

Acknowledgements

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Bypassing IgE and targeting T cells for specific immunotherapy of allergy

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Specific immunotherapy (SIT) is a common treatment for allergic diseases. Despite its usage in clinical practice for nearly a century, more-rational and safer allergen preparations are required. Here, the underlying mechanisms and principles of allergen modification for the future use of SIT in the treatment of allergy are discussed.

IgE-mediated allergy is an immune disorder affecting almost one-third of the population in some countries. Although immediate and late symptoms can be ameliorated by pharmacological treatment, specific immunotherapy (SIT) is the only curative approach against this type of allergy. However, some major problems related to this approach remain to be solved. Currently, SIT is performed with allergen extracts that usually

contain insufficiently characterized allergen mixtures that are not designed for an individual patient's allergen profile and that contain nonallergenic or unwanted toxic proteins. Most importantly, administration of native allergens can cause severe, often life-threatening, anaphylactic reactions. Therefore, the optimally efficient high dose of allergen required for successful SIT can often not be reached. Recent knowledge of the immunological mechanisms underlying SIT should allow such problems to be overcome in the future^{1–3}.

Mechanisms of antigen focusing

The first essential steps in SIT involve the specific recognition of allergen by T cells and the induction of peripheral anergy^{1–3}. Therefore, a basic requirement in

achieving successful SIT without risk of anaphylaxis is for the allergen to express T-cell epitopes that induce T-cell tolerance and lack antibody-binding sites that would otherwise facilitate IgE-mediated allergic responses. An intact three-dimensional structure of an allergen is pivotal in the development of distinct immune response profiles by the preferential usage of particular antigen-presenting cells (APCs) (Fig. 1). Whereas specific B cells most efficiently present conformational allergens at low concentrations, APCs that utilize phagocytosis or pinocytosis for antigen uptake, such as monocytes, macrophages and/or dendritic cells, internalize allergen molecules independently of their structural features^{4,5}. If B cells present low concentrations of antigen to T cells, high interleukin 4 (IL-4) but very little or

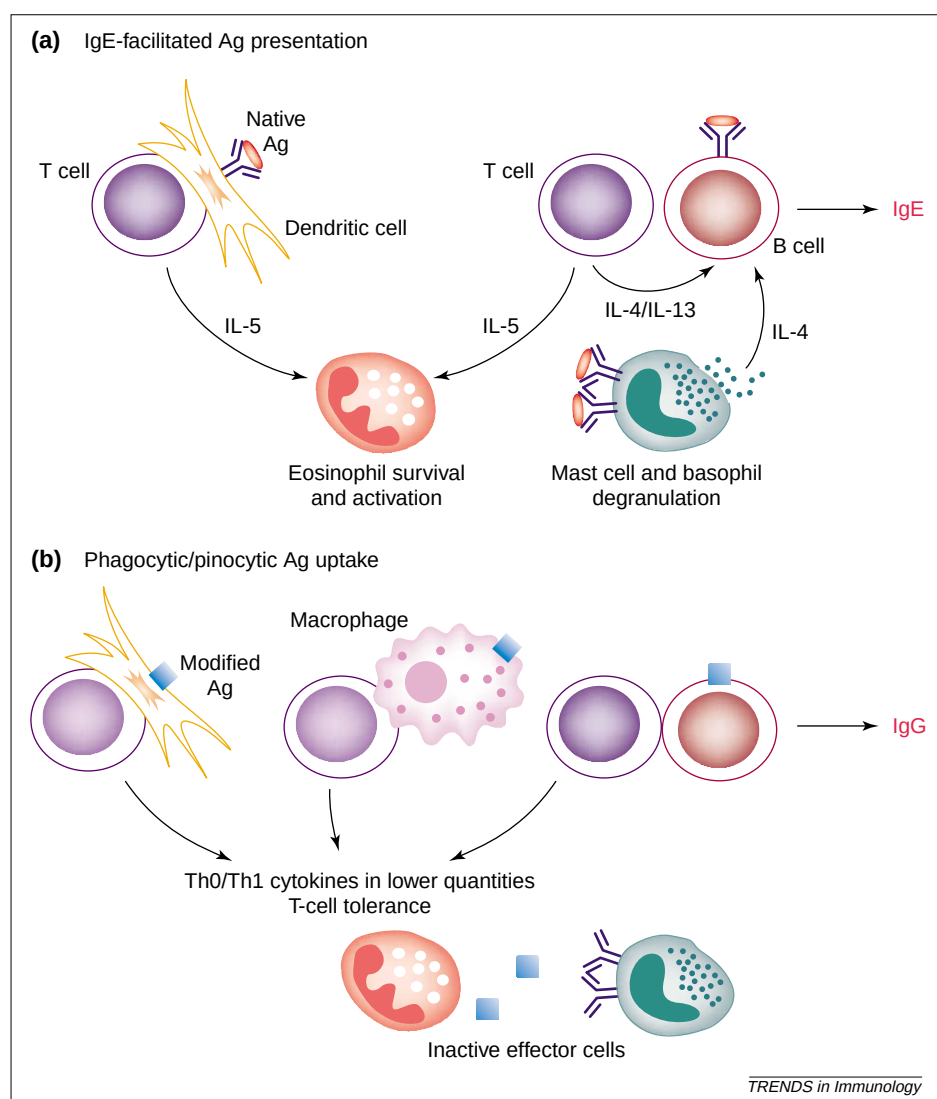


Fig. 1. Native and modified allergens are presented differently and result in different immune responses. (a) Native allergens utilize IgE-facilitated antigen presentation in dendritic cells and B cells, and activate T cells to produce Th2-type cytokines (IL-4, IL-5, IL-13), which aids eosinophil survival. IgE-facilitated presentation also results in the activation of B cells to produce further IgE, a process that is helped by Th2-type cytokines produced by T cells and degranulating mast cells and basophils. Degranulation is initiated on mast cells and basophils by native allergen crosslinking IgE. (b) By contrast, modified allergens are not bound by IgE and do not initiate effector cell degranulation. They utilize phagocytic or pinocytic antigen-uptake mechanisms in dendritic cells, macrophages and B cells. T cells are induced to generate a balanced Th0/Th1-type cytokine pattern in lower quantities as well as T-cell tolerance, resulting in lower IgE and higher IgG production. Abbreviations: Ag, antigen; IL, interleukin; Th, T helper.

no interferon γ (IFN- γ) is produced (Fig. 1a). By contrast, the usage of monocytes and macrophages and high antigen doses results in high IFN- γ but low IL-4 synthesis (Fig. 1b)^{4,5}.

IgE bound to Fc ϵ receptors (Fc ϵ RI and Fc ϵ RII/CD23) on various cells facilitates antigen presentation to T cells⁶. This mechanism allows the presentation of extremely low doses of allergen to CD4⁺ T cells. Following SIT of birch pollen allergy, blocking IgG antibodies were shown to inhibit IgE-facilitated antigen presentation at very low allergen concentrations⁷. In addition, capture of antigen via surface IgE and signal

transduction through the IgE cytoplasmic chain were shown as crucial events in specific IgE synthesis by B cells⁸. Furthermore, studies with neutralizing anti-IgE and anti-CD23 monoclonal antibodies (mAbs) or CD23-deficient mice have suggested that IgE-mediated antigen presentation plays a major role in T helper 2 (Th2)-type cytokine production and lung eosinophilia⁹. Together, these data indicate that IgE also acts as an immunoglobulin that is specialized in antigen capture and focusing, promoting Th2 responses and the further synthesis of IgE. However, it has to be kept in mind that during conventional SIT, IgE-facilitated antigen presentation

might be partially bypassed because of high doses of allergen administration.

Allergen modification strategies for future SIT

Epitopes of allergens recognized by T cells are different to those bound by free and surface-bound immunoglobulins of B cells. T-cell epitopes represent linear peptide sequences (i.e. linear epitopes), whereas B cells recognize allergens in their intact three-dimensional structure (i.e. conformational epitopes). The presence of conformational B-cell epitopes on an allergen provides various possibilities to reduce their IgE-binding properties. It can be expected that the hypoallergenic nature of allergens with altered B-cell epitopes will allow much higher allergen doses containing T-cell epitopes to be administered to allergic patients. This might permit SIT with fewer injections, favoring allergen-specific tolerance in Th2 cells and the development of allergen-specific Th0/Th1 immune response associated with blocking antibodies. Accordingly, several avenues of investigation into the modification of allergens are underway (Box 1). For instance, allergen variants with hypoallergenic activity following chemical modification are already in clinical practice. In addition, modification of allergens by genetic engineering shows great promise and clearly represents the future of SIT. Finally, some clinical studies have been performed on the usage of T-cell epitope peptides that fully lack IgE binding^{10,11}.

Chemical modification

To overcome the principal problems of SIT, earlier studies were mainly based on the chemical modification of allergens. Historically, the first attempts were performed in order to impede the release of allergen from the injection site. Such means of chemical modification included mineral oil and alum precipitation, urea denaturation, and conjugation of antigen either with polyethylene glycol or with poly D-glutamic acid:D-lysine.

In several studies, allergens were modified by means of different aldehydes¹². Formaldehyde- and glutaraldehyde-treated allergen extracts termed 'allergoids' display low IgE-binding capacity, and are better tolerated than the native allergen extract¹². Allergoids are hypoallergenic, but IgE epitopes are not fully destroyed

Box 1. Strategies for allergen modification

- Chemical modification of allergens
- Targeting disulfide bonds in allergens to change their conformation (and therefore B-cell epitopes)
- Mutagenesis or deletion of B-cell epitopes
- Fragmentation of allergens
- Oligomerization of allergens
- Administration of peptides, or altered peptide ligands, based on T-cell epitopes

and they might still retain the risk of anaphylaxis. In addition, it is not clarified whether chemical modification destroys some of the T-cell epitopes of allergoids, and allergoid usage is still hampered by the basic problems of using full allergen extracts.

Bioengineering of allergens

Rapid progress is currently being made using recombinant DNA techniques to modify allergens for SIT, and these are replacing the chemical procedures for preparation of complex allergen extracts. Recombinant allergens represent promising tools for diagnosis and therapy for type I allergy. Primary amino acid sequences of allergens can be identified from cloned genes, and T-cell epitopes can be determined using synthetic peptides. Antibody-binding epitopes are identified by various approaches in order to modify IgE-binding sites of allergens. Site-directed mutagenesis or deletion of B-cell epitopes is one of the most daring of these methods¹³. The deletion mutants show a reduced capacity to bind IgE, but have normal T-cell reactivity. Site-directed mutagenesis of cysteine residues changes the conformation of allergens and represents a favorable approach to find candidate molecules for improved immunotherapy¹⁴.

The presence of conformational B-cell epitopes provides a possibility to reduce anaphylactic activity by fragmentation or oligomerization of allergens. The three-dimensional structure of the major birch pollen allergen Bet v 1 was disrupted by expressing two separate parts of the corresponding cDNA in *Escherichia coli*¹⁵. An interesting approach to generate hypoallergenic derivatives of recombinant allergens was to produce oligomers of

allergens by genetic engineering. Recently, stable recombinant Bet v 1 dimers and trimers have been generated¹⁶. Recombinant fragments or oligomers showed almost no allergenicity but induced proliferation of specific T cells. Skin-test reactivity and histamine release from peripheral blood granulocytes were reduced by about 100-fold in comparison with the native proteins^{15,16}. These results indicate that T-cell epitopes are protected while the anaphylactogenic activity is reduced in recombinant oligomers and fragments.

Targeting T-cell epitopes

It is possible to identify multiple T-cell epitopes with an active core region of 9–11 amino acids within allergen protein sequences. There is considerable rationale for targeting T cells with synthetic peptides based on such T-cell epitopes. To date, clinical trials of peptide immunotherapy (PIT) have been performed in two allergies^{10,11}. Relatively long peptides of 27 and 35 amino acids of the major cat allergen Fel d 1 containing the T-cell epitopes were used to treat allergy to cats and resulted in the induction of tolerance in IL-4-producing cells¹¹. In the other trial, PIT of bee venom allergy was performed with a mixture of short peptides that directly represent the T-cell epitopes (17, 12, 11 amino acids) of the bee venom major allergen, phospholipase A₂. The study showed modulation of the immune response against the whole allergen, inducing specific T-cell anergy and a decrease in the specific IgE:IgG4 ratio¹⁰.

Single amino acid alterations in T-cell epitopes can modify specific T-cell activation and cytokine production¹⁷. Studies with these so-called altered peptide ligands (APLs) of major allergens might enable more-controlled and strengthened PIT in the future.

Concluding remarks

As a consequence of severe side-effects, particularly for food and latex allergies, the high doses required for successful SIT might not be reached using native allergen extracts. Various studies are therefore currently being undertaken with the aim of increasing the success of SIT and decreasing the risk of side-effects. The availability of recombinant allergen technology should allow the generation of modified allergens with decreased

allergenicity yet retained immunogenicity, and will enable standardization of allergen preparations for clinical use. Targeting T cells using modified allergens will enable the administration of higher doses of allergen to induce tolerance in Th2 cells without risk of anaphylaxis.

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Meeting Report

Drugs and the immune system: the emerging era of immunopharmacology

Piero Periti, Luigina Romani, Enzo Bonmassar and Paolo Puccetti

The meeting 'Immunopharmacology and Immunotherapy Today' was held in Florence, Italy, on 10–11 November 2000.

The diverse molecular and cellular mechanisms that regulate immune reactions in response to pharmacological manipulation were discussed at 'Immunopharmacology and Immunotherapy Today', which was held in November 2000. Research in this field is expected to provide a new generation of tools for selective immunotherapeutic intervention in areas as diverse as infection and infection-associated immunopathology, neoplasia, organ transplantation, allergy and autoimmunity.

The complex interaction between the host immune system and xenobiotics is relevant to both health and disease in humans. Although xenobiotics can cause nonselective toxicity to the immune system, they can also provide effective and selective strategies for immunointervention in a variety of pathological conditions. Furthermore, a vast array of endogenous molecules with immune-related function can now be used therapeutically to modulate the activity of the immune system both at the nonspecific (innate) and specific (adaptive) levels. In this post-genomic era, one might assume that the development of new generations of 'drugs' will provide almost limitless opportunities for the rational design of molecules that are unique and selective for specific conditions, such as those occurring in infection-related immunopathology, allergy, autoimmunity and cancer.

Potentiating innate and adaptive immunity
Effects of antimicrobials on the immune response

In the elemental struggle between pathogenic microbes and the host immune system, each strives for a unique advantage and thus each exploits its own unique particularities in pathogenesis and protection. Although antimicrobials were previously considered to act merely by direct interaction with microbes, it is now clear that many affect the pathogen by modulation of host–microbe interactions. A major concept at this meeting was that antibiotics can exert a decisive effect on the host's defense mechanisms and that this effect can be beneficial. Although many beta-lactams are neutral in this respect (i.e. they have neither 'pro-host' nor 'anti-host' activity), some cephalosporins have strong positive modulating effects on such host factors as cytokine production, phagocytic activity, chemotaxis, neutrophil function, IgM production and natural killer cells (J.M.T. Hamilton-Miller, Royal Free and University College Medical School, London, UK). Of similar interest appears to be the immunomodulating activity of quinolones. Although the mechanism of the interaction of these drugs with T cells is not completely clear, the resulting immunomodulation by fluoroquinolones warrants further investigations (K. Riesbeck, Lund University, Malmö, Sweden). The effect is not confined to antibacterial agents, as many antifungals also exert a profound impact on immune responsiveness. The immunomodulatory effects might not be satisfactorily described as generic immunopotentialization,

but might rather involve a redirection of the specific response towards a type 1 pattern of reactivity (L. Romani, University of Perugia, Perugia, Italy).

Effects of cytokines on the immune response

More specifically related to the issue of immunomodulation was the discussion of clinical and pathological responses induced by neoadjuvant treatment with a cytokine mixture (IRX-2) in oral cavity squamous cell carcinomas of the head and neck. J.W. Hadden (University of South Florida, Tampa, FL, USA) reported that IRX-2 immunotherapy induced lymphocyte mobilization in those cancers associated with clinical and histological tumor responses indicative of regression. Interleukin 2 (IL-2) adjuvant treatment was also found to be effective in operable squamous cell carcinomas of the oral cavity and oropharynx (A. De Stefani, IRCC, Turin, Italy). The effects of cytokines might go well beyond their adjuvant activity, to include a specific effect on selected cellular and molecular functions. T helper (Th)-cell activity might be recruited by proinflammatory cytokines, such as IL-12, to weakly immunogenic tumors (F. Fallarino, University of Perugia, Perugia, Italy). A major effect of cytokines might also be exerted via CD1-restricted T-cell responses (G. Graziani, University of Rome 'Tor Vergata', Rome, Italy).

Effects of gene and/or cell therapy and vaccines on the immune response

The state of the art in gene therapy and vaccination in cancer was illustrated by