

Molecular mechanisms in allergy and clinical immunology

Series editors: William T. Shearer, MD, PhD, Lanny J. Rosenwasser, MD, and Bruce S. Bochner, MD

Mechanisms of immunotherapy

Stephen J. Till, MD, PhD, James N. Francis, PhD, Kayhan Nouri-Aria, PhD, FRCPATH, and
Stephen R. Durham, MD *London, United Kingdom*

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Specific allergen injection immunotherapy is highly effective in IgE-mediated diseases, such as allergic rhinitis and venom anaphylaxis. Immunotherapy inhibits both early and late responses to allergen exposure. Immunotherapy is accompanied by increases in allergen-specific IgG, particularly the IgG4 isotype, which blocks not only IgE-dependent histamine release from basophils but also IgE-mediated antigen presentation to T cells. Immunotherapy acts on T cells to modify peripheral and mucosal T_H2 responses to allergen in favor of T_H1 responses. Recent studies have identified increased IL-10 production in peripheral blood and mucosal surfaces after immunotherapy. IL-10 has numerous potential antiallergic properties, including suppression of mast cell, eosinophil, and T-cell responses, as well as acting on B cells to favor heavy chain class switching to IgG4. These IL-10-producing cells might be so-called regulatory T cells and appear to be identified by the CD4⁺CD25⁺ phenotype. Studies in mice suggest that dendritic cells play a vital role in induction of regulatory T cells. Novel approaches to immunotherapy currently being explored include the use of adjuvants, such as monophosphoryl lipid A or nucleotide immunostimulatory sequences derived from bacteria that potentiate T_H1 responses. Alternative strategies include the use of allergen-derived peptides or modified recombinant allergen vaccines that act on T cells while minimizing the IgE-dependent mast cell activation that is dependent on the native allergen conformation. (*J Allergy Clin Immunol* 2004;113:1025-34.)

Key words: Immunotherapy, allergy, IgE, IL-10, regulatory T cells

Allergen injection immunotherapy is highly effective in carefully selected patients with IgE-mediated disease and represents the only routinely administered antigen-specific immunomodulatory treatment given for immu-

Abbreviations used

ITIM: Immunoreceptor tyrosine-based inhibition motif
MPL: Monophosphoryl lipid A

nologic disease of any kind. Immunotherapy has been shown to be effective for venom anaphylaxis and for rhinoconjunctivitis and asthma caused by inhalant allergens.^{1,2} Immunotherapy is particularly effective in seasonal pollinosis.³ Immunotherapy improves seasonal asthma, inhibits seasonal increases in bronchial hyper-responsiveness, and improves quality of life in patients with hay fever.⁴ Immunotherapy confers long-term benefit for at least 3 years after discontinuation.^{5,6} In children immunotherapy has been shown to prevent onset of new sensitizations⁷ and to reduce progression of rhinitis to physician-diagnosed asthma.⁸

Elucidation of mechanisms of immunotherapy has potential implications, not only for the refinement of allergy vaccines but also for the development of tolerogenic vaccines in other branches of medicine.

The allergen specificity of allergic disease and immunotherapy also means that effects of the allergen exposure might be observed either during experimental provocation in a clinical laboratory or during controlled natural exposure. This is in contrast to other immune diseases in which the antigen might be unknown or endogenous.

The natural history of an experimental allergen challenge to the nose, eyes, or bronchi is the immediate development of mast cell-dependent sneeze, itch, watery discharge, or wheeze—chest tightness that was maximal at 15 to 30 minutes and resolved within 1 to 3 hours. A proportion of subjects have a late allergic response that manifests in the nose as nasal obstruction and in the bronchi as airway obstruction. Classically, late responses are maximal at 6 to 12 hours and resolve within 24 hours. The mechanism is distinct from that of the immediate response, being characterized by the recruitment, activation, and persistence of eosinophils, basophils, and activated T cells at the sites of allergen exposure. The immunopathologic changes in the mucosal tissues of subjects chronically exposed to inhalant allergens resemble those seen during the late response.

From Upper Respiratory Medicine, National Heart and Lung Institute, Imperial College.

Disclosure of potential conflict of interest: S. J. Till—none disclosed. J. N. Francis—none disclosed. K. Nouri-Aria—none disclosed. S. R. Durham receives grants/research support from ALK-Abelló.

Received for publication March 14, 2004; revised March 14, 2004; accepted for publication March 16, 2004.

Reprint requests: Stephen R. Durham, MD, Upper Respiratory Medicine, Imperial College, National Heart and Lung Institute, Imperial College School of Medicine, Dovehouse St, London SW3 6LY. E-mail: stephen.till@imperial.ac.uk.

0091-6749/\$30.00

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doi:10.1016/j.jaci.2004.03.024

Cytokines produced by T cells probably play a major role in orchestrating allergic inflammation. T_H1 cells produce IFN- γ and IL-2 but not IL-4 or IL-5 after activation. T_H2 cells produce mainly IL-4, IL-13, and IL-5 but not IL-2 or IFN- γ . T_H2 cells characterize human allergic responses and are present at mucosal surfaces during the late but not immediate response to allergen exposure. T_H2 cells can also be expanded from peripheral blood of allergic patients by means of stimulation with specific allergen *in vitro*. The proallergic effects of IL-4, IL-13, and IL-5 produced by T_H2 cells are numerous and described elsewhere.

Factors that determine the evolution of either T_H1 responses, T_H2 responses, or both include the route and dose of antigen and the nature of the antigen-presenting cell. For example, high doses of allergen might preferentially favor the induction of T_H1 -type responses.⁹ Antigen-presenting cell subsets might direct the development of T_H1 and T_H2 responses. For example, DC1 and DC2 dendritic cell subsets have been implicated in the development of T_H1 and T_H2 responses, respectively.¹⁰ DC2-type dendritic cells have been identified in atopic subjects,¹¹ and their ability to drive T_H2 responses appears to relate to low levels of IL-12 expression.

Early research into immunotherapy mechanisms examined circulating antibody responses. More recently, the focus has been on T-cell responses. Most work has examined the effect of subcutaneous immunotherapy rather than immunotherapy by alternative routes. Mechanisms might have a degree of heterogeneity, reflecting variability in the types of allergic diseases treated, the different patient populations, the use of different adjuvants, and the route, dose, and duration of treatment.

SERUM ANTIBODY RESPONSES

In conventional pollen immunotherapy, serum IgE concentrations show little response,¹² although seasonal increases in IgE are blunted.¹³ A potential untoward effect of immunotherapy is the emergence of novel IgE responses to hitherto unrecognized allergenic components of the extract used for treatment,¹⁴ although the clinical significance of this phenomenon is unknown.

Immunotherapy with inhalant allergens is associated with increases in serum allergen-specific IgG1, IgG4, and IgA levels.^{12,15} In dust mite and venom immunotherapy, specific IgG4 responses are detectable within 60 days of starting treatment.^{15,16} IgG4 antibodies do not activate complement and have little or no inflammatory activity. IgG antibodies have therefore been proposed as blocking antibodies by competing with IgE for allergen binding to mast cells, basophils, and other IgE receptor-expressing cells. For example, IgG4 induced by means of immunotherapy blocks allergen-induced IgE-dependent histamine release by basophils¹⁷ and suppresses allergen-specific T-cell responses *in vitro* by inhibiting binding of IgE-allergen complexes to antigen-presenting cells.^{18,19}

Another potential anti-inflammatory effect of IgG could be mediated through the coaggregation of inhibitory Fc γ RIIb receptors with high-affinity Fc ϵ RI IgE receptors.²⁰ Fc γ RIIb receptors possess an immunoreceptor tyrosine-based inhibition motif (ITIM) that can be phosphorylated by Fc ϵ RI-associated kinases. Phosphorylated ITIM in turn binds intracellular phosphatases that mediate reciprocal inhibition of Fc ϵ RI signaling.²¹ Thus Fc ϵ RI can cause ITIM-dependent autoinhibition of its own signaling. Potentiation of Fc γ RIIb-Fc ϵ RI interaction by a chimeric Fc γ -Fc ϵ construct resulted in inhibition of allergen-IgE-dependent histamine release by human basophils.²² Whether immunotherapy results in formation of multivalent IgE-IgG allergen complexes that mediate a similar effect is unknown. The particular properties of allergen-specific IgA antibodies induced by means of immunotherapy have also yet to be determined.

A traditional objection to the blocking antibody model is the weak correlation between IgG concentrations and the clinical response to treatment.^{23,24} For example, immunotherapy in rush protocols is effective long before any changes in antibody synthesis are detected. However, this interpretation might be overly simplistic: Michils et al²⁵ observed the usual increase in IgG titers to venom immunotherapy but showed that this was preceded by a change in the fine specificity of IgG antibodies. Remarkably, changes in allergen-IgG binding were apparent within the first few hours of ultrarush immunotherapy²⁶ and were sustained when patients were followed up at 6 months.²⁵ Similarly, in murine models of immunotherapy, high concentrations of allergen have been shown to alter antibody affinity and specificity, as well as quantity.²⁷ Immunotherapy could act to change both the character and overall amounts of allergen-specific IgG. These observations stress the importance of studying the activity of allergen-specific IgG, as a blocking antibody or otherwise, as opposed to measuring crude levels in sera.

T-LYMPHOCYTE RESPONSES

Peripheral responses

The central role of T cells in directing allergic responses has generated a number of hypotheses regarding their role in immunotherapy. Investigations have addressed mechanisms such as immune deviation away from a T_H2 phenotype toward a more nonpathogenic T_H1 phenotype, inhibition of antigen presentation to allergen-specific T cells, and, more recently, suppression of responses by T cells with regulatory activity.

Most studies addressing *in vitro* T-cell responses to allergen have examined cells isolated from peripheral blood. Early studies of patients treated with venom or inhalant allergen immunotherapy reported a reduction in proliferative responses to allergen,^{15,28-31} with an overall shift away from a T_H2 to a T_H1 response.^{28,30,32} However, other studies, including 3 of our own based on different cohorts of patients with pollen immunotherapy, have not

reproduced these findings.³³⁻³⁵ A possible explanation is that inhibition of peripheral T-cell proliferation and T_H2 cytokine production is not the fundamental event in immunotherapy. Cultures of PBMCs might provide only a crude reflection of immune interactions and responses in lymphoid tissues, mucosal tissues, or both.

In contrast, the detection after immunotherapy of peripheral blood T cells that produce IL-10 in response to allergen stimulation has emerged as a highly consistent finding. Bellinghausen et al³⁶ were the first to describe IL-10 production after venom immunotherapy. Akdis et al³⁷ similarly described an increase in IL-10 production in response to venom immunotherapy, and this was superimposed on a global suppression of T-cell cytokine and proliferative responses to stimulation with venom allergen *in vitro*. The same investigators observed a similar IL-10 response to venom allergen *in vitro* in beekeepers who became naturally tolerized to venom after repetitive stings.

An important question concerns whether IL-10 plays a role in the induction of clinical tolerance observed with immunotherapy to inhalant allergens, and 2 recent studies have specifically addressed this issue. Jutel et al¹⁵ investigated immunotherapy with house dust mite. Allergen-induced IL-10 and TGF- β production by T cells occurred in parallel to a global suppression of T_H2 proliferative responses and cytokine production. IL-10 and TGF- β were detectable at 7 days into a semirush immunotherapy protocol, and this activity copurified with CD4⁺CD25⁺ T cells. Similar IL-10 responses were observed in healthy nonatopic subjects exposed to allergen. The implication was that immunotherapy restored a tolerant T-cell response in atopic subjects similar to that observed in healthy individuals. The second study also identified CD4⁺CD25⁺ T cells as a source of IL-10 after grass pollen immunotherapy, whereas no changes in grass pollen-induced proliferation or T_H2 cytokine production were observed.³⁵ Although allergen-dependent IL-10 production is detectable in peripheral blood T cells, it is likely that in mucosal diseases, such as allergic rhinitis, the clinically important effects of IL-10 occur within the mucosa or possibly within the draining lymphoid tissue. Local factors to be considered include the ratio of IL-10-producing cells to other cells, cell-cell contact, the effect of microenvironmental cytokines, the presence of mature antigen-presenting cells, and cytokine trapping by extracellular matrix proteins.

An important question is how allergen-specific T cells are recruited to allergic tissue sites. A possible mechanism underlying immunotherapy might be altered chemokine receptor expression by peripheral blood T cells. Chemokines are small chemoattractant molecules that act through specific receptors on leukocytes. Different subsets of immune cells, such as T_H2 cells, might be subject to selective recruitment as a result of restricted expression of certain chemokine receptors. Recent studies have identified the presence of functional CXCR1 on CD4⁺ T cells from allergic patients but not on those from control subjects. CXCR1 is a receptor for IL-8 and has traditionally been associated with high surface expression

on neutrophils. However, as a result of screening atopic allergic subjects and nonatopic control subjects for T-cell surface expression for a whole panel of known chemokine receptors, CXCR1 emerged consistently as a prominent T-cell surface receptor associated with allergic inflammation, including allergen-induced late asthmatic responses.³⁸ When compared with current symptomatic patients with allergic rhinitis, CXCR1 expression on circulating CD4⁺ T cells from patients receiving grass pollen immunotherapy was low or absent.³⁸ The effect of immunotherapy on other T-cell chemokine receptors remains to be determined.

T-lymphocyte responses in tissue

T-cell responses after grass pollen immunotherapy have been examined in nasal mucosal and skin tissue after grass pollen immunotherapy. Nasal and cutaneous biopsy specimens were collected from patients and allergic control subjects during randomized controlled trials of immunotherapy, either after allergen challenge or during natural seasonal exposure. Cytokine production was examined *in vivo* by use of *in situ* hybridization with specific riboprobes, identifying specific cytokine mRNAs. Although treatment appeared to be associated with reduced accumulation of T cells in skin and nose after allergen challenge, there was no attenuation of T-cell numbers in the nasal mucosa during natural seasonal exposure, suggesting that factors other than a reduction in total T cells must account for clinical efficacy.

The first study to describe a shift of allergen-induced T-cell responses in favor of T_H1 cytokine synthesis was published by Varney et al in 1993.³⁹ After 1 year of a controlled trial of grass pollen immunotherapy, intradermal challenge with grass pollen extract was associated with a reduction in the cutaneous late-phase response. When this site underwent biopsy at 24 hours, contrary to expectations, a significant reduction in numbers of IL-4 or IL-5 mRNA-expressing cells was not observed. On the other hand, modest but significant increases in IFN- γ and IL-2 mRNA-expressing cells were observed, consistent with local immune deviation in favor of a T_H1 response. Subsequently, skin biopsy specimens collected after 4 years of immunotherapy were examined for expression of mRNA encoding IL-12, a potent regulator of T_H1 responses.⁴⁰ IL-12 mRNA expression did indeed increase after immunotherapy and correlated positively with IFN- γ mRNA expression and inversely with IL-4 expression.⁴¹

It seems likely that in allergy to inhaled allergens, it is studies of the immunologic changes within the respiratory mucosa (ie, the site of disease) that are of greatest relevance. Nasal mucosal biopsy specimens were therefore collected 24 hours after intranasal allergen provocation from a cohort of immunotherapy- and placebo-treated patients after 1 year's treatment. Consistent with the skin model, immunotherapy was associated with increases in allergen-dependent IFN- γ mRNA expression within the nasal mucosa, without reductions in IL-4 and IL-5 mRNA. Furthermore the increases in IFN- γ observed after allergen

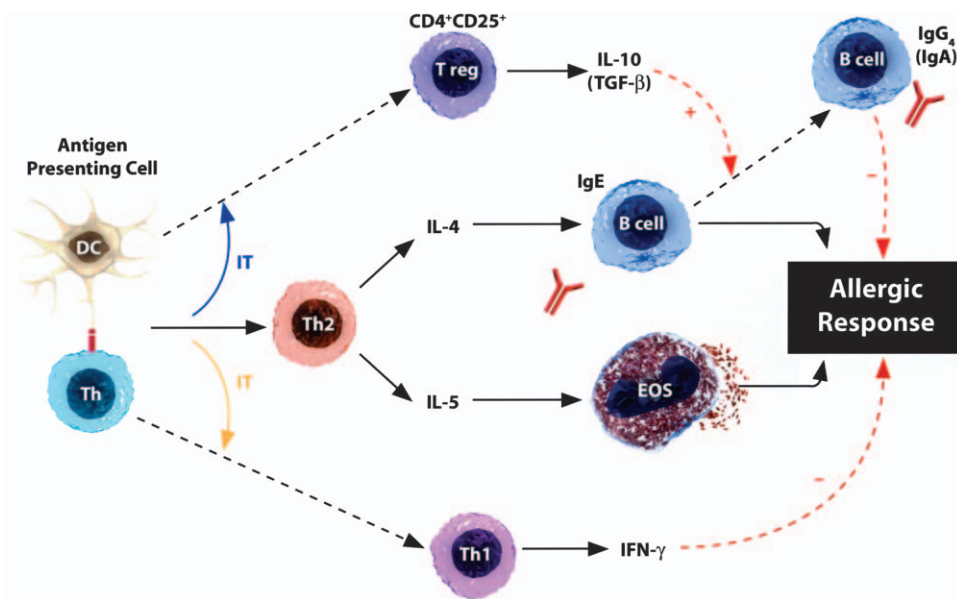


FIG 1. Summary of the effects of immunotherapy on T-cell responses. Immunotherapy readdresses the balance between T_H2/T_H1 responses in favor of T_H1 responses. An increase in IL-10–producing T cells, possibly regulatory T cells is also seen. The relationship between these events remains controversial. *T reg*, T regulatory cell; *DC*, dendritic cell; *EOS*, eosinophil.

challenge outside the pollen season correlated closely with the clinical response to treatment.⁴² Subsequently, cytokine mRNA expression has been examined in nasal biopsy specimens of patients receiving grass pollen immunotherapy in response to natural pollen exposure (ie, during the summer pollen season).⁴³ Seasonal increases in nasal mucosal IFN- γ and IL-5 mRNA expression were only observed in immunotherapy- and placebo-treated patients, respectively, and the ratio of IFN- γ /IL-5 expression was significantly higher in the immunotherapy-treated patients.³⁴ A further study examined the effect of immunotherapy with modified birch pollen allergens on cytokine concentrations in nasal lavage fluid during the pollen season and found changes in protein concentrations consistent with our findings at the cytokine mRNA level.⁴⁴ Although nasal IFN- γ concentrations were increased and IL-5 levels were decreased in the actively treated group, these investigators were also unable to identify any modulation of peripheral blood T-cell cytokine responses after immunotherapy in the same subjects. These findings further support the concept that local, rather than peripheral, immune modulation is necessary for clinically successful immunotherapy.

Local nasal IL-10 responses have also been examined during the pollen season. IL-10 mRNA–expressing cells were found to increase in the nasal mucosa of grass-sensitive patients during the pollen season after immunotherapy. Expression of IL-10 was allergen driven, as well as immunotherapy dependent, because IL-10 mRNA–positive nasal mucosal cells were not identified in untreated atopic subjects nor in immunotherapy-treated patients outside the pollen season.⁴⁵ Moreover, the expression of IL-10 did not appear to occur as part of

a restoration of the normal mucosal immune response because IL-10 expression was minimal in nasal biopsy specimens obtained from nonatopic control subjects during the pollen season. This observation of IL-10 within allergen-exposed tissue is not unique: IL-10 mRNA–positive cells have been described in the skin after intradermal allergen injection in wasp-sensitive patients after venom immunotherapy.⁴⁶ Furthermore, in patients who received intranasal immunotherapy with weed extracts, an increase in IL-10 concentration was detected in nasal lavage fluid during the pollen season.⁴⁷

An important unanswered question concerns the relationship between a T_H2 to T_H1 shift and induction of IL-10 (Fig 1). Akdis and Blaser⁴⁸ noted that addition of IL-2 to venom-stimulated T cells from immunotherapy-treated patients restored proliferation and T_H1 but not T_H2 cytokine production. IL-10 might block B7/CD28 costimulation.⁴⁹ When the B7/CD28 costimulation pathway is blocked by antibodies, IL-2 can rescue allergen-dependent T-cell proliferation but not IL-5 production.⁵⁰ Thus microenvironmental IL-2 produced in the nasal mucosa could act in combination with IL-10 to cause T_H2/T_H0 to T_H0/T_H1 immune deviation. An alternative explanation is that these changes might occur through independent but superimposed mechanisms. For example, immunotherapy might induce IL-10–producing regulatory T cells (discussed below) but also separately modify antigen-presenting cell–T-cell interactions so as to increase T_H1 cytokine expression by allergen-specific T cells.

IL-10 and regulatory T cells

IL-10 is an 18.7-kd protein expressed by a variety of human immune cells, including both T_H1 and T_H2 cells, B

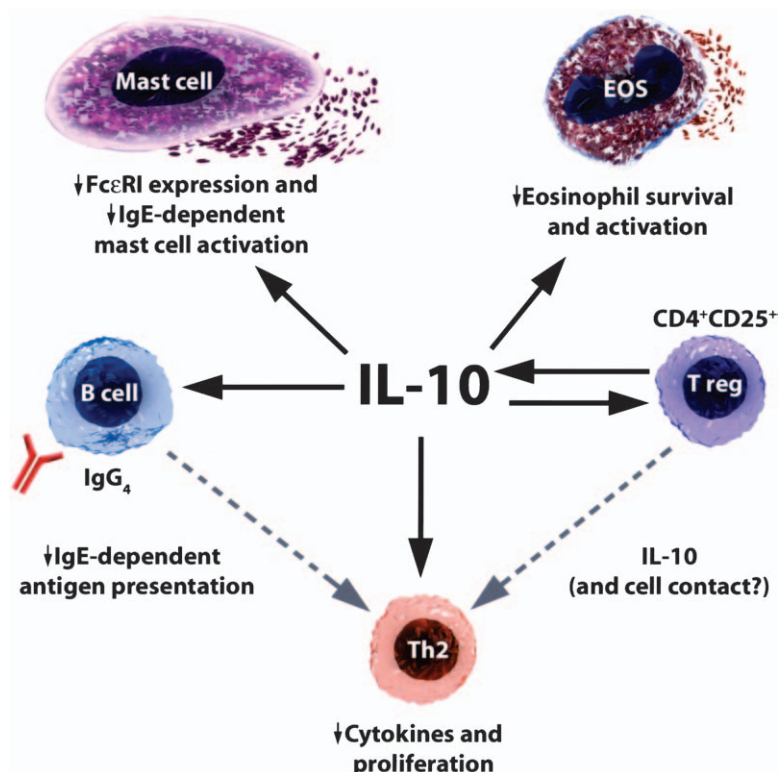


FIG 2. Summary of the potential antiallergic properties of IL-10 on different limbs of the allergic immune response. EOS, Eosinophil; T_{reg}, T regulatory cell.

cells, monocytes-macrophages, dendritic cells, mast cells, and eosinophils. In mouse models IL-10 has been associated with suppression of schistosomal egg-induced delayed-type hypersensitivity,⁵¹ graft rejection,⁵² inflammatory arthritis,⁵³ experimental autoimmune encephalomyelitis (an animal model of multiple sclerosis),⁵⁴ colitis,⁵⁵ and allergic inflammation.^{56,57} IL-10 has a number of documented antiallergic properties that might be important to immunotherapy (Fig 2, reviewed by Bellinghausen et al⁵⁸). These include modulation of IL-4–induced B-cell IgE production in favor of IgG₄,⁵⁹ inhibition of IgE-dependent mast cell activation,⁶⁰ and inhibition of human eosinophil cytokine production and survival.⁶¹ In human T cells IL-10 suppresses production of proallergic cytokines, such as IL-5,³⁵ and is able to induce a state of antigen-specific hyporesponsiveness or anergy.⁵⁵ This might occur as a result of IL-10 receptor–dependent blockade of CD28 T-cell costimulation because CD28 tyrosine phosphorylation and subsequent signaling in T cells in response to ligation by B7 molecules on antigen-presenting cells is inhibited by IL-10.⁴⁹

The properties of T cells producing IL-10 after immunotherapy are contentious, including the immunologic mechanisms that give rise to them. However, studies in mice and to a lesser extent in human subjects have suggested the presence of various types of T cells with regulatory activity (ie, T cells with inhibitory effects on immune responses), including T_R cells (producing IL-10

and possibly TGF-β), CD4⁺CD25⁺ T cells (possibly TGF-β), and T_H3 cells (also TGF-β).⁶² Suppression might be mediated by cell-cell contact (eg, through CTLA-4), as well as through the production of IL-10, TGF-β, or both. The relationships between these different regulatory cell populations are also controversial. T_H3 cells seem likely to be involved in the regulation of gastrointestinal immune responses, whereas CD4⁺CD25⁺ cells might be involved in inducing T_R differentiation.⁶² The findings from bee venom,^{36,37,46} conventional house dust mite,¹⁵ and grass pollen immunotherapy,³⁵ do not, however, fall clearly within this proposed framework. IL-10–producing cells have been described after each of these treatments. In studies based on peripheral blood T cells in which IL-10 could be easily colocalized to phenotypic markers, production was identified almost exclusively with CD4⁺CD25⁺ T cells.^{28,35,37} In the case of house dust mite immunotherapy, these CD4⁺CD25⁺ T cells produced both IL-10 and TGF-β and suppressed immune responses to allergen *in vitro*.¹⁵ Moreover, in Varney et al's³⁹ original immunohistochemical description of biopsy specimens taken from allergen-challenged skin, an increase in CD25⁺ cells was observed in grass pollen immunotherapy–treated patients but not control subjects. Therefore it seems likely that the production of IL-10 and TGF-β by T cells represents an important component of successful immunotherapy or at least is a marker of successful downregulation of allergen-specific T-cell

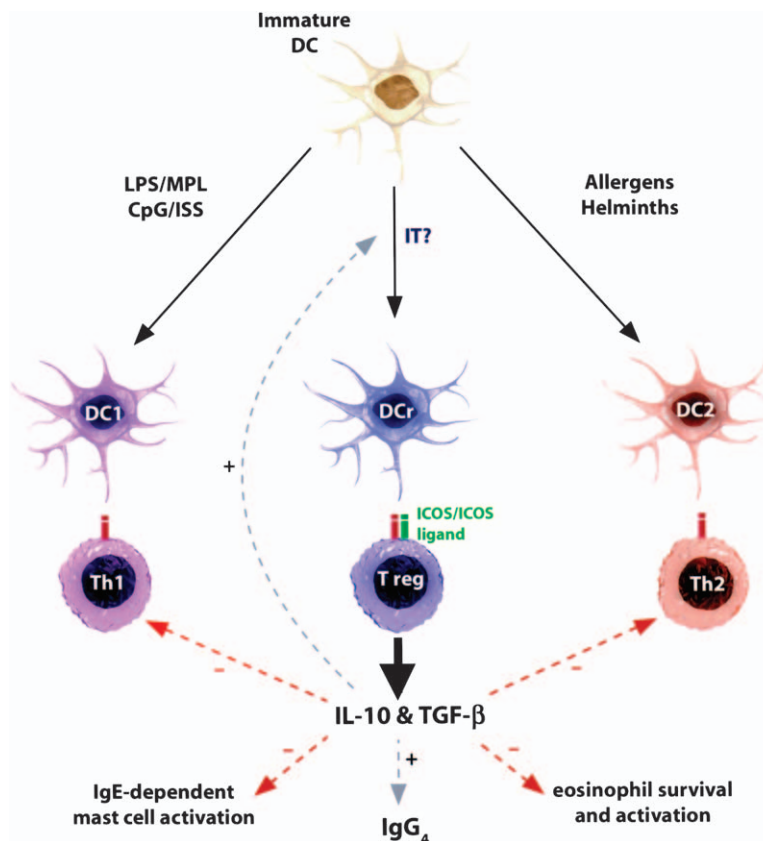


FIG 3. Proposed model for the role of dendritic cells in directing T-cell responses. Allergens and parasites might promote development of DC2 dendritic cells through unknown mechanisms. DC2 dendritic cells direct T_H2 responses and allergic inflammation. LPS and CpG-rich DNA derived from bacteria promote differentiation of DC1 cells that direct T_H1 responses. Monophosphoryl lipid A (MPL) and immunostimulatory sequences (ISS) derived from each of these are being used as novel adjuvants for immunotherapy, with the aim of augmenting T_H1 responses. Immunotherapy might direct the development of protolerogenic regulatory dendritic cells (DCr) that stimulate the development of regulatory T cells (Treg) in a mechanism requiring IL-10 production by DCr and ICOS/ICOS-ligand interaction. Formation of dendritic cells with proregulatory activity might itself be augmented by IL-10. Adapted from Curotto de Lafaille and Lafaille.⁶⁹

responses after immunotherapy. If these T cells are regulatory T cells, are they truly defined by a $CD4^+CD25^+$ phenotype? Because CD25 is also expressed by activated T cells, it is tempting to view $CD4^+CD25^+$ cells as the coincidental result of T-cell activation because T cells are in many cases stimulated *in vitro* to elicit cytokine production. However, since Jutel et al¹⁵ showed that suppressive activity and IL-10 and TGF- β production compartmentalized to peripheral $CD4^+CD25^+$ rather than $CD4^+CD25^-$ cells fractionated before stimulation, it seems likely that this phenotype represents more than an artifact, although this requires confirmation.

The biology of regulatory T-cell induction is relatively poorly understood and the subject of intense ongoing research. Dendritic cells seem likely to play a critical role. In murine asthma models T-cell tolerance and protection against airway pathology can be achieved by means of intranasal exposure to aeroallergen and is associated with the development of IL-10-producing pulmonary dendritic cells (Fig 3).⁵⁷ These dendritic cells could adoptively transfer protection against allergen to other

animals and stimulated development of IL-10-producing regulatory T cells in a mechanism involving T-cell costimulation through the ICOS/ICOS-ligand pathway.⁶³ The primary mechanism that gives rise to these tolerogenic dendritic cells is therefore of great interest because this might represent the fundamental mechanism that underlies allergen immunotherapy. There is some evidence that this process might itself also be IL-10 driven because dendritic cells exposed to IL-10 *in vitro* can inhibit T-cell responses in human subjects⁶⁴ and mice.⁶⁵ Nevertheless, this still does not account for the initial source of IL-10 that would be necessary to initiate the response. It has, however, recently emerged that specific antigen proteins can interact with murine dendritic cells to direct de novo IL-10 synthesis and subsequent development of regulatory T cells.⁶⁶ It is therefore possible that the allergen extracts used for immunotherapy could act directly on dendritic cells to induce a tolerogenic phenotype. Nevertheless, there remain other possibilities: induction of human regulatory T cells has been associated with stimulation by immature dendritic cells,^{67,68} perhaps

through incomplete T-cell stimulation. By analogy, could repeated cutaneous injections of allergen be taken up and presented by immature dendritic cells in the skin? Furthermore, induction of regulatory T cells has been associated with high-avidity T-cell receptor stimulation in mice (see Curotto de Lafaille and Lafaille⁶⁹ for review). By analogy, might repeated high-dose allergen exposure modify human antigen-presenting cell–T-cell interactions in such a way as to induce regulatory T-cell differentiation?

NOVEL STRATEGIES FOR IMMUNOTHERAPY

Modern immunotherapy with standardized extracts in a specialist setting is a safe form of treatment. However, the administration of native allergen might occasionally give rise to severe IgE-mediated systemic reactions and, rarely, anaphylaxis. Consequently, there has been interest in the development of vaccines that reproduce the modulation of T-cell responses seen with conventional immunotherapy without the potential to cross-link IgE on mast cells, thereby eliminating the risk of anaphylaxis. One approach has been to develop recombinant genetically modified allergen proteins that show reduced IgE binding while still containing the relevant T-cell epitopes. For example, genetically engineered recombinant fragments and a concatemeric trimer of the major birch pollen allergen Bet v 1 have been shown to have hypoallergenic activity when tested in the skin⁷⁰ and nose,⁷¹ although their efficacy in immunotherapy has yet to be evaluated. A theoretic additional advantage of using recombinant allergen proteins for immunotherapy is the avoidance of the development of new IgE responses to allergenic components of the crude whole pollen extract used for treatment. On the basis of the same rationale, other investigators have proposed using allergen-derived peptides that do not bind IgE because of the absence of tertiary structure but that do stimulate T cells. Muller et al¹⁶ administered bee venom–derived peptides to a few patients, although in the absence of a placebo control, claims of efficacy can only be regarded as anecdotal. However, treatment with allergen peptides did inhibit T-cell responses *in vitro*, and after undergoing a sting challenge, patients had IgG4 responses similar to those seen in patients treated with whole venom allergen. These observations suggest that development of IgG4 responses after immunotherapy might require exposure to T-cell and B-cell epitopes as in conventional immunotherapy vaccines or through a combination of a peptide vaccine and subsequent natural allergen exposure. In patients with allergy to cats, 27 amino acid peptides derived from the cat allergen Fel d 1 were given subcutaneously, and results showed evidence of modest efficacy.⁷² Others have extended this work to look at smaller peptide vaccines administered through the intradermal route. In a trial of cat allergen peptides, inhibition of peripheral blood T-cell responses *in vitro* was accompanied by modest reductions in early and late cutaneous responses to allergen⁷³ but did not result in

a significant improvement in symptoms over placebo treatment, possibly because of the low number of subjects studied. On the other hand, as a side effect of administration of cat peptides, up to half of asthmatic patients had isolated late asthmatic responses, as evidenced by a decrease in FEV₁ after several hours, without a preceding classic IgE-dependent early response.⁷³

Alternative strategies for immunotherapy include the use of novel adjuvants to potentiate the ability of allergen vaccines to induce T_H2 to T_H1 immune deviation. One such adjuvant is 3-deacylated monophosphoryl lipid A (MPL), which is derived from LPS. MPL is a promoter of T_H1 responses, perhaps through induction of IL-12 production by antigen-presenting cells,⁷⁴ and has been successfully used as an adjuvant in viral vaccines.⁷⁵ In a double-blind placebo-controlled trial a tyrosine-absorbed glutaraldehyde-modified grass pollen extract containing MPL reduced hay fever symptoms and medication requirements and increased allergen-specific IgG levels.⁷⁶ However, further studies are needed because it is unclear whether this vaccine offers a significant advantage over conventional non-MPL–containing extracts in terms of efficacy and safety. Similarly, immunostimulatory sequences of DNA containing CpG motifs stimulate T_H1 responses by means of a mechanism that probably involves induction of macrophage IL-12 production, dendritic cell IL-12 production, or both⁷⁷ and inhibit airway inflammation in murine models of asthma.⁷⁸ Immunostimulatory sequences appear to be even more effective as an adjuvant for murine and human T_H1 responses when directly conjugated to allergen.^{79,80} An immunostimulatory sequence–ragweed allergen (Amb a 1) conjugate suppressed murine airway eosinophilia and hyperresponsiveness.⁸¹ A short course of 6 escalating doses of the conjugate in ragweed-sensitive adults was associated with reduced nasal mucosal eosinophilia, reduced IL-4 expression, and increased IFN- γ expression (ie, T_H2 to T_H1) on nasal rechallenge with ragweed allergen.⁸²

THERAPEUTIC IMPLICATIONS

1. Immunotherapy is highly effective in selected patients with IgE-mediated disease and sensitivity to one or a limited number of allergens.
2. Immunotherapy is the only antigen-specific immunomodulatory treatment in routine use.
3. Immunotherapy has been shown to provide long-term benefit and is the only currently available treatment that modifies the natural history of allergic disease for at least several years after discontinuation.
4. Immunotherapy inhibits allergen-induced late responses in the skin, nose, and lung.
5. Immunotherapy increases serum allergen-specific IgG levels, particularly IgG4. These antibodies block the biologic effects of IgE *in vitro*, although the clinical importance of these effects remains to be evaluated.

6. Immunotherapy alters the T_H2/T_H1 balance in favor of T_H1 responses.
7. Immunotherapy induces IL-10-producing T cells, which might be regulatory T cells. They have been detected in both the peripheral blood and in the nasal mucosa after immunotherapy. IL-10 has numerous potential antiallergic properties against mast cells, T cells, and eosinophils. It also promotes IgG4 production by B cells.
8. The efficacy and safety of immunotherapy might be improved by novel strategies that directly target the T-cell response. These include genetically modified non-IgE-binding recombinant allergens, allergen-derived peptides, and novel T_H1 -promoting adjuvants derived from bacteria, such as MPL and immunostimulatory sequences. Results of further controlled trials are awaited.

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