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Review Series

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Tregs and allergic disease

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Allergic diseases such as asthma, rhinitis, and eczema are increasing in prevalence and affect up to 15% of populations in Westernized countries. The description of Tregs as T cells that prevent development of autoimmune disease led to considerable interest in whether these Tregs were also normally involved in prevention of sensitization to allergens and whether it might be possible to manipulate Tregs for the therapy of allergic disease. Current data suggest that Th2 responses to allergens are normally suppressed by both CD4⁺CD25⁺ Tregs and IL-10 Tregs. Furthermore, suppression by these subsets is decreased in allergic individuals. In animal models, Tregs could be induced by high- or low-dose inhaled antigen, and prior induction of such Tregs prevented subsequent development of allergen sensitization and airway inflammation in inhaled challenge models. For many years, allergen-injection immunotherapy has been used for the therapy of allergic disease, and this treatment may induce IL-10 Tregs, leading to both suppression of Th2 responses and a switch from IgE to IgG4 antibody production. Improvements in allergen immunotherapy, such as peptide therapy, and greater understanding of the biology of Tregs hold great promise for the treatment and prevention of allergic disease.

Allergic disease

Atopic allergic sensitization is defined by production of IgE against environmental antigens such as house dust mites, grass pollen, and animal proteins and can lead to diseases that include asthma, rhinitis and atopic dermatitis (1). These disorders affect 10–15% of Western populations, and their prevalence has doubled in the last 10–15 years. Triggering an immune response through allergen-specific IgE on the surface of mast cells and basophils can lead to immediate symptoms through release of histamine and other mediators while eosinophilic airway inflammation contributes to airway hyperresponsiveness (AHR) in asthma (1). Switching of B cells to IgE production and accumulation of eosinophils are under the control of Th2 lymphocytes through production of IL-4 and IL-5. These cells also produce IL-9 and IL-13, which contribute to AHR in asthma (2, 3).

Regulatory or suppressive T cells have increasingly been defined as important in the prevention of autoimmune disease and other immunopathologies (4); the biology and mechanism of suppression by these cells is reviewed elsewhere in this series. Whether Tregs normally also prevent atopic sensitization and how this regulatory process becomes defective or is bypassed in those individuals who develop allergic disease are areas of much current research. Additionally, the potential for manipulation of Tregs for therapy is clearly attractive for many disease types. Allergen-injection immunotherapy has been used for control of allergic disease for many years and appears to act through modulation of the Th2 response to the allergen, either by immune deviation of allergen-specific Th2 responses in favor of Th1 responses and/or through the induction of Tregs. Understanding the mechanism of therapeutic benefit in this treatment may hold important lessons for immunoregulation in other diseases.

Interest in active T cell suppression as a mechanism for immunological tolerance was reawakened by the demonstration by

Sakaguchi and others that neonatal thymectomy of mice leads to organ-specific autoimmune pathology that can be prevented by transfer of CD4⁺CD25⁺ T cells from nonirradiated animals (5, 6). Shevach and coworkers developed an in vitro system, which has been used widely, showing that mouse CD4⁺CD25⁺ T cells do not proliferate in response to either anti-CD3 or antigenic stimulation and, furthermore, can inhibit the proliferative responses of CD4⁺CD25⁻ T cells (7). Use of this in vitro assay for the study of T cell regulation allowed demonstration of suppression by human peripheral blood and thymic CD4⁺CD25⁺ T cells (8–13). Thymus-derived CD4⁺CD25⁺ regulatory cells have been termed naturally occurring Tregs. A number of other regulatory subsets have been described (see Table 1). These are largely T cell populations induced by in vitro or in vivo manipulation and have been termed adaptive Tregs (14), but it is of note that several reports also suggest the existence of naturally occurring Tregs within the CD4⁺CD25⁻ T cell population (15). Currently there is intense interest in the interrelationship of naturally occurring and induced/adaptive CD4⁺CD25⁺ T cells, IL-10-producing Tregs, and other regulatory subsets, such as Th3 cells described as occurring after induction of oral tolerance (16). It will be important to determine whether these cell types are distinct and how data from mouse models can be related to the development or treatment of human disease.

The mechanism of suppression by different Treg subsets remains controversial; different in vivo and in vitro studies raise possible roles for the immunosuppressive cytokines IL-10 and TGF- β (including a membrane-bound form), the negative costimulatory molecules CTLA4 and PD-1, and the glucocorticoid-induced TNF receptor (6–12, 17–25). However, in vitro suppression by both mouse and human CD4⁺CD25⁺ T cells can be detected in the absence or blockade of these pathways. An important advance in the understanding of the biology of regulation by CD4⁺CD25⁺ T cells occurred in the demonstration of the dependence of their suppressive phenotype on expression of the transcription factor Foxp3. Gene deletion of *Foxp3* abrogated suppression by CD4⁺CD25⁺ T cells whereas ectopic gene expression in CD25⁻ T cells rendered these cells suppressive (26–28). In addition, acquisition of regulatory phenotype by CD4⁺CD25⁻ T cells, either

Nonstandard abbreviations used: AHR, airway hyperresponsiveness; LAR, late asthmatic reaction; PLA₂, phospholipase A₂; TLR, toll-like receptor.

Conflict of interest: D.S. Robinson is a consultant to Lorantis Ltd. S.R. Durham is a consultant to ALK Abello and has received honoraria and research funding from ALK Abello, a manufacturer of allergy vaccines.

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**Table 1**

Different regulatory cell types implicated in the prevention of atopic sensitization in mouse models and humans

Type of Treg	Mechanism of suppression	Allergy active in (refs.)
CD4 ⁺ CD25 ⁺ (naturally occurring, i.e., thymically derived)	Foxp3 contact dependent, IL-10, TGF- β , CTLA4, PD-1 ^A	Mouse (41) Human (52–55)
CD4 ⁺ CD25 ⁺ (induced)	IL-10, TGF- β	Mouse (29, 44) Human (52–54)
Tr1	IL-10, TGF- β	Mouse (45)
IL-10 Treg	Contact dependent, IL-10 (in vivo)	Mouse (46–48) Human (55)
Th1 cells	IFN- γ , IL-10	Mouse (33)

It remains unclear how these subtypes are interrelated and whether human CD4⁺CD25⁺ T cells represent naturally occurring or adaptive Tregs (see ref. 14). Which subtypes are induced (if any) by whole allergen immunotherapy or peptide immunotherapy also remains to be determined. ^AIL-10, TGF- β , CTLA4, and PD-1 are implicated in suppression by naturally occurring CD4⁺CD25⁺ T cells in some, but not all, reports.

through the action of TGF- β or coculture with CD4⁺CD25⁺ Tregs, was associated with acquisition of Foxp3 expression (29). Mutation of *Foxp3* was described in patients with immune dysregulation, polyendocrinopathy, enteropathy, and X-linked syndrome (IPEX syndrome), a syndrome which includes the development of atopic dermatitis; thus Foxp3 Tregs may be involved in the prevention of allergic sensitization (30). It has been suggested that Foxp3 acts as a master switch for the regulatory phenotype in much the same way as do GATA3 and T-bet in Th2 and Th1 T cell phenotypes respectively (31). However, it is of note that IL-10-producing Tregs do not show overexpression of *Foxp3*, which suggests that regulation can occur through different pathways (32).

In addition, Th1 cells have inhibitory effects on Th2 function (partially through production of the immunomodulatory cytokine IL-10) and have been shown to prevent or diminish airway inflammation in at least some mouse allergen challenge models (33). This was also shown by in vitro overexpression of the Th1-associated transcription factor T-bet, which was reported to reduce expression of GATA3 and Th2 cytokines in Th2 cells and is underexpressed in the airway in asthmatics compared to control subjects (34, 35). Thus in the context of allergic disease, Th1 cells may be suppressive, and immune deviation from a Th2 to a Th1 response may be a legitimate regulatory strategy for treatment of allergic disease. In addition, NK T cells and TCR $\gamma\delta$ -bearing cells have also been suggested as having regulatory roles (36).

Tregs in mouse models of allergic disease

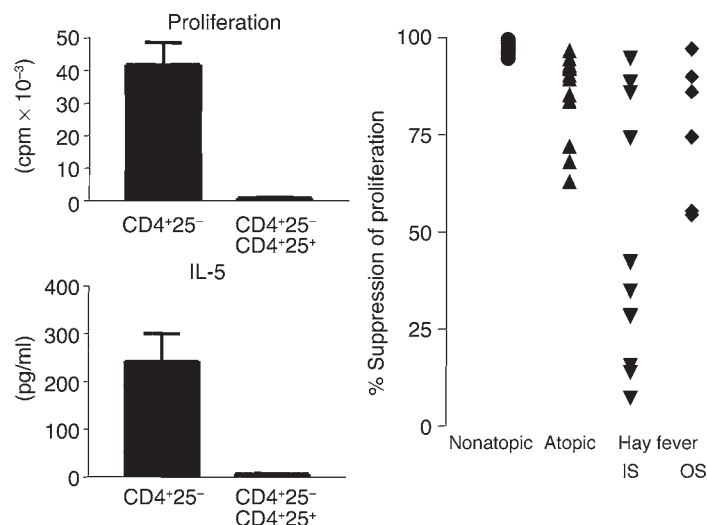
A number of reports of experiments using mouse models have suggested a potential role for Tregs in the prevention and control of Th2-mediated pathology.

Mouse CD4⁺CD25⁺ T cells were shown to suppress the in vitro differentiation of Th2 cells from naive CD4⁺ T cells but required preactivation to inhibit cytokine production from differentiated Th2 cells (37). In transgenic mice with monoclonal populations of T and B cells, a single immunization of antigen resulted in very high serum IgE levels, which could be prevented by both CD25⁺ and CD4⁺CD25⁺ T cells. This suggested that Tregs normally prevent IgE responses (38). However, depletion of CD4⁺CD25⁺ T cells actually reduced airway inflammation in a murine model of allergic sensitization (39), and transfer of CD4⁺CD25⁺ T cells had no effect on inflammation or AHR

in another report (40). In a double-transgenic model, CD4⁺CD25⁺ OVA-specific TCR transgenic T cells infiltrated the lungs of animals expressing the OVA antigen under a lung-specific promoter. Although these cells suppressed airway inflammation, they did not prevent AHR in response to inhaled OVA (41). These data suggest that, at least in animal models, naturally occurring CD4⁺CD25⁺ T cells which develop during normal thymic maturation may not be sufficient to prevent allergic airway disease. In other models, CD4⁺CD25⁺ Tregs have been induced from CD4⁺CD25⁺ T cells. In one report, these adaptive CD4⁺CD25⁺ T cells were derived after antigen exposure in the presence of

TGF- β and expressed the transcription factor Foxp3. These cells could prevent house dust mite-induced airway inflammation in an inhaled allergen challenge model, although no data was presented on AHR (29). Repeated low-dose inhaled exposure to antigen induced tolerance to subsequent Th2 sensitization and airway pathology in mouse models (42, 43). Recently this was shown to depend on the induction of Foxp3⁺ CD4⁺ T cells that expressed surface TGF- β and suppressed development of airway inflammation in vivo, and antigen-induced T cell proliferation in vivo, through a contact-dependent mechanism (44). In human peripheral blood one might expect that the CD4⁺CD25⁺ T cell population will contain both naturally occurring and adaptive Tregs, and it will be of interest to determine how such subsets develop upon natural allergen exposure (see below).

In addition to the roles for CD4⁺CD25⁺ T cells, the use of mouse models suggests potential roles for IL-10-producing Tregs in the suppression of allergic pathology. These include Tr1 clones derived by antigen stimulation in the presence of IL-10, which could prevent Th2 sensitization and IgE production if adoptively transferred prior to sensitization (45). Tolerance to inhaled antigen challenge can also be induced by high-dose intranasal antigen delivery to the respiratory tract in mice. This tolerance was shown to be dependent on IL-10 production by mature dendritic cells, which in turn induced development of IL-10-producing regulatory CD4⁺ T cells (46, 47). In addition, prior exposure to heat-killed *Mycobacterium vaccae* reduced both Th2 airway inflammation and AHR to subsequent allergen challenge, and this was mediated by CD4⁺CD45RB^{lo} T cells acting through both IL-10 and TGF- β (48). IL-10-producing Tregs have also been derived by antigen stimulation of either mouse or human naive CD4⁺ T cells in the presence of immunosuppressive drugs, dexamethasone, and vitamin D3 (49), although these have not been tested in mouse models of allergic disease. A further possible means of inducing regulation was demonstrated by Hoyne and colleagues. House dust mite-pulsed antigen-presenting cells that had been engineered to overexpress the Notch ligand Jagged-1 were injected into mice and shown to induce CD4⁺ T cells that could prevent house dust mite-induced airway pathology in an allergen challenge model (50). The Notch signaling system is involved in cell fate decision (including in the immune system) and may influence whether a proinflammatory or anti-inflammatory response is mounted.

**Figure 1**

Peripheral blood CD4⁺CD25⁺ T cells from atopic volunteers show reduced suppressive ability in allergen-stimulated T cell cultures. CD4⁺CD25⁻ T cells were separated from peripheral blood by immunomagnetic separation, then cultured with allergen extracts either alone or mixed with CD4⁺CD25⁺ T cells. Proliferation was assessed by incorporation of tritiated thymidine (shown as cpm), and IL-5 was measured in supernatants at day 6 of cultures by Luminex bead array. Data shown are means and standard errors for cpm and IL-5 from 9 separate nonatopic donors, showing almost complete suppression of responses of CD4⁺CD25⁻ T cells when CD4⁺CD25⁺ T cells were added. When these data were expressed as percentage suppression (reduction in counts in the mixed culture compared with those in CD4⁺CD25⁻ T cells alone), suppression was significantly less when cells were obtained from atopic donors or volunteers with hay fever studied in or out of season (IS or OS). Suppression out of season was significantly more than that seen in season but still significantly less than in nonatopic controls. Figure adapted from *Lancet* (54), with permission from Elsevier.

Thus murine models of allergic disease demonstrate that Tregs have potential in preventing allergic sensitization and that such cells can be induced in vivo. However, which regulatory pathways act in allergic disease and how these cells suppress Th2 responses clearly remains controversial.

Tregs and allergic responses in humans

There have been few reports of activity of human Tregs on Th2 cells in vitro. Human thymocyte Th2 clones were relatively resistant to suppression of allergen-induced activation by autologous CD4⁺CD25⁺ thymocytes (51). However, human peripheral blood CD4⁺CD25⁺ T cells suppressed proliferation and Th2-type cytokine production by CD4⁺CD25⁻ T cells stimulated in vitro with allergen (52, 53). Thus Tregs may act to prevent inappropriate Th2 responses to environmental allergens. One possible reason for development of atopic sensitization would therefore be a deficiency or failure of such regulation. Although initial reports found no clear difference in the suppressive ability of CD4⁺CD25⁺ T cells between atopic allergic and nonatopic control subjects (52, 53), a more recent study showed that the suppressive ability of CD4⁺CD25⁺ T cells from atopic subjects in cocultures with allergen-stimulated autologous CD4⁺CD25⁻ T cells was significantly less than that seen for cells from nonatopics (54) (Figure 1). There was no difference between atopic and nonatopic donors in the

ability of their CD4⁺CD25⁺ T cells to suppress CD4⁺CD25⁻ T cells activated via anti-CD3 and anti-CD28 monoclonal antibodies, suggesting that atopy is not associated with a generalized defect in regulatory capacity of CD4⁺CD25⁺ T cells (54). One possibility was that the CD4⁺CD25⁺ T cells isolated from the atopic subjects contained fewer Tregs and more activated effector T cells, since CD25 is also expressed by recently activated or memory effector T cells. However, no differences in other phenotypic markers were detected to suggest an excess of activated T cells, and differences in CD4⁺CD25⁺ T cell suppression of grass pollen-stimulated cultures between patients with hay fever and nonatopic controls persisted even outside the pollen season (Figure 1). Human CD4⁺CD25⁺ T cells were confirmed to express mRNA for the Foxp3 transcription factor, which is consistent with a role for Foxp3 in the regulatory phenotype of CD4⁺CD25⁺ T cells in humans as well as mice (54). These findings show that human CD4⁺CD25⁺ T cells can indeed suppress T cell activation by allergens and that this process may be deficient in atopic subjects.

In addition to CD4⁺CD25⁺ T cells, IL-10-producing Tregs have been implicated in the prevention of atopic sensitization. Akdis et al. showed an increased frequency of allergen-specific IL-10-producing CD4⁺ T cells in the blood of nonatopic individuals when compared to atopics and, furthermore, that these IL-10-producing T cells could specifically inhibit allergen-activated IL-4-producing T cells. Suppression was reversed by blocking antibodies to IL-10 and TGF- β (55). The cells were isolated after allergen stimulation and were CD25⁺. How these cells relate to the CD4⁺CD25⁺ T cells studied by others remains unclear; it is of note that suppression by induced CD4⁺CD25⁺ T cells (derived from coculture of CD25⁻ T cells with CD4⁺CD25⁺ T cells in vitro) was dependent on IL-10, in contrast to CD4⁺CD25⁺ T cells freshly isolated from blood, in which suppression was not reversed by blocking antibodies to IL-10 or IL-10 receptor (54, 56).

Development of allergic sensitization: suppression versus activation of Th2 responses

The factors determining the balance between a suppressive or regulatory response to allergen and activation of a Th2 response either during maturation of the immune system or after allergen sensitization are uncertain. One hypothesis for the increasing prevalence of Th2-associated allergic disease, the hygiene hypothesis, is that Western lifestyles reduce contact with environmental microorganisms. Such microorganisms are thought to modify antigen-presenting cells (in part via toll-like receptors [TLRs]) so that they induce either immune deviation in favor of Th1 responses or the development of Treg responses to allergens. Susceptibility to such factors may explain genetic associations of atopic disease with polymorphisms such as those described for the LPS receptor CD14 and TLR2 (57–59). Different concentrations of LPS could favor either Th1 or Th2 responses, and activation of TLR4 via LPS overcame regulation by CD4⁺CD25⁺ T cells (60–62). Route, dose, and timing of allergen exposure, in addition to coexposure to pathogens or environmental bacteria, are all probably important in allergic sensitization. However, it is of note that allergic sensitization can occur in adulthood, so it is not the prerogative of the developing immune system. Both prior inhaled allergen exposure and exposure to mycobacterial antigens were shown to suppress



subsequent allergic sensitization in mice through mechanisms that include induction of IL-10-producing Tregs (46–48). Such mechanisms may explain the apparent protective effect of exposure to cats during early childhood on the later development of atopic sensitization to cat allergens. High-dose exposure to cat allergen has been associated with a dominant IL-10 response in peripheral blood mononuclear cells cultured with cat allergen and elevated serum concentrations of cat allergen-specific IgG4; this has been termed a “modified Th2” response (63). Whether this relates to the development of Tregs is at present unclear. In contrast, house dust mite exposure showed no such protective effect. It will clearly be of interest to determine whether this high-exposure tolerance is akin to high-dose tolerance in mouse models, which is dependent on IL-10-producing Tregs, and whether the normal nonatopic state results from low-dose chronic tolerance as described in mouse models dependent on surface TGF- β ⁺ Tregs (44). Recent data suggest that both IL-10-producing T cells and CD4⁺CD25⁺ T cells may contribute to the development of tolerance since children lose atopic sensitization to cows’ milk allergens (64, 65).

Further understanding of the maturation of regulatory responses will be required if preventive vaccination strategies are to be developed for allergic disease. The data from animal models of allergic airway sensitization suggest that naturally occurring CD4⁺CD25⁺ T cells derived from the thymus may not be sufficient to prevent airway inflammation and that more than one type of Treg may be required.

Influence of allergy treatment on Treg populations

Corticosteroids. How might Tregs be induced by therapy to control or prevent allergic disease? Corticosteroids are widely used as topical or systemic anti-inflammatory treatment for allergic disease and inhibit T cell activation and Th2 cytokine expression both in vitro and in vivo. In addition, corticosteroids increase IL-10 production by T cells and macrophages. In vitro exposure of human CD4⁺CD25⁺ T cells to corticosteroids have been shown to increase their suppressive capacity in subsequent allergen-stimulated cultures, in part through an increase in IL-10 production (66). In addition, corticosteroids with vitamin D3 could induce an IL-10-producing Treg phenotype in vitro in both mouse and human CD4⁺ T cells, and these IL-10-producing Tregs inhibited experimental allergic encephalomyelitis (49) and suppressed human allergen-specific Th2 cells in vitro (Catherine Hawrylowicz, King’s College, London, United Kingdom; personal communication). Whether such strategies can be modified for in vivo use in treating human allergic disease remains to be determined. Current clinical experience indicates that, although corticosteroids can control allergic inflammation effectively, symptoms recur if they are discontinued. The development and duration of regulatory memory is poorly understood, but current steroid regimens do not appear to alter underlying sensitization. In contrast, allergen immunotherapy modulates Th2 responses to allergen, and this effect can persist for at least 3–4 years following discontinuation of therapy (67).

Allergen immunotherapy. Allergen immunotherapy involves the subcutaneous injection of incrementally increasing doses of allergen in order to suppress symptoms on subsequent reexposure to that allergen (68). In patients with severe seasonal pollinosis, grass pollen vaccination is highly effective (67–70). In children, 3 years of house dust mite extract immunotherapy prevented the onset of new allergen sensitivities (71) and resulted in a 2- to 3-fold reduction in the risk of developing physician-diagnosed asthma (72).

Some (73, 74), but not all (75) studies have described decreased peripheral blood T cell responsiveness to allergen and/or immune deviation in favor of Th1 responses (76, 77). In some early reports, evidence of the induction of regulatory responses, for example, increased expression of IL-10, was not sought. Increased proportions of CD4⁺CD25⁺ cells were found in grass pollen-stimulated peripheral blood mononuclear cell cultures following immunotherapy (78); these could represent a Treg population which might act to suppress Th2 T cells. In support of this hypothesis, grass pollen stimulation of PBMCs led to increased IL-10 production, which was colocalized to CD4⁺CD25⁺ T cells. These findings are consistent with previous observations in venom-sensitive patients, which also suggest a role for IL-10 in suppression of allergen-specific Th2 responses (73, 79). A report of immunotherapy in patients with house dust mite allergy demonstrated increased IL-10⁺ CD4⁺CD25⁺ cells in peripheral blood after 70 days of treatment compared with before therapy (80). Whether these CD4⁺CD25⁺ T cells arise from the expansion of naturally occurring CD4⁺CD25⁺ T cells or are analogous to IL-10-dependent CD4⁺CD25⁺ T cells induced in vitro (56) remains to be determined.

Effective suppression of allergen-responsive T cells in target organs (such as the lung) requires trafficking of Tregs. Studies of in vivo suppression in animal models of inflammatory bowel disease suggest a role for locally produced IL-10 or TGF- β (17). Allergen immunotherapy treatment is associated with reduced local nasal tissue infiltration by eosinophils, basophils, and Th2 T cells after allergen challenge together with local increases in T cells producing IL-2 and IFN- γ (Th1 cells), as detected by in situ hybridization (75, 81–83). In the same nasal biopsies, obtained during the peak pollen season, there were increases in the number of IL-10⁺ and TGF- β ⁺ cells, detectable at both mRNA and protein levels (84). These increases in IL-10 and TGF- β were not detectable outside the pollen season, suggesting that local mucosal contact with allergen was necessary for these effects. Neither were these cells detectable in normal nonatopic subjects, which suggested that these IL-10 and TGF- β responses were not a feature of the normal mucosal response to pollen nor due to a toxic or other effect of pollen per se, in the absence of allergic sensitization. Colocalization studies confirmed that 15–20% of these nasal IL-10- and TGF- β -producing cells were T cells, raising the possibility of an immunotherapy-induced, local adaptive mucosal T regulatory response following natural allergen exposure. Clearly the development of specific markers for Tregs is an urgent requirement for their tissue localization or isolation. However, IL-10 and TGF- β also colocalized to macrophages (40%) and alternative sources, including B cells and dendritic cells. It is therefore possible that local increases in IL-10 and TGF- β from these innate immune cells may also result from the observed local increases in IFN- γ . Whether due to an immunotherapy-induced, allergen-specific T regulatory response (for example, production of IL-10 and/or TGF- β) and/or Th1 immune deviation (Th2 to Th1 shift in cytokine expression patterns), the production of these inhibitory cytokines within the nasal mucosa has the potential to suppress local allergen-specific Th2 cell responses and redirect antibody class switching in favor of IgG4 (IL-10 isotype switch factor), IgG (IFN- γ isotype switch factor), and IgA (TGF- β isotype switch factor).

In agreement with previous studies, our observations showed 40- to 100-fold increases in serum allergen-specific total IgG and IgG4 subclass antibodies and blunting of seasonal increases in IgE (84). It is possible that such antibody changes are important for

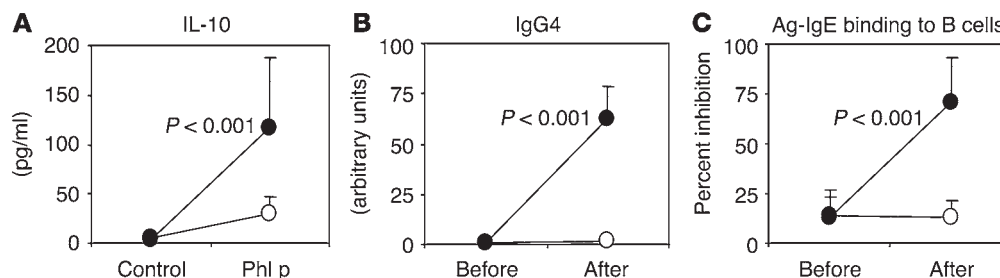


Figure 2

Immunological changes after allergen immunotherapy. Following 2-year grass pollen immunotherapy (closed circles), there were significant increases in (A) allergen-stimulated PBMC production of IL-10 (78); (B) serum concentrations of grass pollen (Phleum P5) allergen-specific IgG4 (84); and (C) serum inhibitory activity for allergen-IgE binding to B cells (88) compared with controls (open circles). These changes were accompanied by a reduction in symptoms and rescue medication use during the pollen season (83); inhibition of the allergen (grass pollen) induced late cutaneous response.

clinical efficacy of immunotherapy. For example, allergen-IgE-IgG complexes may coaggregate inhibitory Fc γ RIIb receptors with high-affinity Fc ϵ RI IgE receptors (85), resulting in the inhibition of Fc ϵ RI signalling (86, 87). Alternatively, they may inhibit IgE-facilitated allergen presentation to T cells (88), a rate-limiting step in allergen-specific Th2 T cell-driven allergic responses (89). Increases in peripheral IL-10 production, serum allergen-specific IgG4, and IgG-dependent serum-inhibitory activity for allergen-IgE binding to B cells after immunotherapy are shown in Figure 2. A hypothesis concerning the putative mechanism of allergen-injection immunotherapy is summarized in Figure 3.

Modifications of allergen immunotherapy

Despite the impressive efficacy of allergen-injection immunotherapy with whole allergen extracts, its widespread usage is confined to specialist centers in view of the risk of occasional IgE-mediated adverse events, which include systemic anaphylaxis. A number of strategies aim to modify immunotherapy for allergic disease in order to separate allergenicity (IgE cross-linking) from immunogenicity (induction of protective, non-IgE immunity).

Approaches have included the generation of allergoids through disruption of surface B cell epitopes of the protein. This has been achieved with amino-cross-linking agents such as glutaraldehyde

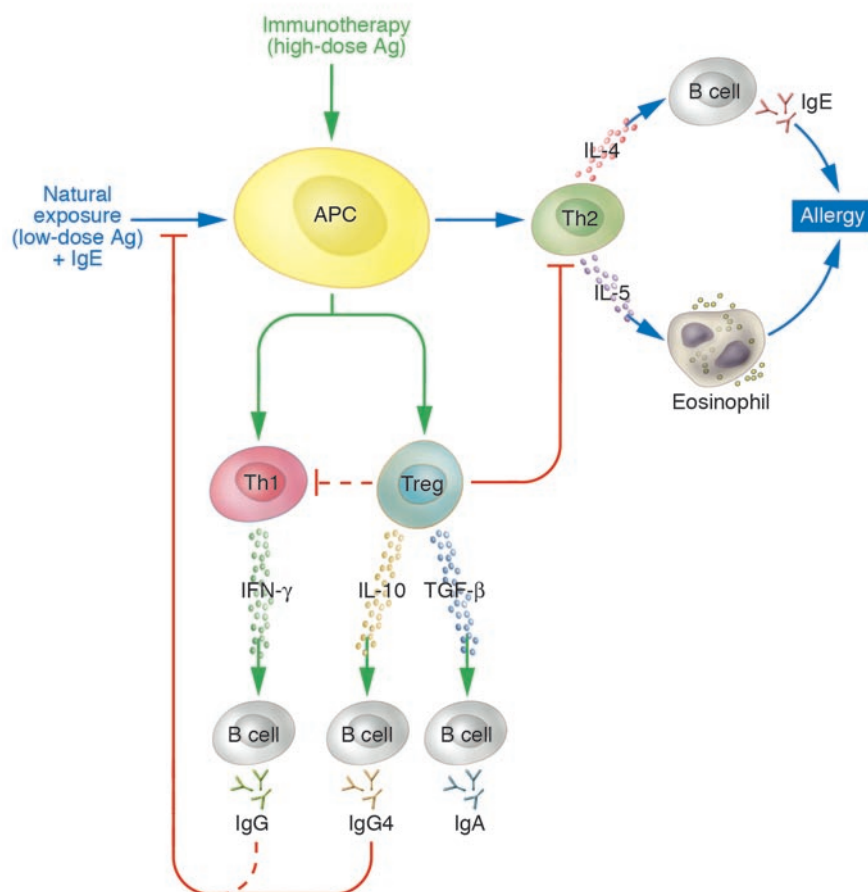


Figure 3

Potential mechanisms of conventional allergen immunotherapy. High-dose allergen exposure during immunotherapy results in both immune deviation of Th2 responses in favor of a Th0/Th1 response and in the generation of IL-10- and TGF- β -producing CD4⁺CD25⁺ T cells, possibly Tregs. IFN- γ -induced activation of bystander macrophages and/or other cells represents an alternative source of these inhibitory cytokines. During subsequent natural environmental exposure to allergens, the activation and/or maintenance of the usual atopic Th2 T cell response is inhibited. Additionally, these cytokines induce preferential switching of B cell responses in favor of IgG and IgG4 antibodies (and possibly IgA antibodies under the influence of TGF- β). IgG may also inhibit IgE-facilitated allergen binding to antigen-presenting cells with subsequent downregulation of IgE-dependent Th2 T lymphocyte responses. Blue arrows represent immune response pathway to natural exposure (low-doses Ag and IgE); green arrows represent immune response pathway to immunotherapy (high-dose Ag); red blocked lines represent inhibition (high-dose Ag); dotted lines represent possible means of action not yet proven.

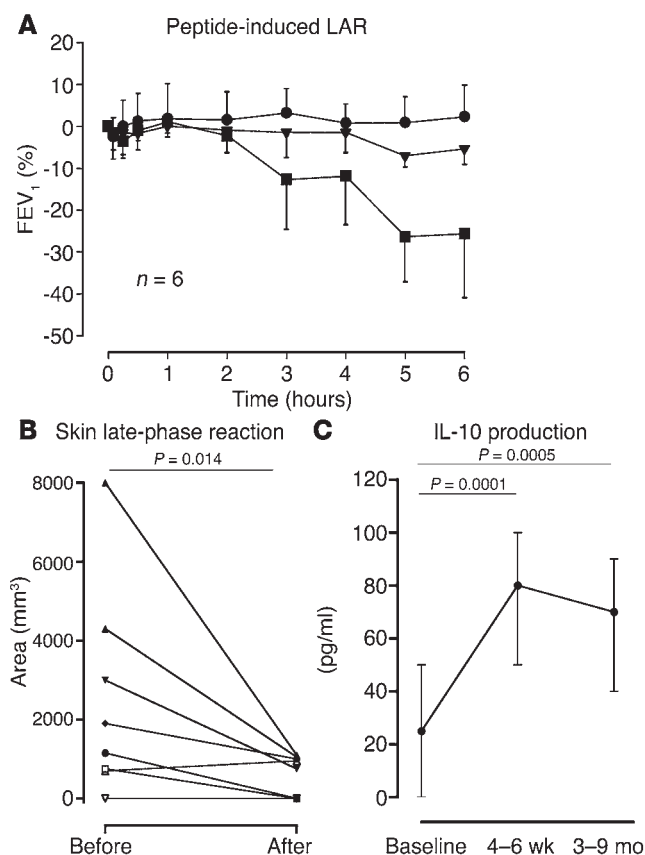


Figure 4

Peptide immunotherapy is associated with allergen-specific hyporesponsiveness and the induction of IL-10. **(A)** Cat-allergic asthmatic subjects ($n = 6$) were challenged intradermally with a mixture of 12 peptides ($5 \mu\text{g}$ each) from the sequence of Fel d 1 or vehicle alone. Lung function was measured by spirometry for 6 hours. Challenges were separated by 14 days or more. Challenge with vehicle (circles) did not significantly modify forced expiratory volume in one second (FEV₁). Initial peptide challenge (squares) resulted in an isolated LAR that significantly differed from baseline ($P = 0.02$ area under the curve; AUC). A second challenge (triangles) with the same dose of peptide was associated with an attenuated or absent LAR. Values are mean of 6 individuals with standard error. **(B)** Cat-allergic asthmatic subjects underwent intradermal allergen challenge (volar aspect of the forearm) before and after administration of a mixture of 11 peptides in incremental divided doses. Peptide treatment significantly reduced the magnitude of the cutaneous late-phase reaction. **(C)** Treatment of 16 cat-allergic asthmatic individuals with a mixture of 12 peptides (incremental divided doses) resulted in elevated production of IL-10 by peripheral blood mononuclear cells at both 4–6 weeks and 3–9 months after the completion of treatment. Data presented as median and interquartile range.

(or formalin). In general, allergoids have demonstrated efficacy and safety profiles similar to those described for unmodified allergen extracts (90). In other studies, the introduction of point mutations or deletions has been used to reduce the number of conformational B cell epitopes displayed by an allergen (91). More recently, allergen has been coupled to synthetic bacterial DNA sequences (92). In the case of DNA-allergen conjugates, the proposed mechanisms of action are 2-fold: steric hindrance of the interaction between allergen and IgE together with modulation of T cell responses toward a Th1 phenotype through activation of the TLR-9 molecule by bacterial DNA CpG motifs (93). Clinical studies evaluating the safety and efficacy of genetically engineered allergens (trimers and fragments of the major birch pollen allergen Bet v 1) and allergen-DNA conjugates (for example, immunostimulatory sequence-conjugated ragweed allergen) have thus far only been reported in abstract form.

During the last decade, synthetic peptides derived from the amino acid sequences of allergens have been evaluated for the treatment of allergic individuals. The majority of data has been obtained from the use of peptides derived from the major cat allergen Fel d 1, although more recently, studies have been performed with peptides from the bee venom allergen phospholipase A₂ (PLA₂). The potential of peptide immunotherapy to reduce IgE-mediated allergenicity while maintaining immunogenicity has been examined in a number of clinical studies in recent years.

Peptide immunotherapy

Peptide therapy has been used in several animal models of human disease in an attempt to modulate T cell responses (94–99). Peptides from a variety of antigens have been administered intra-

nasally, orally, or by injection, leading to reduced proinflammatory responses accompanied by the induction of immunoregulatory cytokines such as IL-10 and/or TGF- β . Evidence for the functional existence of Tregs has been obtained in some murine models. For example, intramolecular suppression was demonstrated following intranasal administration of house dust mite peptides (100). Amelioration of disease was achieved following adoptive transfer of cells (101). Recently, oral administration of peptides to subjects with rheumatoid arthritis (phase I, open study) was associated with reductions in the PBMC production of IL-2, IFN- γ , and TNF- α as well as an increase in the production of IL-10, and an increase in the expression of mRNA encoding *Foxp3* (102).

Short peptides (16/17 residues) representing T cell epitopes of the major cat allergen Fel d 1 did not trigger basophil histamine release or early asthmatic reactions upon intradermal injection or inhaled challenge in cat allergic asthmatic subjects (103–106). However, intradermal injection of allergen peptides did, in some individuals, lead to the induction of isolated late asthmatic reactions (LARs), which were MHC class II-restricted (103). Subsequent injection of peptides did not reproduce the response, implying the induction of tolerance (Figure 4). Development of peptide therapy based on these observations has enabled the safe delivery of molar doses of peptides far in excess of those possible with intact allergen molecules (103, 105).

The mechanism of abrogation of LAR and reduction of late-phase skin reactions to allergen challenge was investigated by analysis of in vitro responses of PBMCs to allergen before and after peptide therapy (106). Supernatants harvested from cultures of PBMCs of cat-allergic subjects receiving Fel d 1 peptides or bee venom-allergic subjects receiving PLA₂ peptides showed reductions in proliferation of CD4⁺ T cells and both Th1 and Th2 cytokine production, with a significant increase in IL-10 production. Similar results were reported by Müller and colleagues following PLA₂ peptide therapy (107).

Preliminary data from clinical studies with Fel d 1 peptides supports a central role for Tregs in the downregulation of allergic responses. An antigen-specific regulatory activity was present within the CD4⁺ peripheral T cell population after Fel d 1 peptide therapy (108). Comparison of peripheral blood CD4⁺CD25⁺ T



cell suppression of cat allergen-stimulated CD4⁺CD25⁻ T cells before and after peptide immunotherapy did not show any change in the suppressive ability of the CD4⁺CD25⁺ T cell subset despite significant reduction of CD4⁺ T cell responses to allergen in this double-blind study (109). Thus peptide therapy may induce a regulatory subset separate from CD4⁺CD25⁺ T cells, in other words, an adaptive rather than a naturally occurring regulatory population. However, analysis of 24-hour biopsy samples taken from allergen-induced cutaneous late-phase reactions to whole cat dander allergen extract showed increases in the number of CD4⁺CD25⁺ cells, increases in the number of CD4⁺IFN- γ cells, and increases in TGF- β expression following peptide therapy (110). Thus evidence for both immune deviation (increased IFN- γ expression) and an indication of regulation (TGF- β) were found in this study.

Conclusion

In summary, current evidence suggests that human CD4⁺CD25⁺ T cells and IL-10-producing Tregs have the capacity to suppress Th2 responses to allergen and that this process may be defective in those who develop allergic sensitization. Allergen immunotherapy modifies T cell responses to allergen and may do so through induction of IL-10-producing Tregs, although other mechanisms, including Th1 immune deviation, are probably involved. Controversy remains about the nature and interrelation of different Treg populations; it is likely that CD4⁺CD25⁺ T cells isolated from human adult blood represent a mixed population of naturally

occurring Tregs analogous to those studied in mice and adaptive Tregs induced by low- or high-dose allergen exposure. The exact mechanism of suppression also remains controversial and may differ for different regulatory populations. Further work is required to determine the phenotype and derivation of such cells and to determine whether modification of existing immunotherapy can make such treatment more widely applicable and more effective. Greater understanding of regulatory mechanisms in the development of allergic sensitization and their manipulation by immunotherapy holds the promise of vaccination strategies to prevent allergic disease.

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