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Immunoregulation by targeting T cells in the treatment of allergy and asthma

Mark Larché^{1,2}

T-cell responses to allergens are crucial in determining the choice between health and disease. Th2 responses drive synthesis of IgE and the recruitment, maturation, survival and effector function of accessory cells such as eosinophils, basophils and mast cells. Allergen-specific strategies for targeting T-cell responses in established allergic diseases have been employed with success for almost a century. Recently, new insight into the mechanism of action of such approaches has revealed modulation of the delicate and complex regulatory mechanisms including suppression of Th2 responses through antagonistic Th1 responses and regulation through IL-10 and TGF β production.

Addresses

¹ Immunology and Allergy Division, Department of Medicine, McMaster University, 1200 Main Street West, Hamilton, ON L8N 3Z5, Canada

² Allergy and Clinical Immunology, Faculty of Medicine, Imperial College, London SW7 2AZ, United Kingdom

Corresponding author: Larché, Mark (larche@mcmaster.ca)

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Introduction

Allergic hypersensitivity is associated with both immunoglobulin E (IgE) and T helper 2 (Th2) responses to environmental allergens and is increasing in prevalence. In allergic individuals, priming of allergen-specific CD4⁺ Th2 cells by antigen-presenting cells (APCs) results in the production of Th2 cytokines (such as interleukin-4 [IL-4] and IL-13) that are responsible for the initiation of immunoglobulin heavy chain switching to epsilon and for the subsequent production of allergen-specific IgE. IgE binds to high-affinity receptors (Fc ϵ RI) on mast cells and basophils. Cross-linking of cell surface IgE leads to activation of effector cells and to the release of mediators such as histamine, prostaglandins, cysteinyl leukotrienes, cytokines and chemokines, which results in immediate allergic reactions such as the wheal and flare response in the skin. IgE also binds to Fc ϵ RI on dendritic cells and monocytes and to the low-affinity IgE receptor Fc ϵ RII (CD23; mainly on B cells). Specific IgE on the cell surface

enhances uptake of allergen and presentation of allergen-derived peptides to CD4⁺ T cells that drive late-phase allergic reactions. The production of IL-4, IL-5, IL-9 and IL-13 by allergen-specific Th2 cells also contributes to the development, survival and recruitment of eosinophils, the differentiation of mast cells, and mucous hypersecretion. Direct activation of allergen-specific T cells *in vivo* with synthetic T-cell epitopes leads to delayed symptoms of asthma and rhinitis [1]. Furthermore, the activity of both allergen-specific IL-10-secreting T regulatory (T_{Reg}) cells and CD4⁺CD25⁺ T_{Reg} cells is compromised in allergic disease [2,3]. Thus, the pivotal role of T cells in the development of allergic disease identifies this cell as a target for therapeutic intervention.

Allergen-specific treatments for allergic diseases are based on specific immunotherapy (SIT), which has been practiced for almost a century. SIT involves the administration (generally by subcutaneous injection but also by mucosal application) of increasing doses of extracts of the allergen to which the patient is allergic. Typically, a dose escalation phase is initially employed for safety and is followed by a maintenance phase of monthly injections generally lasting several years. An increase in the Th1:Th2 ratio has been reported after SIT, both through analysis of tissue samples obtained before and after therapy [4–6] and through *in vitro* studies of peripheral blood mononuclear cells (PBMCs) [7–9]. Serum levels of total and allergen-specific IgG are increased following SIT; however, the isotype of these antibodies does not have characteristics of a purely Th1 response that would favour IgG₂.

The causal association of Th2 responses and allergic diseases and the shift from Th2- to Th1-dominant responses following SIT have encouraged the development of novel strategies to induce immunoregulation through the promotion of Th1 responses. To this end, a variety of adjuvants have been employed including, most recently, components of bacterial cell walls and bacterial DNA sequences. Short-term therapy results in the induction of IgG₁ responses that, in the longer term, are superseded by IgG₄, which has been linked to the regulatory cytokine IL-10. SIT induces epitope-specific T-cell tolerance, which might be IL-10-dependent [10], increases IL-10 production by T cells and APCs [11,12] and reduces the number of Th2 cells and eosinophils at sites of allergen challenge [5]. Several studies have now demonstrated induction of IL-10 mRNA and protein from both T cells and APCs following SIT [9,11–17]. Most recently, increases in allergen-specific IgA have

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been linked to the induction of transforming growth factor β (TGF β) during SIT [15].

Thus, immunotherapy has been associated with several forms of immunoregulation: the induction of Th1 responses, antagonizing the existing Th2 response; the induction of IL-10, suppressing cellular responses to allergen and driving IgG₄-blocking antibody production; and the induction of TGF β , suppressing cellular responses, driving the generation of Fox p3⁺ T_{Reg} cells and allergen-specific IgA. This short review will address the evidence in favor of these forms of immunoregulation following allergen-specific treatment strategies that target T cells.

Novel approaches to therapy: evidence for immunomodulation

Recombinant allergens

Recombinant allergens offer advantages over natural allergen extracts because they are defined molecules and can be formulated in a reproducible and standardized manner. In the first study to evaluate unmodified recombinant proteins, grass pollen-allergic individuals were treated with a mixture of five recombinant grass (*Phleum pratense*) allergen molecules (total protein content of maintenance dose, 40 μ g) adsorbed to aluminium hydroxide [18^{••}]. Treatment was clinically efficacious, providing an improvement in combined symptom and medication scores of the same order as current best practice with allergen extracts. Interestingly, the recombinant preparation contained only low levels of endotoxin, excluding a substantial role for endotoxin in modulation of the Th2 response in this study. Treatment was accompanied by a gradual reduction in allergen-specific IgE over the 18 month course of treatment together with substantial induction of IgG₄ in response to allergen (~4000-fold increase) and a weaker induction of IgG₁ (60-fold increase). Because no other IgG isotypes were measured, it is not possible to evaluate the induction of Th1-associated isotypes such as IgG₂ and IgG₃ in the relative absence of endotoxin. No T-cell studies were performed and thus definitive statements cannot be made about the nature of the immune deviation achieved. However, the induction of high levels of IgG₄ is suggestive of a substantial IL-10 response to therapy. Further studies are required to determine Th1/Th2 and regulatory responses to treatment with low-endotoxin recombinant allergen preparations.

Hypoallergenic recombinant allergens

Hypoallergenic molecules have reduced IgE reactivity, either through engineering or as a result of natural polymorphism. Although many hypoallergenic molecules have been developed, few have progressed to human *in vivo* studies. Evaluation of the allergenicity of a genetically engineered hypoallergenic apple protein (Mal d 1) showed reduced basophil histamine release, decreased

skin-prick test response and reduced allergenicity in double-blind placebo-controlled food challenge, but this molecule has not been used for therapy [19[•]]. A trimeric Bet v 1 molecule and a mixture of two recombinant fragments also showed reduced skin-prick test reactivity [20]. The two preparations were evaluated in a multi-center double-blind placebo-controlled trial [21]. Analysis of PBMC responses to allergen stimulation was performed in subjects at one of the three study centers. Responses were compared before and after therapy with the Bet v 1 trimer and Bet v 1 fragments; this demonstrated reduced numbers of IL-5-secreting and IL-13-secreting cells following treatment in the trimer-treated group, but not in the fragment-treated group. IL-12-secreting cells were increased in number, but this failed to achieve statistical significance. Numbers of IL-10-secreting cells were variable and showed no clear pattern of change in either treatment group.

Increased levels of allergen-specific IgG (IgG₁, IgG₂ and IgG₄) were induced in the serum by both treatments. Induction of IgG responses was transient, with levels returning to near baseline after one year. Serum from treated individuals was able to reduce basophil histamine release *in vitro*.

In a sub-analysis of randomly selected patients at one center, nasal lavage revealed significantly increased levels of allergen-specific IgG₄. Induction of IgG in nasal secretions correlated with serum levels of antibody and was associated with improved tolerance of nasal allergen challenge [22[•]]. Specific IgE was also higher following therapy, and a modest increase was also observed in the levels of IgA. Treatment with both allergen preparations was associated with reduced immediate skin reactions within the trimer- and fragment-treated groups, but not the placebo group. No comparison between treatment groups and placebo was provided [23]. Induction of IgG₁, IgG₂ and IgG₄ in this trial is indicative of a mixed Th1/Th2 response. Furthermore, although the frequency of IL-10-secreting cells did not change in enzyme-linked immunospot assays, the induction of IgG₄ implies a role for IL-10 in this system, perhaps through induction of greater quantities of IL-10 from the same number of IL-10-secreting cells. The induction of IgG₂ is indicative of a Th1 response and might have been related to the increased frequency of IL-12-secreting cells resulting from the increased immunogenicity of the trimer in particular, by virtue of its polymeric structure. A weak induction of IgA responses suggests a limited Th3 response.

T-cell peptides

Synthetic peptides containing T-cell epitopes retain the ability to induce immune modulation in T cells, although they are unable to cross-link specific IgE molecules and to activate effector cells. Peptide vaccines from the major

cat allergen (Fel d 1) and from the bee venom-derived phospholipase A₂ (Api m 1) have been developed and clinically evaluated (reviewed in [24]). Initial peptide therapy trials in cat-allergic subjects showed modest clinical efficacy, but also frequent adverse events [25]. More recent studies with larger mixtures of shorter peptides demonstrated reduced early- and late-phase allergic skin reactions following allergen challenge, accompanied by downregulation of Th1- and Th2-cell responses in peripheral blood and enhanced production of IL-10 [26].

In contrast to findings in peripheral blood, allergen challenge and biopsy of skin sites revealed the recruitment of increased numbers of interferon (IFN) γ ⁺ CD4 T cells after peptide therapy. No increase in IL-10-secreting cells was observed. However, a significant increase in TGF β was observed, although this could not be localized to specific cell types for technical reasons [27]. Using a novel *in vitro* assay for functional allergen-specific T-cell suppression, peptide therapy was demonstrated to induce a population of CD4⁺ regulatory cells capable of suppressing proliferation of PBMCs obtained before therapy [28^{*}]. Peptide therapy for bee venom allergy was associated with protection from cutaneous allergen challenge and partial protection from live sting challenge [29]. Cutaneous late-phase reactions to allergen challenge were reduced. Peripheral blood Th1- and Th2-cytokine responses to allergen were decreased together with a concomitant increase in IL-10 production [16]. A potential explanation for reduced IFN γ levels in peripheral blood was provided by the observation that mRNA encoding suppressor of cytokine signaling-3 was induced during therapy. Suppressor of cytokine signaling-3 is known to be induced by IL-10 and to suppress IL-12 signaling. Evidence was also provided for the induction of allergen-specific IgG antibodies following peptide immunotherapy for bee venom allergy [16,29,30].

The use of larger peptides resulted in changes in antibody levels that were more consistent with whole-allergen therapy, whereas induction of IgG and IgG₄ antibodies in studies with short peptides was variable and might have been related more closely to challenge of patients with the whole molecule after peptide treatment, rather than antibody induction by the peptides themselves. Thus, peptide therapy results in the induction of functional allergen-specific regulatory cells and is associated with enhanced production of IL-10 in the blood, but increased expression of IFN γ and TGF β at skin sites of allergen challenge.

Amb a 1-immunostimulatory phosphorothioate oligonucleotide conjugate

Modifying the function of APCs through the use of Toll-like receptor ligands in combination with allergens has been proposed as a strategy for shifting the Th1/Th2 balance. Synthetic DNA sequences that contain CpG

motifs have been covalently linked to Amb a 1 to generate allergen immunostimulatory conjugates (AICs).

Methods using AICs demonstrated reduced allergenicity in skin-prick tests compared with those using native allergen. Treatment of ragweed-allergic subjects with the CpG DNA-allergen complexes resulted in suppression of Th2 responses and in upregulation of Th1 responses to allergen challenge, both *in vitro* and *in vivo*. In a small placebo-controlled study, PBMCs from ragweed-allergic individuals treated with Amb a 1-immunostimulatory phosphorothioate oligonucleotide conjugate showed significantly reduced IL-5 production, an increase in IFN γ production, and a reduction in the IL-5:IFN γ ratio. Similarly, Th2 chemokines CCL17 and CCL22 were decreased, whereas Th1 chemokines CXCL9 and CXCL10 were increased and there was an increase in the CXCL10:CCL17 ratio. Interestingly, IL-13 production was unaltered by treatment. IL-10 production was significantly elevated 2 weeks after the end of treatment, but 14 weeks later there was only a trend for an increase in this cytokine. Global responses to control antigen streptokinase and mitogen phytohemagglutinin were unaffected by treatment. Antibody responses and the clinical response to treatment were not reported [31].

In a larger study that employed a similar dosing regimen, nasal biopsies were taken from a subset of subjects at baseline and following nasal allergen challenge two weeks after the completion of therapy (before the ragweed season); biopsies were taken again after the ragweed season. Interestingly, clinical benefit was only reported for the following ragweed season in a subset of patients analyzed longitudinally. Following the ragweed season (but not before the season), allergen challenge in the placebo group resulted in a significant increase in IL-4 and IL-5 mRNA⁺ cells, and there was a trend for an increase in major basic protein⁺ cells in the nasal mucosa. In the active treatment group, there was no significant increase in IL-4 and IL-5 mRNA⁺ cells, but a significant increase in IFN γ mRNA⁺ cells was observed. Again, antibody responses were not reported [32]. Taken together, these two studies in AIC-treated individuals suggest that a marked deviation of the allergen-specific immune response from Th2 to Th1 occurs with this form of therapy. Transient induction of IL-10 might indicate a role for this regulatory cytokine, but the lack of information regarding induction of IgG isotypes (particularly IgG₄) does not allow firm conclusions to be drawn.

Mixed grass pollen allergen extract with monophosphoryl lipid A

Monophosphoryl lipid A is a glycolipid isolated from the cell wall of *Salmonella minnesota* and has been used as a Th1-inducing adjuvant (Toll-like receptor-4 agonist) for both prophylactic and therapeutic vaccines in infectious

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diseases and cancer. A double-blind placebo-controlled clinical trial of tyrosine-adsorbed grass pollen allergoid (glutaraldehyde-treated) extract demonstrated clinically significant improvements in combined symptom and medication scores after a course of four pre-seasonal injections [33]. The treatment effect size was moderate and was accompanied by increases in allergen-specific IgG (no isotypes were measured) and a modest reduction in the seasonal increase in IgE, which was not statistically significant between groups.

A more recent study in a smaller group of individuals treated with the same preparation analysed antibody isotype induction before treatment, after treatment, during the pollen season, and after the pollen season [34]. The majority of treated individuals responded by induction of allergen-specific IgG₁ responses and IgG₄ responses. IgM and IgG₂ were induced by treatment in some individuals. Seasonal increases in allergen-specific IgE were blunted in the active treatment group. Serum from actively treated subjects was able to inhibit *in vitro* histamine release from basophils of sensitized subjects. No analysis of T-cell and cytokine responses has been applied with this form of treatment to date. Based on the isotypes of allergen-specific antibodies induced it is probable that treatment was associated with induction of both Th1 cells and IL-10-secreting T_{Reg} cells. Further studies are required to investigate modulation of T-cell responses.

Conclusions

Targeting of T cells through allergen-specific therapy results in modification of the balance between Th1, Th2 and T_{Reg} cells. Modified T-cell responses lead to qualitative changes in B-cell responses, most notably through the induction of allergen-specific IgG responses and (when looked for) less-robust IgA responses. Different approaches yield subtly different results. However, in general terms, an increase in the Th1:Th2 ratio is observed together with the induction of T_{Reg} cell responses, characterised by increased IL-10 and TGFβ production. Allergen-specific IgG responses are dominated by IgG₁ and particularly IgG₄, the amplification of which is driven by IL-10. Future strategies for treatment could focus on optimising the induction of regulatory T- and B-cell responses to restore immune regulation.

Update

Immunological changes associated with conventional injection SIT for aeroallergen sensitivity appear to require a period of days to weeks to take effect. For this reason, the immunological mechanisms of RUSH (rapidly delivered therapy with multiple allergen administrations within a short period of time) immunotherapy have been somewhat enigmatic. A recent study in wasp venom 'ultra-RUSH' immunotherapy has shed new light on these changes [35]. Peripheral blood was sampled before and immediately after the ultra-RUSH injection protocol

in which patients received six incremental doses of venom over a 3.5 hour period. Further samples were obtained before booster injections at days 15 and 45. Culture of whole peripheral blood revealed an increase in IFNγ and a decrease in IL-4, the latter achieving statistical significance within the first day. Furthermore the percentage of peripheral blood T cells staining for IFNγ was significantly increased after the first day, an effect that persisted to day 45. The percentage of IL-13⁺ T cells was reduced at days 15 and 45. In addition, the authors evaluated the percentage of peripheral blood T cells expressing the phenotype of naturally occurring regulatory T cells (CD4⁺CD25^{hi}) and also of CD3⁺IL-10⁺ T cells. Both populations were significantly elevated by the end of the first day of therapy, and frequencies continued to increase during the course of therapy. Interestingly, the magnitude of immunological changes associated with deviation from the allergic phenotype appeared to be greatest in the patients with less severe sensitization implying that it might be easier to achieve clinical efficacy in mild disease. Thus, RUSH immunotherapy appears to be associated with a rapid Th2 to Th1 shift in the balance of cytokine production, together with a concomitant increase in the frequency of cells that have characteristics of both naturally occurring and induced regulatory T cells.

Evidence exists for both high and low zone (high and low antigen dose) tolerance in models of allergic contact dermatitis. Protection from contact hypersensitivity following epicutaneous treatment with sub-immunogenic doses of hapten was shown to be associated with the induction of CD4⁺ IL-10-secreting T cells that were required for the generation of CD8⁺ Tc2 cells that inhibited disease [36]. Recently, comparison of epicutaneous administration of the tolerogenic dose with administration via the oral and intravenous routes revealed equivalent efficacy [37]. All routes of administration appeared to be associated with the same CD4 and CD8 cell-dependent mechanism, and both CD4⁺ and CD8⁺ regulatory T cells were distributed systemically. These studies suggest that immunological tolerance through targeting of T-cell responses might be achieved through a variety of routes, creating wider possibilities for clinical intervention in the future.

Acknowledgements

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