergens are proteins in most cases and have two characteristics; they induce IgE responses in sensitization phase and a clinical response on subsequent exposures (Figure 1). Despite the wide investigations on allergens, their structures and their sequences, researches on their allergenicity still are ongoing [4].

Physiopathology of Allergic Immune Responses

Allergic immune response consists of sensitization and development of specific immune response toward the allergen. During sensitization, the following events occur: priming of allergen-specific CD4+ T_H2 cells, production of T_H2 cytokines (IL-4 and -13), and thus class switching for IgE production, activation of endothelial cells, eosinophil migration to tissues, mast cell and basophile degranulation. Subsequent and frequent exposures lead to changes in target organs and remodeling.[5]

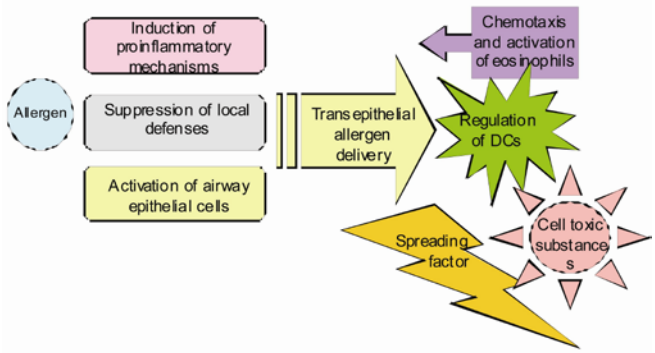

Figure 1. Features of allergens contributing to allergenicity

T-cell Phenotypes in Allergic Response

T cell is known to be the principle conductor of the allergic orchestra. The T-helper cell response is mainly the T_H2 type which is mostly associated with the allergic response. However, newly discovered cells also contribute to the pathogenesis. In fact, the ratio of allergen-specific IL-10-secreting cells (Treg cells) to IFNγ-secreting cells (T_H1 cells) and IL-4-secreting cells (T_H2 cells) and their continuous balance at the onset or during the course of response determines the outcome to be normal or develop allergic immune response.[6]

In atopic disorders, T_H2 cells produce IL-4, IL-5 and IL-13[7] which result in allergen-specific IgE production by B cells, eosinophil activation and recruitment, mucous production, bronchial hyperreactivity and allergic tissue homing of T_H2 cells. T_H1 subset might lead to chronicity and effector phase in allergic diseases. On the contrary, Treg cells inhibit the development of T_H1 and T_H2 cell responses.[6] Treg cells act by engaging inhibitory cell surface molecules, producing inhibitory cytokines, as well as cytolysis and disrupting metabolism.[8]

Up to now, published data regarding T_H17 cells favor their role as proinflammatory by inducing proinflammatory cytokines (TNF-α, IL-1β, and IL-6) and chemokines (CXCL1, 2 and 8), and neutrophil recruitment.[2] IL-17 has also been indicated as a marker for allergy severity.[9]

Specific Immunotherapy (SIT)

Allergen-specific Immunotherapy which is also called allergen immunotherapy, hyposensitization therapy or desensitization, involves administration of increasing concentrations of antigen-specific extracts to allergic patients with the goal of inducing a state of immunologic tolerance.[10] The aim is to alleviate symptoms during exposure to the allergen. It is an FDA-approved, clinically effective method and induces long-term remission of allergic rhinitis and allergic asthma, with improvement in clinical symptoms.[11-13] Successful immunotherapy results not only in the increase of allergen concentration necessary to induce immediate or late-phase reactions, but also in the decreased responses to nonspecific stimulation.[14] Therefore, in contrast to symptomatic treatment, it can reduce the likelihood of developing additional sensitizations by interrupting the so-called “atopic march” and patients may benefit from persistence of

alleviation of clinical symptoms.[11,14-16] However, only less than 5% of patients choose immunotherapy as a treatment option for their allergy.[16] As allergen immunotherapy reaches its 100th anniversary, attempts for safer methods and more convenient procedures continue.[14]

Natural Immunomodulation

Naturally occurring immunomodulation can induce humoral and cellular features that we try to imitate in SIT, as in the case of beekeepers. Beekeepers' lymphocytes do not proliferate in response to allergens and do not produce IL-2.[17] Heavily stung beekeepers have specific IgE as well as positive prick test for bee venome, but their partial tolerance is due to the existence of bee venom specific IgG and mainly IgG4[18] and allergen-specific IL-10-secreting T cells.[19,20] The level of specific IgG_4 primarily reflects exposure and correlates with the number of annual stings and years spent in bee-keeping.[6] It should be kept in mind that the beekeepers are at risk of bee-venom allergy and anaphylaxis if they have fewer than 10 annual stings and high serum-specific IgE and low serum-specific IgG. On the other hand they can better tolerate immunotherapy.[18]

As another example, exposure to large concentrations of animal dander in children also induces IL-10 and IgG4 production and protection from allergy.[21] This IgG can compete with IgE for allergen binding and can reduce IgE-mediated degranulation of mast cells and basophils.[1]

Recent studies imply the possibility that maternal farm exposure and consumption of farm milk can increase microbial exposure which might lead to a form of natural immunotherapy. In this setting, the environmental factors redirect the immune response to aeroallergens from Th2- to Treg-responses.[22]

Sequential Events in SIT

In allergen-specific immunotherapy, very early effects are attributed to mast cell and basophile desensitization (very early desensitization effect). Intermediate effects are due to changes in allergen-specific T cells (generation of Treg cells and peripheral T cell tolerance), and late effects are related to B cells and IgE (modulation of allergen-specific IgE and IgG subtype responses) and also mast cells, basophils, and eosinophils (suppression of effector cells and inflammatory responses).[5]

Mechanisms of SIT

Although due to different methodologies in different studies, the exact mechanisms of SIT have not been fully described, some features seem to be in common. These include: change of antigen presenting cell (APC) function, T cell responses, immunoglobulin responses and response of other cells.[1]

APCs in SIT. APCs and especially dendritic cells (DCs) initiate the immune response by delivering the environmental signals to other cells. Their lineage and their own maturation status can influence the ultimate result to be peripheral tolerance or immunity. While performing SIT, since we do not have pro-inflammatory signals and innate immunity is not triggered, DCs show partially mature phenotype, thus they have tolerogenic interaction with lymph node T cells. As a result, IL-10 secreting Treg cells develop.[23,24]

T-cell responses in SIT. Having a small number of Treg cells and a large number of T_H2 cells results in an allergic response. It has been shown that SIT can restore the activity of allergen-specific IL-10-secreting Treg cells.[5,20,25-27]

SIT affects T-cell responses to allergen by employing several mechanisms, including the following: by increasing the allergen-induced ratio of T_H1 cytokines to T_H2 cytokines, by inducing epitope-specific T-cell anergy that can be blocked by neutralization of IL-10, by generating allergen-specific Treg cells that can suppress the responses of effector T cells and by increasing the production of cytokines with regulatory activity.[1]

One study based on mathematical modeling of allergy and specific immunotherapy has suggested that the crucial event in immunotherapy is proliferation of Treg cells and suppression of Th2 cells. High-dose injections with short intervals can augment these effects.[28]

Immunoglobulin responses in SIT. IgG blocking antibodies compete with IgE for allergen binding.[5,10,20] Their functional activity rather than the absolute amount determines the outcome. Allergen-specific IgG might be directed against the same epitopes as allergen-specific IgE (blocking the effect of IgE), against different epitopes (no effect) and in some cases it can amplify cross-linking of allergen-IgE-FcεRI complexes (increasing effector function and adverse effects).[29]

SIT-induced IgG in addition to competing with IgE and preventing degranulation of mast cells and

basophils, can inhibit IgE facilitated allergen presentation to T cells, thus decreasing late-phase reactions, and can reduce the number of allergen-specific memory B cells by preventing activation signals for affinity maturation, memory induction and differentiation.[1,30] In addition to the above-mentioned mechanisms, IgG can also deliver a negative inhibitory signal to mast cells via FcγRIIB.[31] However, it has recently been shown that IgG induction alone is not sufficient to desensitize the patients.[32]

SIT induces ten to hundred fold increase in IgG1 and -4 and a modest increase in IgG2. It has been observed that IgG4 exerts inhibitory effects on binding of IgE- FcεRII complexes on B cells[33] and this might be due to its unique structural characteristics of its hinge region and its ability to separate and re-pair (forming monomeric antibodies)[6,34] and also the fact that it does not fix complement. Furthermore, IL-10 generated during SIT not only elicits anergy in T cells but also decreases IgE: IgG4 ratio in peripheral blood.[20] Interestingly, IFNγ suppresses IgG1 production while increases IgG2. Recently, it has been shown that allergen specific IgA in response to TGFβ increases during SIT.[1]

Changes in other cells. Allergen-specific immunotherapy can reduce the number of tissue mast cells as well as eosinophils,[26,30] while decreasing the mediator release in mast cells, basophils and eosinophils. Release of proinflammatory cytokines also decreases during the course of SIT. IL-10 is the cytokine responsible for downregulation of eosinophil activity, causing reduced proinflammatory cytokine release, inhibition of GM-CSF production and CD-40 expression, ultimately eosinophil cell death.[26]

New Strategies in SIT

To standardize SIT, many strategies have been employed and these attempts would try to maximize the safety as well as efficacy (Table 1).

Allergens

Recombinant Allergens and Allergen Derivatives

Using recombinant/engineered allergens, possibly modified by site-directed mutagenesis, represents an exciting alternative approach which is directed at maintaining the immunogenicity of a vaccine while reducing the capacity to bind allergen-specific IgE.[11,35] The results related to their use, hold promise that recombinant allergen–based immunotherapy will improve current immunotherapy practice and may open possibilities for prophylactic vaccination,[36] although no clinical efficacy has been documented yet. Side effects occurring in this type of immunotherapy highlight the necessity of determining maximum tolerated dose in these molecules which are thought to be hypoallergenic.[32]

T-cell-epitope Containing Peptide Approaches

Peptide fragments corresponding to T cell epitopes, have been used in experimental models of allergy and autoimmunity. Their secondary and tertiary structures and their sizes, help them to lessen cross-linking of IgE. Adverse events occur as a result of residual IgE reactivity in larger peptides and activation of allergen-specific affector T cells at high peptide doses. Therapeutic effects are caused by induction of allergen-specific Treg cells.[37] These vaccines can also be taken orally to induce mucosal immunization as a replacement for systemic inoculation.[38]

B-cell-epitope Containing Approaches

Synthetic peptides with the potential to induce the production of blocking IgG have been defined from IgE-binding epitope-mapping data and from analysis of the three-dimensional structure of an allergen. Vaccination of animals with such peptides has shown success.[1]

Table 1. Alternative strategies in Allergen Extract Immunotherapy

Allergens	Delivery routes	Extracts
- Adjuvants and carriers	- Epicutaneous/ transcutaneous	- Recombinant allergens
- Allergoids/polymerized extracts	- Intralymphatic	- Frangments or folding variants of recombinant allergens
- Immunostimulatory sequences	- Subcutaneous	- T-cell-epitope containing peptides
- Fusion proteins	- Local nasal	- B-cell-epitope containing peptides
	- Oral	- DNA vaccines
	- Sublingual-swallow	
	- Sublingual-spit	

DNA Vaccination

New developments in this field include plasmid injection, oral delivery of genes using chitosan-DNA nanoparticles and replicon-based DNA vaccines (targeting DCs). Allergen-encoding DNA induces allergen-specific T_H1-cell response but has the potential to cause anaphylaxis because of systemic production of allergen by transfected cells.[1]

Delivery Routes

Epicutaneous/Transcutaneous Immunotherapy

Transcutaneous immunization (TCI) or epicutaneous immunotherapy (EPIT) refers to the application of vaccines to the skin. By using microneedle or needle-free patches, antigens along with adjuvants are delivered to the potent APCs of the skin and also allergen specific IgE on Langerhans cells. These antigens are subsequently delivered to T cells. Keratinocytes also play an active role by creating an inflammatory environment favoring the induction of allergy-protective immune responses. This method, with shorter duration and lasting effects, so far has found to be efficacious and safe. Extensive clinical trials are needed to support the preliminary findings and optimize the delivery systems and adjuvants used.[16]

Intralymphatic Immunotherapy

In an attempt to decrease the frequency of allergen administration, Senti et al, have used the intralymphatic approach for allergen immunotherapy. In a randomized (not blinded) controlled trail, allergens were delivered via superficial inguinal lymph nodes under ultrasound guidance. The method was proven to be safe and effective but future investigations are needed to approve the method.[13,16,39,40]

Non-injection Immunotherapy

In order to increase the safety of immunotherapy and provide an easier administration, non-injection routes such as nasal, oral, sublingual-swallow and sublingual-spit techniques were developed. In nasal immunotherapy, an aqueous solution or powder in capsules (which are to be broken later), are sprayed into the nose through an appropriate devise to avoid inhalation into the deep airways. Thus, by introduction of sublingual immunotherapy, the use of nasal immunotherapy is declining.[41]

In oral immunotherapy (SLIT), which is shown to be effective and relatively safe,[42] antigens in aqueous solutions, tablets or gastroresistant capsules are swallowed. In sublingual swallow technique antigen is held for 1 to 2 minutes under the tongue and then is swallowed, while in sublingual spit technique the antigen is spit out after 1 to 2 minutes under the tongue.[43]

Sublingual pharmacokinetics of non-injection routes do not seem to differ between allergic and healthy individuals or between modified and native allergens. The rapid binding of allergen to oral mucosa is the reason why sublingual-swallow and –spit technique show no significant differences in local pharmacokinetics.[43] The differences in sublingual and subcutaneous methods may be due to differences between oral APCs and Langerhans cells and their skin counterparts.[44] However; similar to subcutaneous immunotherapy, sublingual method in allergic subjects causes a reduction in proliferative responses of T cells, their cytokine production, in ICAM-1 expression on epithelial cells, the number of eosinophils and neutrophils, induction of systemic Treg cells, an increase in IL-10 production, and decreased bronchial reactivity to methacoline. It also causes antigen-specific IgE decrease, early rise in IgG1, late increases in IgG4. The majority of publications comparing active sublingual immunotherapy with placebo or controls showed efficacy of this method for rhinitis, conjunctivitis, and/or asthma. Contrary to subcutaneous immunotherapy that has a high potential for severe reactions, sublingual route has minimal risk.[22,13,45] To date, no severe or life-threatening adverse events have been reported and the majority of events (asthma exacerbation, rhinoconjunctivitis, oral cavity pruritis, throat irritation, rhinitis, itchy eyes, nausea, and GI complaints) have been mild and self-resolving requiring symptomatic medications or dose-adjustments.[43] The safety of this technique will likely provide increased opportunities for higher risk patients, such as asthmatic persons and children under the age of 5.[43,44] SLIT can be used co-seasonally, pre-seasonally or continuously.[13] Furthermore, the effectiveness of this method in allergic rhinitis and asthma and also for prevention of new sensitizations is noteworthy[46] (Table 2).

Allergen Extracts for Immunotherapy

Allergen extracts are used for diagnosis and specific immunotherapy of allergies. These extracts contain allergenic and non-allergenic constituents.

Table 2. Comparison of subcutaneous and sublingual immunotherapy

Topics	Sublingual immunotherapy	Subcutaneous immunotherapy
Administration	Oral	Injection
APCs involved	Oral APCs and Langerhans Cells	Skin APCs and Langerhans Cells
Efficacy	++++	++++
Safety	++++	+++
Severe adverse reactions	-	+

APC: Antigen Presenting Cell

The concentration of allergens is influenced by biovariability, different production processes and genetic diversity of the affected patients.[47] The route of immunotherapy also affects antigen preparation. For example, in sublingual immunotherapy, antigen is first exposed to oral mucosa and due to limited absorption of allergens from mucosal surfaces, high-dose regimens likely facilitate capture of allergens by sentinel dendritic cells, which represents a critical step to induce adequate and long-lasting T cell responses.[44] Thus, the need for standardization of these extracts in order to avoid large differences among different manufacturers and to ensure a consistent composition and potency are essential and are at most importance,[30,44,47]

In recent years, for each medicinal product, including allergen products, quality, safety and efficacy have to be proven by the manufacturer to obtain a marketing authorization (MA).[48] Allergen products according to Directive 2001/83/EC refer to "any medicinal products, administered to human beings, which are intended to identify or induce a specific acquired alteration in the immunological response to an allergizing Agent". A number of guidance documents exist that concentrate on specific aspects of allergen products such as "the Note for Guidance on Allergen Products" and "the Monograph on Allergen Products of the European Pharmacopoeia".[48]

Alternatives for Currently Available Extracts

Adjuvants and Carriers

Use of adjuvants such as alum and more recently L-tyrosine reduce the rate of dissemination from the injection site and reduce systemic reactions. Another approach is to provide T_H1 adjuvant effects as with monophosphoryl lipid A.[49] The use of liposomes to encapsulate allergens for SCIT has been proposed and in one study showed clinical efficacy compared with traditional vaccines as well as a good safety profile.[11] Mycobacterium adjuvants have also been shown to inhibit the development of allergic responses in animals.[50] Carrier Molecules combined to allergens such as virus-like particles are under investigation for the design of new vaccines. These highly-immunogenic molecules are fused to allergens with low or no allergenicity. This technology might be applied to any allergen with supposedly long-lasting effects.[32]

Allergoids/Polymerized Extracts

These approaches can reduce allergenicity of the extract while retaining its immunogenicity.

Immunostimulatory Sequences

Synthetic cytosine phosphorothionate guanosine DNA chains, when covalently linked to allergens, have the advantage of being recognized by toll-like receptor 9 and directing the response toward a regulatory and T_H1 response[9,45] and also of sterically interfering with the reaction of IgE with the allergen, thus reducing its allergenicity.

TH1 responses to allergens can be augmented by direct linkage of immunostimulatory sequence of DNA (ISS) to the protein. Favorable results have been obtained by this method.[45]

Fusion Proteins

Mast cells and basophils express FcγRIIb, which contains an immunoreceptor tyrosine-based inhibition motif within its cytoplasmic tail. Aggregating FcγRIIb to the major IgE receptor, FcεRI, leads to inhibition of FcεRI signaling.[14]

CONCLUSION

Specific Immunotherapy for respiratory allergy and severe allergic reactions is used for about one century and there is now solid documentation of its efficacy and safety.[30,51]

Subcutaneous immunotherapy is generally available in Europe, the United States and in most countries.

Many extracts are standardized either biologically or immunologically. The clinical efficacy of SCIT is now well established[11] and regarding safety, though systemic reactions are not frequent, careful administration is recommended.[11,52]

Sublingual immunotherapy is currently marketed in several European countries and is also available in other countries (eg, Argentina, Brazil, the Gulf States, and South Africa). Extracts may be standardized either biologically or immunologically. Efficacy has been shown in several studies. It was well tolerated with local and self-limiting side effects. However, there still remains some questions to be answered regarding SLIT.

Despite the wide administration of allergen specific immunotherapy in developed countries, its use in developing countries such as Iran is still limited. Few numbers of specialists in the field of allergology and poor definition of allergens in these countries partly explain this limitation. Because of limited resources, at first it was believed that SIT should be contraindicated in developing countries, but it has been now proposed that its use may lead to economic savings.[11] So it is recommended that each country should evaluate the cost-effectiveness of SIT and especially SLIT in its health care system and one rule cannot be applied in general.[11] Besides the economic considerations, cultural level is also a determining factor for the decision to perform SIT and there is evident need for educational programs in this regard.[53] The globally growing anti-vaccination movement is also discouraging patients from accepting SIT and certainly informing physicians about these associations will help overcome this problem.[54] Developing biomarkers that determine patients' responsiveness to SIT and also showing clinical effectiveness of this modality, would also be beneficial.[55]

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