

4 Immunologic Responses to Subcutaneous Allergen Immunotherapy

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INTRODUCTION

Immunotherapy is highly effective in appropriately selected patients with allergic disease. Whereas conventional vaccination strategies are employed to initiate and then boost immunologic memory, allergen immunotherapy aims to subdue established immune responses mediated by IgE and allergen-specific memory T cells through controlled exposure to the offending allergen. Since subcutaneous injection [subcutaneous allergen immunotherapy (SCIT)] of sufficient native allergen to invoke immunoregulatory mechanisms can trigger unwanted IgE-mediated reactions, the amounts of allergen contained in injections are increased incrementally from low levels until a safe but sufficient maintenance dose can be achieved. Patient selection is important and the risk/benefit ratio must be assessed on an individual basis. The underlying mechanisms are important since they provide insight into the mechanisms of allergic disease and induction of clinical tolerance. In addition, allergen injection immunotherapy is allergen-specific. This enables one to observe the effects of specific modulation of the immune response in a patient in whom the provoking factor(s) (common aeroallergen or venom) are known. The effects of the allergen exposure may be observed either during experimental provocation in a clinical laboratory or during natural environmental conditions. Similarly, the influence of immunotherapy on clinical, Immunologic, and pathologic changes may be observed under controlled conditions. This is in contrast to other Immunologic diseases where the antigen is unknown and no antigen-specific treatment is available. Sublingual allergen immunotherapy (SLIT) is emerging as a viable clinical alternative to the traditional subcutaneous route, although there are very few published, well-controlled mechanistic studies (Chap. 2). This chapter will therefore focus entirely on mechanisms of injection immunotherapy, reflecting a collective research effort spanning several decades.

SCIT results in a rapid inhibition of allergen-induced late responses, with a slower and proportionately smaller decline in early responses. Biopsies taken from skin and nasal mucosa reveal reductions in inflammatory cell numbers, including mast cells, basophils, and eosinophils. Around six to eight weeks after starting weekly immunotherapy up dosing injections, increases in allergen-specific IgG, particularly of IgG4 isotype, are observed. These antibodies block IgE effector mechanisms, including basophil histamine release and IgE-facilitated antigen presentation to T cells. Induction of allergen-specific IgA is also observed, and these antibodies can induce monocytic cells to produce interleukin-10, an immunoregulatory cytokine. These humoral responses likely reflect modulation of allergen-specific T-cell responses. Immunotherapy modifies peripheral and mucosal Th2 responses to allergen in favor of Th1 cytokine and IL-10 production (Fig. 1). The latter may be a key early event and IL-10-producing T cells are detectable within a few weeks of the first injection. IL-10 inhibits mast cell, eosinophil, and T-cell responses, as well as acting on B cells to favor IgG4 production. These IL-10 producing T cells may be so-called T_{R1} -type inducible regulatory T cells. The mechanism leading to development of these cells is yet to be elucidated, though similar populations can be experimentally induced by tolerogenic dendritic cells.

While current treatment regimes are effective, refinement of immunotherapy, both in terms of efficacy and safety profile, remains an important goal. In addition, the possibility of developing immunoregulatory vaccines for nonallergic immune diseases ensures considerable ongoing interest in underlying mechanisms. Novel approaches being clinically tested include the combination of allergens with immunomodulatory adjuvants to potentiate responses. These include bacterially derived modified lipid compounds or CpG-rich immunostimulatory

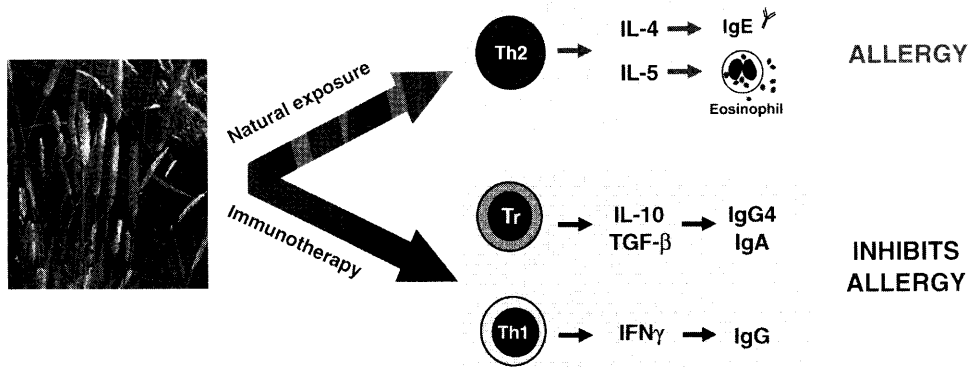


Figure 1 The effects of immunotherapy on T-cell responses: immunotherapy readdresses the balance between Th2/Th1 responses, in favor of Th1 responses. An increase in IL-10 and TGF- β -producing T cells, possibly regulatory T cells, has also been reported. IL-10 directly inhibits activation of inflammatory leukocytes including T cells, mast cells, and eosinophils. Additionally, IL-10 favors IgG4 production, which can block IgE-dependent pathways of effector cell activation.

oligodeoxynucleotides, which act through toll-like receptor-4 and receptor-9, respectively. Alternative strategies include the use of allergen-derived peptides or modified recombinant allergen vaccines. These aim to maintain the beneficial effects of vaccines while minimizing the immediate IgE-dependent complications, which currently require SCIT to be conducted cautiously and under specialist supervision.

THE ALLERGIC RESPONSE

Early and Late Responses

Experimental allergen exposure in the nose, eyes, or bronchi leads to development of mast cell-dependent sneeze, itch, watery discharge, and bronchospasm, maximal at 15 to 30 minutes and resolving within 1 to 3 hours. This "early response" is triggered by activation of mast cells through cross-linking of allergen-specific IgE molecules pre-bound to high-affinity IgE receptors (Fc ϵ RI). The sequela of this activation is the release of numerous preformed and newly synthesized mediators, including histamine, tryptase, TAME-esterase, bradykinin, leukotrienes (including LTC₄, LTD₄, and LTE₄), prostaglandins [including PGF_{2 α} and PGD₂ (specific for mast cells)], and platelet-activating factor. These mediators collectively induce vasodilatation, increased vascular permeability, mucosal edema, increased mucus production from submucosal glands and goblet cells within the respiratory epithelium, and smooth muscle contraction in the lower respiratory tract.

In a proportion of individuals, the early response resolves to be followed by a late response. Airflow obstruction is usually the predominant symptom in both upper and lower airways. Skin challenge testing provokes similar cutaneous early and late responses with wheal and flare followed by late-onset localized edema and tenderness. Late responses are maximal at 6 to 12 hours and resolve within 24 hours. The late response is characterized by recruitment of eosinophils, basophils, activated T cells, and dendritic cells to the site of allergen exposure. These activated leukocytes are a rich source of potentially pathogenic mediators such as Th2 cytokines (IL-4, IL-5, IL-9, and IL-13), which modulate inflammatory cell function and upregulate adhesion molecule expression and B-cell IgE synthesis. The late response is also at least partially dependent on IgE-mediated mechanisms since anti-IgE therapy (omalizumab) is associated with inhibition of cutaneous allergen-induced late responses in addition to early responses (1). The immunopathologic changes in the mucosa during the late response are largely representative of those seen during "natural" chronic allergen exposure (e.g., during the pollen season).

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Figure 2
The allergic
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Effects of SCIT

SCIT inhibits the late responses in the skin (2), nose (3), and lung (4), though it has not been established that this effect is predictive of clinical efficacy. In comparison, the effect on the early response appears to be relatively modest (and variable). For example, certain investigators describe only temporary inhibition of the early response in the skin (5) and no inhibition in the lung (4). Within a group of house-dust mite-sensitive children, suppression of the early skin response appears to be predictive of prolonged suppression following discontinuation of treatment, though this requires confirmation in a prospective study (6). The evolution of early and late response inhibition has been examined during a conventional grass pollen immunotherapy regime (7) (Fig. 2). Remarkably, late responses to intradermal challenge testing are reduced two weeks into treatment, by which time patients had received less than 1% of the total cumulative allergen dose administered weekly during the two-month up dosing phase. It is established that immunotherapy with low doses of allergens is ineffective. Therefore, this finding suggests that late-response suppression alone is insufficient to account for the clinical efficacy of immunotherapy. In this same study, the decline in the sizes of early responses is proportionally much less but evolves slowly over a time frame more in keeping with clinical protection from symptoms.

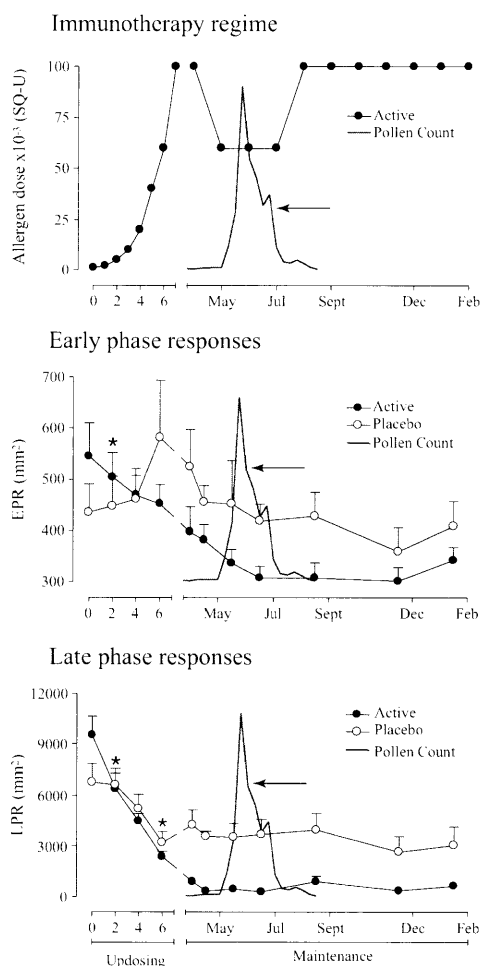


Figure 2 Time course analysis of clinical measurements during the first year of grass pollen immunotherapy with the dose of grass pollen allergen administered at each visit represented in the top panel. Early and late skin responses were assessed at 15 minutes and 24 hours following intradermal challenge with grass pollen allergen. Data are expressed as mean $\pm$ standard error. The solid line (see arrows) represents pollen counts in London, United Kingdom. * $p < 0.05$ versus pre-immunotherapy value. Source: From Ref. 7.

symptoms on exposure with lower measurable bronchial hyperactivity, eosinophil counts, and ECP concentrations.

ANTIBODY RESPONSES

Isotype Changes

Conventional pollen immunotherapy appears to have little effect on allergen-specific serum IgE concentrations (16), although increases in IgE during the pollen season are blunted (17). Following treatment with birch pollen extract, serum immunoblotting analysis suggests that a proportion of individuals developed new IgE responses to components of the vaccine (18). Nevertheless, the clinical effect of these new sensitizations is unknown, and the concentrations of IgE produced are relatively low. The functional significance of the humoral response to immunotherapy was first addressed in the 1930s by Cooke, who was able to demonstrate that transfer of sera from immunotherapy patients inhibited allergic responses. Platts-Mills and colleagues identified that antibodies from nasal lavage of immunotherapy patients inhibit histamine release from basophils in vitro (19). Allergen-specific IgG1, IgG4, and IgGA isotypes are all increased by immunotherapy with inhalant allergens (16,20). Of these, IgG4 shows the greatest proportionate increase. IgG4 antibodies are not proinflammatory in humans leading to the proposal that they act as "blocking antibodies" by competing for binding sites on allergens with surface-bound IgE (i.e., bound to FcεRI high-affinity receptors). IgG4 produced in response to immunotherapy blocks allergen-induced IgE-dependent histamine release by basophils in vitro (21). The properties of IgA antibodies induced by immunotherapy have also been examined (22). Cross-linking of polymeric IgA2 is a potent inducer of IL-10 expression by monocytes. IL-10 favors B-cell production of IgG4 and is an important immunoregulatory cytokine (discussed below).

Effects on Antigen Presentation

In addition to blocking allergen binding by cell surface FcεRI-bound IgE, IgG4 antibodies also inhibit formation of IgE-allergen complexes in solution. Formation of these multivalent complexes is required for IgE binding to low-affinity FcεRII (CD23) IgE receptors expressed by antigen-presenting cells (APC), including B cells, dendritic cells, and monocytes. This process appears to allow these cells to bind and process low concentrations of allergen for presentation to T cells by human leukocyte antigen class II molecules. van Neerven and colleagues demonstrated that sera from patients allergic to birch pollen facilitates antigen binding to B cells and ensuing activation of birch-specific T cells (23). This process is inhibited by sera from birch and grass pollen immunotherapy patients and the inhibitory activity co-purified with the IgG fraction (24-26). In contrast, IgA fractions do not demonstrate significant blocking activity in cellular assays (22). When the development of grass pollen allergen-specific IgG4 and corresponding blocking activity were studied over a detailed time course, responses took six to eight weeks to achieve significant levels during a conventional up dosing regime (7).

A common objection to the blocking antibody model is the weak correlation between allergen-specific IgG concentrations and the clinical response to immunotherapy (27-29). However, data from our group show that two years following discontinuation of grass pollen immunotherapy, grass pollen allergen-specific IgG4 levels decline by approximately 80% (unpublished observations), though functional blocking activity in vitro persists during this period, as does clinical remission. It is possible that a population of long-lived high-avidity memory IgG+ B cells selectively persists following immunotherapy withdrawal, perhaps because of ongoing low-dose natural antigen exposure.

T-CELL RESPONSES

Considerable attention has focused on characterizing T-cell responses to SCIT. These studies have led to two major mechanistic hypotheses: first, that deviation of Th2 responses occurs in favor of a Th0/Th1 phenotype (assumed to be less pathogenic), and second, that allergen-specific T-cell

responses are suppressed by newly induced regulatory T cells or through inhibition of antigen presentation by IgG.

IMMUNE DEVIATION

A body of evidence suggests that immune deviation of T-helper responses occurs in successful immunotherapy, though no consistent pattern of change has emerged that is common to all studies. Many *in vitro* studies of peripheral blood T cells from immunotherapy patients have identified reductions in proliferative responses to allergen, usually in conjunction with a shift in the patterns of cytokine production from a Th2/0 toward Th1/0 profile (30–33). Others have not succeeded in reproducing these findings, reporting no changes in proliferative responses or cytokine production following SCIT (34–37). Potential reasons for these discrepancies include variations in laboratory methodology and lack of standardisation of allergen extracts used for SCIT. Another possible explanation is that inhibition of *peripheral* T-cell proliferation and Th2 cytokine production is not required for immunotherapy, and these responses poorly reflect the critical immune interactions and responses in lymphoid and mucosal tissues.

The effects of immunotherapy on the cytokine profile of T cells recruited into tissue have been investigated (35,36). Nasal mucosal biopsies performed during the allergen-induced late response of grass pollen immunotherapy patients reveal increases in IFN- γ mRNA-expressing cells (13). The relevance of this finding is supported by the inverse correlation between numbers of IFN- γ mRNA+ cells and clinical symptoms. A potential inducer of IFN- γ expression by mucosal T cells is IL-12 (38), and there is evidence that this mechanism may operate in grass pollen immunotherapy. Skin biopsies collected during the cutaneous late response were examined for IL-12 mRNA by *in situ* hybridization (39). In immunotherapy but not placebo-treated patients, there is concomitant late-response suppression and IL-12 mRNA expression. The latter correlates directly with IFN- γ , and the principal sources of IL-12 mRNA are the CD68+ macrophages. Similar studies demonstrate that immunotherapy significantly inhibits seasonal increases in IL-5- and IL-9 mRNA-expressing cells in the nasal mucosa (10,14). These studies underline the probable relevance of studying "end organ" immune responses rather than in the peripheral blood, particularly for diseases induced by inhalant allergens such as grass pollen.

Regulatory T Cells and IL-10

Studies also suggest that SCIT induces regulatory T cells that are able to modify allergen-specific T-helper cell responses. One subset of regulatory cells, first identified as CD4+CD25+ cells by Sakaguchi (40), appear to arise predominantly in the thymus and express the transcription factor Foxp3 (41). The inhibitory properties of human CD4+CD25+ cells *in vitro* and *in vivo* are described in numerous studies (42) and depend, at least in part, on cell-cell contact. Functional roles for membrane cytotoxic T-lymphocyte-associated protein 4 (43), surface-bound transforming growth factor beta (TGF- β) (44), and the glucocorticoid-induced tumor necrosis factor receptor (GITR) (45) have been proposed. These cells appear to regulate allergen-specific T-cell responses in healthy nonatopic individuals. In contrast, in subjects with allergic rhinitis, purified CD4+CD25+ T cells fail to suppress activation of CD4+CD25- T cells by grass pollen allergen (46). An additional population of "inducible" regulatory cells, often referred to as T_R1 cells, has also been described. Inducible regulatory T cells are generated *in vitro* through stimulation (47–49) and are classically described as not expressing Foxp3 (50), suggesting that these cells represent a subset distinct from the naturally occurring CD4+CD25+ regulatory T cells. Inducible regulatory T cells can produce cytokines such as IL-10 and TGF- β , both of which have important immunomodulatory properties.

Expression of IL-10 by T cells after SCIT is a consistent finding of several studies (31,37,51). IL-10 is expressed by both Th1 and Th2 cells, B cells, monocytes, dendritic cells, mast cells, and eosinophils and is associated with protection from immune-mediated pathology in diverse murine disease models, including pulmonary allergy (52). IL-10 acts on B cells to induce production of IgG4 in the presence of IL-4 (53). In addition, it has numerous potential antiallergic properties, including inhibition of mast cell activation by IgE (54), Th2 cytokine production, including IL-5 (37), and induction of T-cell hyporesponsiveness (55) through IL-10 receptor-dependent blockade of CD28 phosphorylation (56). The latter is an essential costimulatory pathway for T cells during antigen-induced activation by APC.

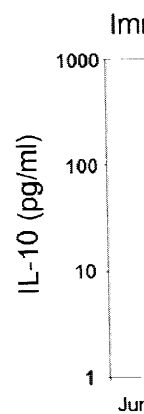


Figure 4 Effect of immunotherapy on IL-10 production by Jurkat cells. Jurkat cells were stimulated with grass pollen extract (10⁶ units/ml) for 24 hours. IL-10 production was measured by ELISA. Source: Adams et al.

IL-10 is also evident to be added (26). The response is only 15% accounted for by mRNA levels. It is possible that the methodology used for B cells, and cultures analyzed associated with house-dust T cells produced important of "natural" difficulty being expected many studies CD4+CD25+ in vitro studies.

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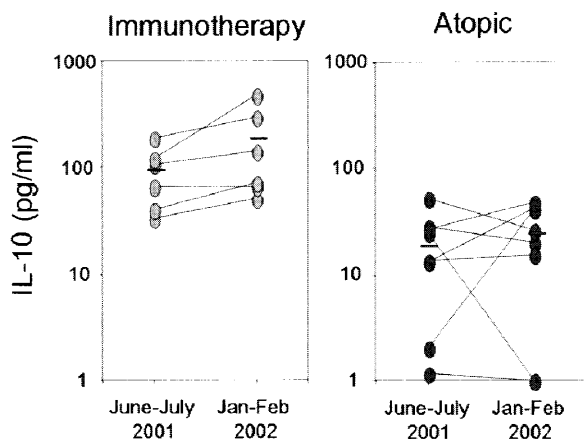


Figure 4 Effect of grass pollen immunotherapy on IL-10 production by peripheral blood T cells. Peripheral blood mononuclear cells were isolated from patients who had received at least 18 months of conventional grass pollen immunotherapy (light grey circles) and untreated hay fever subjects (dark grey circles) during and after the 2001 grass pollen season in London. Peripheral blood mononuclear cells were stimulated for six days with *Phleum pratense* (timothy grass) extract and culture supernatants tested for interleukin-10 concentrations by ELISA. Source: Adapted from Ref. 37.

IL-10 production by peripheral blood T cells is described following immunotherapy with bee venom (31), house-dust mite (20), and grass pollen (37) (Fig. 4). IL-10 mRNA expression is also evident in nasal biopsies of grass pollen immunotherapy patients, and expression appears to be additionally dependent on concurrent antigen exposure, that is, during the pollen season (26). The mucosal IL-10 response does not represent a restoration of the "normal" immune response as expression is not seen in pollen-exposed nonatopic control subjects. Surprisingly, only 15% of IL-10 mRNA signals colocalized to CD3+ T cells, with a further 35% being accounted for by CD68+ tissue macrophages (26). However, numbers of cells expressing IL-10 mRNA need not necessarily correlate with secretion of bioactive IL-10 protein, and it is quite possible that the contribution of T cells to IL-10 production is underestimated by this methodology. In peripheral blood studies following bee venom immunotherapy, monocytes, B cells, and T cells are all cell sources of IL-10 (51). The endogenous IL-10 produced in these cultures suppresses CD4+ T-cell responses. Subsequently, T-cell phenotypic subsets were analyzed for IL-10 production by flow cytometry. Using this approach, IL-10 production is associated with CD4+CD25+ T cells following SCIT with bee venom (50), grass pollen (37), or house-dust mite (20). In the case of house-dust mite immunotherapy, these CD4+CD25+ T cells produce both IL-10 and TGF- β and suppress immune responses to allergen *in vitro*. An important issue is how this phenotype of IL-10-producing T cells fits within the current model of "naturally occurring" CD4+CD25+ and "inducible" T_R1 regulatory cell subsets. One difficulty is that CD25 is not exclusively a marker of naturally occurring regulatory T cells, being expressed also by activated T cells. Indeed, *in vitro* stimulation is a common feature of many studies using peripheral blood T cells. However, Jutel and colleagues did separate CD4+CD25+ and CD4+CD25- T cells from house-dust mite immunotherapy subjects before *in vitro* stimulation and IL-10 production compartmentalized to the former subset (20).

How Do Immunotherapy Vaccines Induce IL-10 Responses?

Identifying the mechanism by which immunotherapy induces IL-10-producing T cells could be important in the design of new vaccines. Murine models provide some insights. Nevertheless, important differences exist between SCIT administered to patients to modulate mature Th2 responses and animal models where the emphasis is on tolerizing regimes given before sensitization. Tolerizing animals by oral or intranasal exposure to ovalbumin prior to intraperitoneal sensitization induces IL-10-producing regulatory T cells (57,58). In the intranasal tolerance model, induction of these T_R1 -like cells is dependent on pulmonary lymph node dendritic cells expressing IL-10 and the inducible costimulatory molecule ligand (ICOSL) ligand. Indeed, adoptive transfer of these dendritic cells or T_R1 cells alone is sufficient to confer tolerance on the recipients (57,59). Human peripheral blood plasmacytoid dendritic

cells stimulated with a toll-like receptor 9 agonist express ICOSL ligand and induce differentiation of T_{H1} cells from naïve T cells *in vitro* (60). Furthermore, cross-linking of the high-affinity IgE receptor (FcεRI) on plasmacytoid dendritic cells by allergen also induces IL-10 expression (61). Although these mechanisms are not directly demonstrated in immunotherapy, they do illustrate how dendritic cell subsets could provide the necessary tools to induce T-cell IL-10 responses following allergen vaccination.

NOVEL IMMUNOTHERAPY STRATEGIES

Immunotherapy performed with modern vaccines is a relatively safe form of treatment in trained hands. However, administering native allergen to IgE-sensitized individuals can trigger both local and systemic reactions. Immunotherapy regimes therefore tend to be cautious, involving numerous injections of gradually increasing allergen doses performed under specialist supervision. The aim for novel immunotherapy strategies should be to reduce the potential for IgE-mediated side effects while exposing patients to fewer injections and making fewer demands on resources.

Circumventing IgE-Mediated Side Effects

The first category of novel therapies is based on the hypothesis that immunotherapy works primarily through stimulating T cells at high antigen doses and that this directly leads to regulatory T-cell induction. This class of therapies is also based on the assumption that IgE-dependent mechanisms mediate immunotherapy side effects but are not necessary for vaccine efficacy. Strategies tested include genetically modified allergen proteins with reduced IgE binding but intact T-cell epitopes. For example, recombinant fragments of major birch pollen allergen Bet v1 have been generated with minimal allergenicity in cutaneous and nasal challenge models (62,63). These recombinant proteins successfully induce Bet v1-specific IgG1 and IgG4 responses that block basophil histamine release triggered by exposure to wild-type Bet v1 (64). Another approach is the use of allergen-derived peptides that stimulate T cells but that cannot cross-link IgE. Overlapping peptides representing the cat allergen Fel d1 have been examined in small clinical studies with promising results (65). Finally, a randomized double-blind placebo-controlled trial examined the value of pretreatment with a humanized anti-IgE monoclonal antibody (omalizumab) on side effects in a rapid up dosing ("rush") protocol of ragweed immunotherapy (66). Such accelerated immunotherapy regimes balance increased convenience and compliance with a higher risk of systemic reactions. Omalizumab resulted in a fivefold reduction in the risk of anaphylaxis during the one-day rapid up dosing period.

Adjuvants

The second category is based around the use of adjuvants to potentiate the Immunologic effects of vaccination with whole allergen proteins. Ideally, such adjuvants should not only potentiate Th2 to Th1 immune deviation, but should also favor induction of regulatory T-cell responses. The type-B immunostimulatory phosphorothioate oligodeoxynucleotide 5'-TGACTGTAACGTTTCGAGATGA (ODN-1018) was tested in ragweed-stimulated peripheral blood mononuclear cells (PBMC) responses. It promotes Th1 cytokine and IL-12 responses at the expense of Th2 cytokine production. This activity could be further enhanced by conjugation of ODN-1018 with the major ragweed allergen, Amb a1. (67). A functionally similar type-B phosphorothioate oligodeoxynucleotide (ODN-2006) activates human plasmacytoid dendritic cells through the toll-like receptor-9 and induces regulatory T cells (60,68). A randomized double-blind placebo-controlled phase 2 trial examined the effects of six weekly injections of the ODN-1018-Amb a1 conjugate on ragweed-induced allergic rhinitis (69). Treatment is associated with improvement in peak season visual analogue, nasal, and quality of life symptom scores. Another clinically tested adjuvant is monophosphoryl lipid A, a derivative of bacterial lipopolysaccharide. Monophosphoryl lipid A acts through toll-like receptor-4 to induce IL-12 production and promote Th1 responses to allergen by human PBMC (70). In a randomized double-blind placebo-controlled study, a vaccine containing monophosphoryl lipid A and tyrosine-absorbed glutaraldehyde-modified extracts of grass pollen (Pollinex Quattro[®]) reduces hay fever symptoms and increases allergen-specific IgG when administered as four preseasonal injections (71).

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In addition to guiding development of novel treatment approaches, knowledge of immunotherapy mechanisms is also likely to enable the development of effective biomarkers in order to predict patients who are likely to respond to immunotherapy and to predict relapse following discontinuation. Measurement of the biological activity of so-called "blocking" antibodies holds some promise (72). The sublingual route is also emerging as an effective and safe alternative (73). Meanwhile, the subcutaneous route using standardized natural allergens remains the gold standard against which to test putative biomarkers and novel immunomodulatory approaches.

SALIENT POINTS

1. Allergen injection immunotherapy is effective in selected patients with IgE-mediated disease and sensitivity to one or limited numbers of allergens.
2. Allergic disorders in humans are characterized by expression of IL-4 and IL-5.
3. SCIT inhibits allergen-induced early and late responses in nose, skin, and lung.
4. SCIT inhibits recruitment of mast cells, basophils, and eosinophils to the nose and lung.
5. SCIT increases allergen-specific IgA and IgG, especially IgG4. IgG antibodies inhibit some of the effects of IgE in vitro, but the clinical importance remains to be established.
6. SCIT induces immune deviation in favor of Th1 responses as well as T-cell IL-10 production in the nasal mucosa and peripheral blood. IL-10 has numerous antiallergic properties and promotes IgG4 production by B cells. Dendritic cells are the most likely candidates for inducing IL-10 responses.
7. Novel approaches include non-IgE-binding recombinant allergens, allergen-derived peptides, and combining conventional vaccines with anti-IgE (omalizumab). Bacterial-derived oligonucleotides and lipids acting through toll-like receptors to promote Th1 and regulatory T-cell responses also hold promise.

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