

Current reviews of allergy and clinical immunology

(Supported by a grant from GlaxoSmithKline, Research Triangle Park, NC)

Series editor: Harold S. Nelson, MD

Immunotherapy: 1999-2004

Philip S. Norman, MD *Baltimore, Md*

This activity is available for CME credit. See page 30A for important information.

Allergen immunotherapy continues to be used worldwide in the management of allergic rhinitis and asthma. We continue to refine our knowledge of the indications for immunotherapy, the allergens that can be used successfully, and the requirements for dosage and route of administration. Several additional allergens have been shown to be effective in controlled studies. The sublingual route of administration is safe and can be effective if the dose is large enough. Since reviews in 1998, further studies of immunologic changes after immunotherapy have confirmed that T_H2 lymphocyte-mediated responses are suppressed, and T_H1 responses are stimulated. Eosinophil and basophil inflammatory responses to allergen exposure are downregulated through one or both of these mechanisms. Research continues to seek improvements through allergen vaccine modifications suggested by recent immunologic discoveries. None of these is available in practice. (*J Allergy Clin Immunol* 2004;113:1013-23.)

Key words: Immunotherapy, allergen, T cells, cytokines, mast cell, basophil, CpG motif, peptide, anti-IgE

A review in the Journal in 1998 recapitulated the history of immunotherapy for respiratory allergies since its introduction by Noon in 1911 and examined the current state of its clinical use.¹ A second review described our understanding of underlying mechanisms.² This review will examine human studies reported in the ensuing years, including immunotherapy for food allergy. Protection against insect venom anaphylaxis is a large and separate subject not covered.

MECHANISM

Mechanistic studies continue to support the idea that immunotherapy expands allergen-specific T_H1 immunity and suppresses the T_H2 responses resulting in clinical

Abbreviations used

LAR: Late asthmatic reaction
RI: Rush immunotherapy
SLAM: Signaling lymphocytic activation molecule
SLIT: Sublingual immunotherapy
TCR: T-cell receptor

allergy. Whether the first leads to the second is not clear. The 1998 review by Durham and Till² summarized the biopsy evidence that immunotherapy reduces the CD4⁺ lymphocyte response to allergen challenge in the nose and skin. It also increases cells expressing mRNA for the T_H1 cytokines IFN- γ , IL-2, and IL-12 while reducing cells expressing the T_H2 cytokine IL-4.

T-cell T_H2 and T_H1 cytokine production *in vitro*

In 1998, there were reports that PBMCs taken after immunotherapy showed reduced IL-4 production after allergen stimulation and other reports that showed enhanced IFN- γ expression.² Since then, expression or production of cytokines in patterns considered characteristic of T_H1 or T_H2 cells in cultures of T cells or PBMCs has received much attention, with conflicting results.

Söderlund et al³ studied cytokine expression in PBMCs before and after birch pollen immunotherapy. Spontaneous expression of IL-4 mRNA was detected in most of the allergic patients but not in healthy donors. In immunotherapy-treated patients, IL-4 mRNA decreased during the pollen season compared with at the onset of the study, whereas in placebo-treated patients IL-4 mRNA increased or remained unchanged. Similar results were obtained after *in vitro* stimulation with allergen. In contrast, IFN- γ was readily detected, without significant differences between the groups at either time. IL-5 was increased during the pollen season in both groups and presumably not influenced by immunotherapy.

Van Bever et al⁴ compared treated asthmatic patients sensitive to house dust mites with untreated control subjects. PBMCs of treated patients secreted less IL-2, IL-4, and IL-5 after immunotherapy. They could not confirm any change in IFN- γ . Klimek et al⁵ studied immunotherapy with birch pollen allergoid and found expected

From the Johns Hopkins Asthma and Allergy Center, Johns Hopkins University School of Medicine.

Received for publication March 3, 2004; revised March 3, 2004; accepted for publication March 16, 2004.

Disclosure of potential conflict of interest: P. Norman is a consultant to the Data Safety Monitoring Board, Neurocrine Technologies, Inc.

Reprint requests: Philip S. Norman, MD, Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Circle, Baltimore MD 21224. E-mail: pnorman@jhmi.edu.

0091-6749/\$30.00

© 2004 American Academy of Allergy, Asthma and Immunology

doi:10.1016/j.jaci.2004.03.020

increases in cytokines in nasal secretions. On the other hand, in allergen-stimulated cultures of T cells, they could find no change in cytokine expression for IL-4, IL-5, IL-10, or IFN- γ . Waccholz et al⁶ studied grass pollen immunotherapy and found a seasonal increase in the ratio of IFN- γ to IL-5 in the nasal mucosa but no changes in expression of these cytokines in allergen-stimulated T-cell cultures.

In a somewhat different approach, Moverare et al⁷ followed induction of birch pollen immunotherapy with serial cultures of PBMCs stimulated by birch pollen extract. After a short decrease probably caused by seasonal variation, birch pollen-specific proliferation and IL-4, IL-5, and IL-10 production significantly increased when the maintenance dose had been reached, as well as during the subsequent pollen season. The increase in IL-4 correlated with a temporary increase in serum birch pollen-specific IgE levels.

Majori et al⁸ examined cytokine production in PBMC cultures stimulated by a polyclonal activator. Immunotherapy for dust mites had a marked effect in increasing the IFN- γ /IL-4 ratio in peripheral blood CD4⁺ T cells after 3 months of the dose-increase phase ($5.47\% \pm 1.5\%$ vs $4.07\% \pm 1.49\%$; $P = .03$), with a further increase after 1 year's treatment ($16.12\% \pm 2.8\%$ vs $5.47\% \pm 1.5\%$; $P = .002$). There were no significant changes in the IFN- γ /IL-4 ratio in peripheral blood CD8⁺ T cells at the 3 times of the study.

T-cell lines and clones. Oda et al⁹ developed both T-cell lines and T-cell clones from house dust mite-sensitive asthmatic patients before and at 3 and 18 months after rush immunotherapy (RI). All the *Dermatophagoides farinae*-specific T-cell lines established before RI exhibited a T_H2 pattern characterized by the production of high levels of IL-4 and undetectable or small amounts of IFN- γ . In contrast, 18 months after RI, cytokine production by *D farinae*-specific T-cell lines showed either a T_H0 or T_H1 profile (ie, markedly increased IFN- γ production, reduced IL-4 production, or both). Of interest, 3 months after RI, very few cell lines could be established, and those that were established produced small amounts of both IL-4 and IFN- γ in accordance with a reduced proliferative response to *D farinae*. IL-5 production was also decreased 3 and 18 months after RI.

Similar cytokine profiles were found in *D farinae*-specific T-cell clones. Clones established before RI produced IL-4 and a small amount of IFN- γ , which represented T_H2-like cells. Three months after RI, hardly any mite-specific T-cell clones could be established, and the few that grew produced small amounts of IL-4 and IFN- γ . Eighteen months after RI, *D farinae*-specific T-cell clones had shifted their cytokine-secreting patterns to those of T_H1- or T_H0-like cells.

In contrast, the T-cell clones of control subjects not given RI showed T_H2-type responses throughout. The authors suggest that T_H2 cells disappeared early after starting RI and that T_H1 and T_H0 populations emerged slowly and selectively later. They could not find any differences in IL-10 production.⁹

IL-10 and TGF- β . In studies of immunotherapy for insect venom anaphylaxis, Akdis and Blaser¹⁰ found increased secretion of IL-10 in T-cell cultures, along with decreased allergen-driven proliferation and decreased production of T_H2 and T_H1 cytokines. They attributed the latter effects to the ability of IL-10 to block the costimulatory CD28-B7.1 interaction and subsequent signaling pathways in T cells. A further study by Jutel et al¹¹ from the same group extends these findings to immunotherapy for house dust mite-induced rhinitis and asthma. In particular, they examined T-cell suppression by IL-10 and TGF- β in cultures stimulated with Der p 1. Seventy days after initiation of immunotherapy, the deviated immune response was characterized by suppressed T-cell proliferation accompanied by diminished T_H1 (IFN- γ) and T_H2 (IL-5 and IL-13) cytokine responses and increased IL-10 and TGF- β secretion by allergen-specific T cells. Neutralization of cytokine activity indicated that T-cell suppression was induced by IL-10 and TGF- β . In addition, immunotherapy induced an antigen-specific suppressive activity in CD4⁺CD25⁺ T cells of allergic individuals.

Francis et al¹² reported on IL-10 production in the same year. They took PBMCs from patients who had undergone a year of grass pollen immunotherapy for comparison with untreated allergic control subjects. Cultures from immunotherapy-treated patients stimulated with *Phleum pratense* produced significantly more IL-10 than cultures from atopic control subjects (immunotherapy-treated patients, 116 ± 21 pg/mL [$n = 11$]; control subjects, 30 ± 5 pg/mL [$n = 11$]; $P < .001$). The number of CD4⁺CD25⁺ cells identified after allergen stimulation was also greater in the immunotherapy group. The numbers of CD4⁺CD25⁺ T cells correlated with *P pratense*-induced proliferation in both control groups but not in the immunotherapy group. Moreover, T cells from immunotherapy-treated patients that were positive for intracellular IL-10 were almost exclusively CD4⁺CD25⁺ cells. Significantly higher amounts of the T_H2 cytokines IL-4, IL-5, and IL-13 were produced in both the immunotherapy and atopic groups than in the nonatopic control group ($P < .05$). In contrast, there was no difference in T_H2 cytokines between the immunotherapy-treated and untreated atopic groups. Different patterns of responses were seen for IL-10: production was higher in the immunotherapy-treated patients than in both the untreated atopic and healthy nonatopic groups (for both the healthy and atopic groups vs the immunotherapy group, $P < .01$). No changes in TGF- β levels were detected.

This group also studied IL-10 responses in nasal biopsy specimens in patients receiving grass immunotherapy for 2 years. Local and systemic IL-10 responses and serum antibody concentrations were measured before and after a double-blind trial. They observed local increases in IL-10 mRNA-positive cells in the nasal mucosa after immunotherapy but only during the pollen season. IL-10 protein-positive cells were also increased and correlated with IL-10 mRNA cells. These changes were not observed

in placebo-treated subjects or in healthy control subjects. Fifteen percent and 35% of IL-10 mRNA signals were colocalized to CD3 T cells and CD68 macrophages, respectively, whereas only 1% to 2% of total CD3 cells and 4% of macrophages expressed IL-10. After immunotherapy, peripheral T cells cultured in the presence of grass pollen extract also produced IL-10. Immunotherapy resulted in blunting of seasonal increases in serum allergen Phl p 5-specific IgE, 60- to 80-fold increases in Phl p 5-specific IgG, and 100-fold increases in Phl p 5-specific IgG4.¹³ See below about the possible role of IgG4 antibodies.

In considering these varying results, one must realize that estimating changes in allergen-specific regulatory, T_H1, and T_H2 T cells in peripheral blood presents the investigator with multiple difficulties. T cells with receptors specific for epitopes from an allergen usually represent less than one in a thousand of the lymphocytes in a sample. PBMCs must therefore be stimulated by allergen for days to allow proliferation of the cells of interest, giving them time to change their profile. T_H1 or T_H2 cells are estimated indirectly by cytokine profile because there are no markers specific for these classes of human T cells. Cytokine profiles might be estimated on the basis of either expression (mRNA staining) or production (staining with cytokine-specific antibodies), which are not necessarily the same thing. The timing of specimens might be crucial because some immunologic changes might occur rapidly during immunotherapy, and others might occur gradually over a year or longer and might vary with the dosage regimen. Furthermore, results might be influenced by natural mucosal exposure.

On the other hand, cytokine expression in stained tissues after challenge has been more consistent, possibly because allergen-specific cells become concentrated at challenged sites. No studies seriously contradict the hypothesis that T_H2 allergic responses are suppressed and T_H1 responses are stimulated. The T-cell lines and clones developed by Oda et al⁹ suggest that T_H2 suppression occurs first and T_H1 stimulation occurs later. A study of T_H2 apoptosis tends to support this hypothesis (see below).

T-cell apoptosis. Guerra et al¹⁴ introduced a new concept: that immunotherapy makes allergen-specific IL-4-producing T cells susceptible to apoptosis on exposure to allergen. They examined cultures from grass-sensitive patients treated or untreated with immunotherapy. In allergen-stimulated cultures of cells from untreated patients, IL-4 represented 71% ± 12% of cytokine-producing cells, and IFN-γ represented 7% ± 3%. Immunotherapy-treated patients showed the reverse, with IL-4 cells at 11% ± 7% and IFN-γ cells at 64% ± 18%. To account for this phenomenon, they propose that immunotherapy renders T_H2 cells susceptible to "activation-induced cell death." Using a technique that labels cells for breaks in DNA as a marker for apoptosis, they found that cultures from immunotherapy-treated patients showed a significant proportion (39% ± 14%) of lymphocytes undergoing apoptosis after culture with allergen, whereas no apoptosis occurred in non-immunotherapy-treated

patients. When stained simultaneously for cytokines, apoptotic cells expressed mainly IL-4 (84% ± 11%), whereas fewer cells expressed IFN-γ (4% ± 2%).

Lymphocyte activation. Laksonen et al¹⁵ studied changes in signaling lymphocytic activation molecule (SLAM) during immunotherapy. Before immunotherapy, *in vitro* allergen-induced SLAM mRNA expression in PBMCs was significantly lower in patients with allergic rhinitis than in healthy control subjects. After 1 year of treatment, SLAM mRNA expression was increased in immunotherapy-treated patients and was associated with IFN-γ mRNA expression but inversely associated with symptom improvement. However, in cultures taken earlier, when the maintenance dose was reached, a transient increase in SLAM mRNA expression was associated with the clinical symptom improvement at 1 year. Because SLAM is increased in several T_H1-mediated autoimmune conditions, the authors interpret their findings as additional evidence that immunotherapy initiates an increased T_H1 response.

T-cell receptor V use. Sade et al¹⁶ examined the expression and use of the V-α, V-β, V-γ, and V-δ chains of T-cell receptors (TCRs) in patients allergic to house dust mites. Analyzed before and 6 months after immunotherapy initiation, lymphocyte TCRs showed significantly increased V-β5b, V-β12, and V-α12.1 values compared with control values (without significant changes in other markers). The authors suggest that the repeated allergen administration during immunotherapy caused the proliferation of suppressor T cells that have an oligoclonal TCR-V use.

B cells

Although most studies of peripheral blood cells focus on T cells, Håkansson et al¹⁷ enumerated B cells bearing several markers by means of flow cytometry before and after immunotherapy for birch pollen allergy. In untreated patients seasonal exposure to birch showed an increase in cells with surface expression of CD23, CD40, and HLA-DR at the same time IgE antibodies increased. In patients given immunotherapy, no such increases occurred. The authors hypothesize that a T_H1 T-cell predominance might account for the lack of B-cell activation on allergen exposure.

Wachholz et al¹⁸ have a hypothesis that might account for these effects. They propose that allergen-specific IgG antibodies induced by immunotherapy can disrupt formation of allergen-IgE complexes that bind to antigen-presenting cells and facilitate allergen presentation. They developed a flow cytometric assay on the basis of detection of allergen-IgE binding to the low-affinity IgE receptor on B cells to examine the blocking effects of serum. In 10 patients who received active grass pollen immunotherapy, there was induction of a serum activity that inhibited allergen-IgE binding to B cells ($P = .02$ vs placebo subjects), as well as subsequent allergen presentation to T cells. This activity copurified with IgG and was allergen specific because sera taken from patients treated with grass pollen immunotherapy but who were

also birch pollen sensitive did not inhibit IgE–birch pollen allergen binding to B cells. The authors write, “Further studies are needed to assess whether this activity might predict the clinical response to treatment.” A further study by Nouri-Aria et al¹³ confirmed this response after 2 years of immunotherapy. They propose that “grass pollen immunotherapy may induce allergen-specific, IL-10–dependent ‘protective’ IgG4 responses.”

Inflammatory cytokines

Several of the studies cited above noted reductions in the eosinophil and basophil stimulator IL-5. Meissner et al¹⁹ studied this cytokine in patients given immunotherapy for cat allergy. Before immunotherapy, IL-5 production was significantly higher in cells of allergic donors stimulated with allergen ($P < .005$) or mitogen ($P < .05$). After reaching the maintenance dose for immunotherapy, allergen-induced IL-5 production returned to normal levels. The ability of immunotherapy to reduce inflammatory neutrophil responses was tested by Montseirin et al²⁰ in allergic asthmatic patients. Neutrophil degranulation *in vitro* after stimulation with formyl-methionyl-leucyl-phenylalanine was found to be higher in the asthmatic patients than in healthy control subjects and returned to normal levels after immunotherapy.

Wilson et al²¹ studied patients receiving grass pollen immunotherapy with nasal mucosal biopsy specimens in season and found fewer eosinophils in treated patients. A significant correlation was observed between eosinophils and IL-5 expression ($r = 0.5$; $P < .05$). Both eosinophils ($r = 0.6$; $P < .02$) and IL-5 ($r = 0.6$; $P < .02$) correlated with symptoms after immunotherapy.

De Amici et al²² studied serum cytokine levels (no challenge) before and after 6 months of immunotherapy for dust mite allergy and found the following: IL-1 β and TNF- α levels were increased before immunotherapy. After immunotherapy, they decreased to levels similar to those found in nonallergic control subjects. IL-2 and IL-6 levels, on the other hand, were initially low but increased after immunotherapy. These data indicate a reduction in inflammatory responses.

Mast cells and basophils

Durham et al²³ examined mast cell numbers in skin biopsy specimens from sites of 24-hour late-phase reactions to allergen before and after immunotherapy for grass pollen allergy. Treated patients had significant reductions in symptom scores during the grass pollen season and reduced immediate skin reactivity, as determined with prick tests. After immunotherapy, the numbers of mast cells were on average reduced 5-fold at the challenge sites. The numbers of mast cells showed strong correlations with symptom and rescue medication scores during the season ($r = 0.61$, $P = .001$; $r = 0.75$, $P = .0001$, respectively). The mechanism underlying this change is not understood.

In another study from the same group, Wilson et al²⁴ examined cell-specific markers for basophils, eosinophils, and mast cells in nasal epithelium and submucosa in

subjects receiving immunotherapy for grass pollinosis. The nasal submucosa in placebo-treated patients showed significant seasonal increases in basophils ($P < .01$), mast cells ($P < .05$), and eosinophils ($P = .002$). In immunotherapy-treated patients smaller but still significant increases in basophils ($P < .01$) and eosinophils ($P = .01$) were observed, but not in mast cells ($P = .9$). These differences were significant between groups for eosinophils ($P < .05$). In the epithelium, on the other hand, mast cells and eosinophils showed seasonal increases in both groups (placebo-treated patients: $P < .01$, immunotherapy-treated patients: $P < .05$ for both). The between-group difference was significant for eosinophils ($P = .05$). Basophils were observed in the epitheliums of 6 of 17 in the placebo-treated group and 1 of 20 in the immunotherapy-treated group ($P = .03$). Neutrophil numbers remained constant in both the epithelium and submucosa. These findings extend the anti-inflammatory effects of immunotherapy to basophils.

Antibody binding capacity

Pierson-Mullany et al²⁵ studied allergen-specific IgE and IgG4 antibody affinity and concentration in an index they refer to as antibody binding capacity ($[Ig] \times K_A$). The binding capacity was determined for individuals with ragweed allergy undergoing ragweed immunotherapy in comparison with allergic individuals not receiving immunotherapy. The mean binding capacity for IgG4 after long-term immunotherapy was 1.6 log units higher ($P < .0001$) than for control subjects. The binding capacity for IgE was 1.2 log units lower after long-term immunotherapy ($P < .0001$) compared with control subjects.

Summary of mechanism

Current concepts of the cellular pathogenesis of common respiratory allergies are illustrated in Fig 1. Fig 2 shows how immunotherapy modifies the reactions according to recent findings.

LONG-TERM EFFECTS OF IMMUNOTHERAPY

In the past 5 years, the most important article to clinicians is the report by Durham et al²⁶ on long-term effects. Relief of grass pollen hay fever continued 3 to 4 years after stopping immunotherapy of 3 years' duration. Sixteen patients received maintenance injections for an additional 3 years (monthly injections of alum-adsorbed extract equivalent to 20 μ g of the principal grass allergen), 16 patients received matched placebos, and 15 new patients were followed with no therapy. Over the 3 years of the study, the symptoms reported by the maintenance and placebo-treated patients were similarly suppressed, whereas the new patients reported more severe symptoms. Inhibition of late-phase skin responses continued in both the maintenance and placebo-treated patients. In the placebo-treated patients there was no evidence of return of CD3⁺ or IL-4⁺ cells in allergen-challenged skin biopsy sites. This study confirmed that immunotherapy for

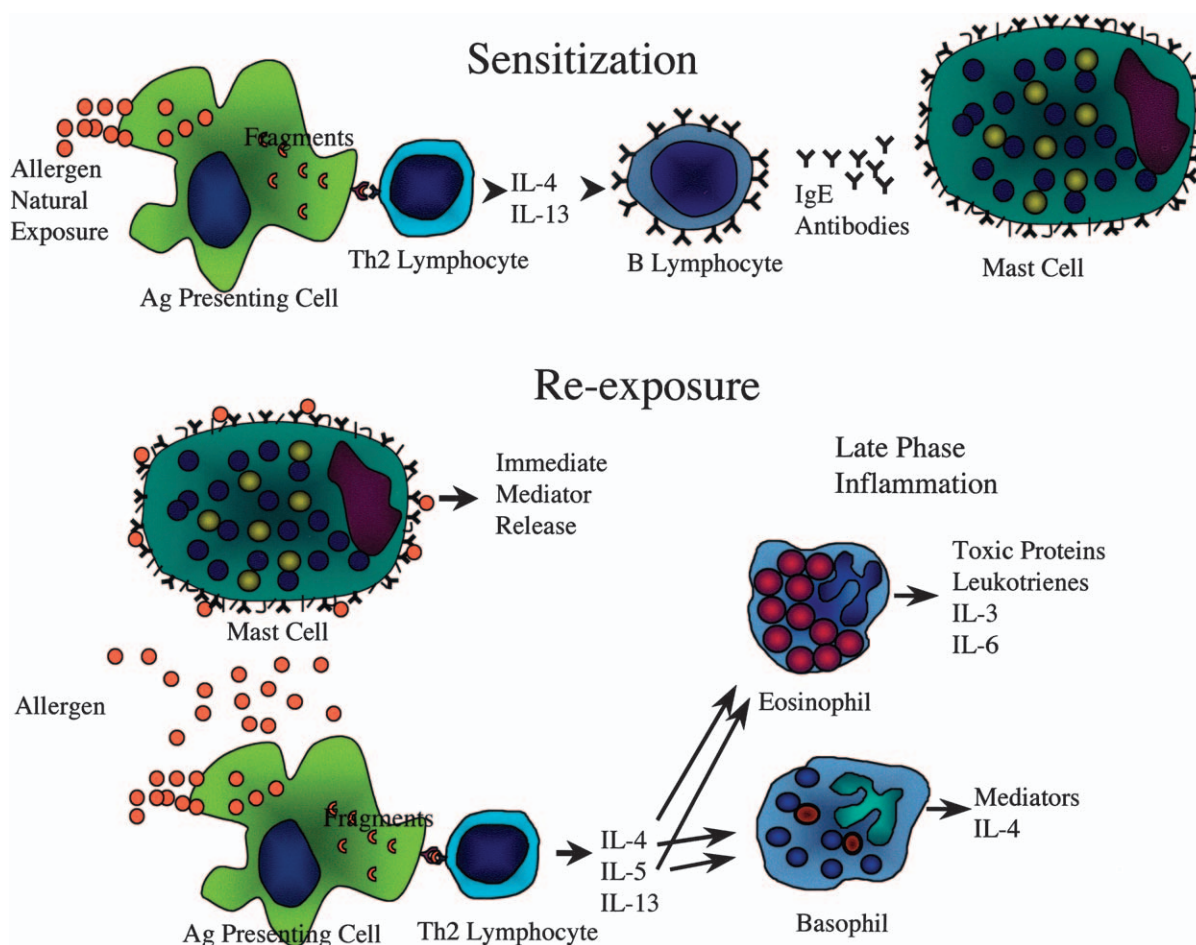


FIG 1. Current concept of the pathogenesis of allergic reactions. In genetically predisposed individuals, primary exposure to an allergen leads to activation of T_H2 lymphocytes and stimulation of IgE synthesis. Later exposures cause immediate mediator release and further activation of T_H2 cells, with resulting eosinophil and basophil inflammation. Ag, Antigen.

respiratory allergies affords long-term improvement just as insect venom immunotherapy protects against anaphylaxis long after it has been discontinued.

SUBLINGUAL IMMUNOTHERAPY

For years generally regarded as an ineffective route for immunotherapy, sublingual (with or without swallowing) administration of allergen extracts is now supported by many studies in Europe with a number of allergens. Locally applied immunotherapy, whether oral (swallowed), sublingual (with or without swallowing), nasal, or bronchial, was extensively reviewed in 2003 by Canonica and Passalacqua,²⁷ whose clinic has carried out many investigations of local, principally sublingual immunotherapy (often referred to as SLIT). According to their review, the nasal and bronchial routes have generally been abandoned because of local side effects. Straight swallow (ie, no retention in the mouth) is less favored than SLIT because the dose required is larger and more likely to induce gastrointestinal side effects. They

reviewed 22 double-blind, placebo-controlled trials of SLIT conducted with adequate methods and analysis that have been published in peer-reviewed journals. All but 3 studies confirmed clinical efficacy in rhinitis induced by common allergens, such as grass, mites, birch, and *Parietaria* species. There were also single positive studies with olive, cypress, and mixed-tree extracts. The magnitude of the clinical efficacy ranged between about 20% and 50% reduction of symptom or medication scores and thus was superior to the placebo effect and close to the effect of subcutaneous immunotherapy. The most frequently reported side effect was oral-sublingual itching after taking the dose, which was described as mild and self-resolving. The authors write, "Noticeably, no severe systemic adverse event was ever reported in the literature during a period of 15 years." The optimum dose is unknown. In the studies cited, effective doses ranged from approximately 3 to 5 times to 375 times the doses used in subcutaneous immunotherapy.

Few direct comparisons of SLIT with subcutaneous immunotherapy are available. Mungan et al²⁸ compared mixed dust mite extracts by the 2 routes with placebo

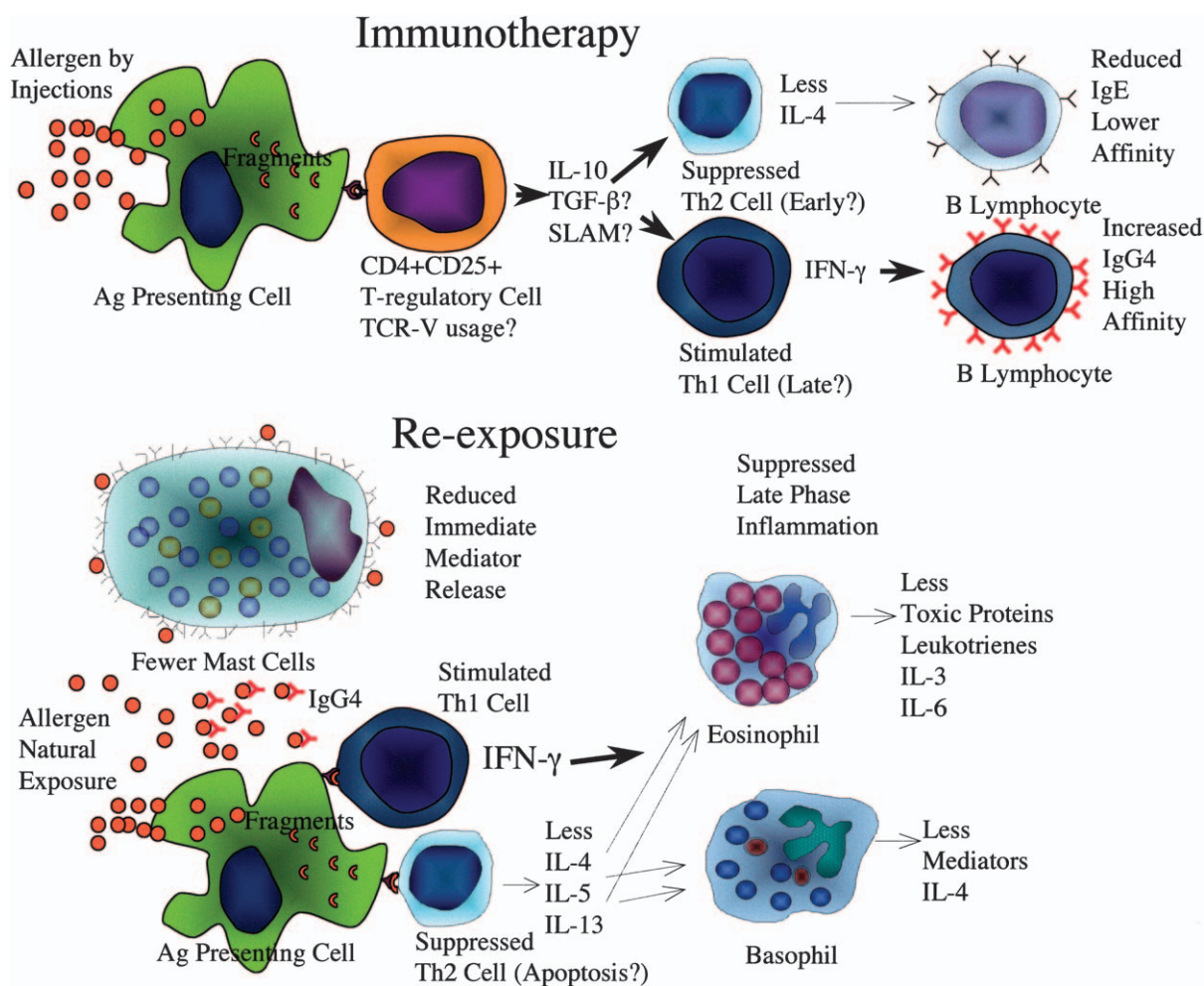


FIG 2. Proposed model of the immunologic effects of allergen immunotherapy. Parenteral introduction of allergen stimulates regulatory T cells to suppress T_H2 responses and stimulate T_H1 responses. These changes partially suppress responses to subsequent exposure, both immediate mediator release and late-phase inflammation. Ag, Antigen.

SLIT in dust mite-induced rhinitis and asthma. The cumulative dose of SLIT was about 86 times greater. Subcutaneous immunotherapy for both rhinitis and asthma was clinically effective. Patients treated with SLIT had decreased rhinitis symptoms ($P < .01$) but no change in asthma scores. Medication scores significantly decreased in both actively treated groups ($P < .01$) at the first year compared with baseline values. When skin prick tests were evaluated, the subcutaneously treated group had a significant decrease in the wheal diameter induced by *Dermatophagoides pteronyssinus* ($P < .01$), *D. farinae* ($P < .05$), and histamine ($P < .05$), whereas the other 2 groups showed no difference. Side effects were minimal in both treated groups.

Khinchin et al²⁹ carried out the only double-blind, double-dummy study reported thus far. In birch pollen allergy the cumulative dose of allergen after the first treatment season was 4717 μ g of Bet v 1 in the SLIT group and 27 μ g of Bet v 1 in the subcutaneous group, implying that SLIT-treated patients on average received 175 times

more allergen than the subcutaneous group. The accumulated mean dose over the 2-year treatment period was 11,182 μ g of Bet v 1 for SLIT-treated and 51 μ g of Bet v 1 for subcutaneously treated patients. Although both treatment groups fared significantly better during 2 birch pollen seasons than the placebo groups on the basis of 2 different methods of estimating symptoms, there was not a significant difference between the 2 active treatments. Five systemic reactions occurred in the subcutaneous group, 2 of which were treated with adrenaline. No systemic reactions of this grade occurred in the SLIT group. In the SLIT group there was an overrepresentation of itching or mild edema in the mouth, throat, or both associated with drop intakes (13 patients vs 2 and 4 patients in the subcutaneous and placebo groups, respectively).

Bagnasco et al³⁰ assessed the pharmacokinetics of sublingual placement in human subjects by using an iodine 123-radiolabeled purified allergen (Par j 1). Sequential scintiscans, plasma radioactivity, and plasma chromatographs were evaluated. No systemic absorption

of the allergen through the mucosae occurred; plasma radioactivity increased only after the allergen was swallowed. Moreover, the allergen was retained for long periods (up to 40 hours) in the oral mucosa. After swallowing, native allergen could not be detected in the bloodstream but only the label. It was not possible to test for allergen in local lymph nodes.^{30,31}

Whether SLIT induces the same immunologic changes as subcutaneous immunotherapy is not clear. Increases in specific IgG4 levels and decreases in IgE levels have been found at times, although not regularly.²⁷ Fanta et al³² found decreased proliferative responses to allergen but no change in cytokine production by allergen-specific T-cell clones with grass pollen extract in a modest cumulative 1-year dose (80 µg of major allergen).

Several commercial preparations of allergen extracts prepared specifically for local use, particularly sublingual use, are available in Europe. No such preparations are approved in the United States. A number of questions remain to be answered about dosage, timing, and immunologic responses. The results of blinded clinical studies thus far, although not uniformly positive, encourage further investigation.

IMMUNOTHERAPY FOR ASTHMA

Immunotherapy for the management of asthma continues to be a matter for discussion. A multicenter study in Europe by Moller et al³³ of children with birch or grass pollen allergies confirms that specific immunotherapy during childhood can reduce the incidence of later asthma. Among 151 patients aged 6 to 14 years, after 3 years of immunotherapy, 19 of 79 treated patients had asthma, whereas 32 of 72 control subjects had asthma (odds ratio, 2.52; $P < .05$).

Cools et al,³⁴ in Belgium, carried out a longer-term follow-up of childhood immunotherapy in a retrospective study. Asthmatic patients allergic to either house dust mite (*D pteronyssinus*; $n = 34$) or to both house dust mite and grass pollen ($n = 14$) and who were treated with specific immunotherapy during childhood (mean duration of immunotherapy, 61 ± 9.70 months) were re-evaluated in early adulthood after mean cessation of immunotherapy for 9.3 ± 2.76 years. A control group of asthmatic patients ($n = 42$) with comparable asthma features had been treated with appropriate antiasthmatic drugs during childhood but never received immunotherapy. At the time of re-evaluation, the mean age in the immunotherapy-treated group was 23.1 ± 3.50 years; in the control group it was 22.7 ± 3.40 years. The risk of frequent asthmatic symptoms was 3 times higher in the control group than in the systemic immunotherapy-treated group (prevalence ratio, 3.43; $P = .0006$). These results tend to substantiate that immunotherapy for rhinitis early in life has useful preventive effects.

A number of studies reviewed above included some patients with allergic asthma along with their rhinitis. In general, the asthmatic symptoms were improved in the

immunotherapy-treated groups along with the rhinitis. A search of the literature on immunotherapy for the past 5 years reveals no new detailed reports of controlled studies specifically addressing asthma in adults, despite many reviews and discussions both pro and con. The most thorough review was by Abramson et al,³⁵ who searched the Cochrane Airways Group trials registry up to June 2001, along with dissertation abstracts, current contents, and reference lists of articles. They selected randomized controlled trials by using allergen-specific immunotherapy to treat asthma and reporting at least one clinical outcome. Three reviewers independently assessed eligibility of studies for inclusion. Two reviewers independently performed quality assessment of studies. Seventy-five trials were included, with 3506 participants (3188 with asthma). There were 36 trials of immunotherapy for house mite allergy, 20 for pollen allergy, 10 for animal dander allergy, 2 for *Cladosporium* species mold allergy, and 1 for latex allergy, and 6 trials looked at multiple allergens. Concealment of allocation was assessed as clearly adequate in only 15 of these trials. Significant heterogeneity was present in a number of comparisons. Overall, there was a significant reduction in asthma symptoms and medication and improvement in bronchial hyperreactivity after immunotherapy. Asthma symptom scores improved significantly (standardized mean difference, -0.72 ; 95% CI, -0.99 to -0.33), and it would have been necessary to treat 4 (95% CI, 3-5) patients with immunotherapy to avoid one deterioration in asthma symptoms. Overall, it would have been necessary to treat 5 (95% CI, 4-6) patients with immunotherapy to avoid one requiring increased medication. Allergen immunotherapy significantly reduced allergen-specific bronchial hyperreactivity, with some reduction in nonspecific bronchial hyperreactivity as well. There was no consistent effect on lung function. The authors concluded that immunotherapy reduces asthma symptoms and use of asthma medications and improves bronchial hyperreactivity. One trial found that the size of the benefit is possibly comparable with that seen with inhaled steroids. The possibility of adverse effects (eg, anaphylaxis) must be considered.

A debate between Adkinson³⁶ (con) and Bousquet³⁷ (pro) reviews the issues in detail and makes interesting reading.

NEW ANTIGENS

Evidence from controlled trials for the efficacy of immunotherapy for allergy to grass pollens, ragweed pollen, birch pollen, mountain cedar pollen, *Parietaria* species pollen, cat and dog danders, and dust mites was reviewed earlier.¹ Since that review, evidence for the efficacy of one additional pollen, latex, and peanut allergens has been published.

Hop Japanese, a weed that grows extensively in Korea, induces a fall pollinosis. An extract of the pollen was given to sensitive patients with a seasonal exacerbation of asthma. The regimen culminated at a maximum tolerated

dose (up to 2500 PNU) and resulted in increases in IgG1 and IgG4 antibodies, along with mild increases in IL-10 and soluble CD23 in serum. Symptom scores for asthma in season were reduced after a year of immunotherapy.³⁸

There have been reports of several individual cases of latex allergy successfully treated with latex extracts.^{39,40} Two placebo-controlled studies used standardized latex extracts prepared by 2 European commercial suppliers. They reported success in evaluating results with latex challenges and symptom diary cards. In both studies systemic reactions occurred during the course of the injections.^{41,42} No commercial standardized extract of latex is available in the United States.

Food allergens might be a new area for immunotherapy. Two early reports indicate that responses to challenges with a small amount of peanut are reduced after a series of injections of peanut extract, although the shots induced a considerable rate of systemic reactions.^{43,44} A review by Bannon et al⁴⁵ makes the case for the use of recombinant peanut allergens in immunotherapy. Currently, there are no other published clinical studies of immunotherapy with food allergens. Covering immunotherapy with concomitant doses of anti-IgE might offer an opportunity to make peanut immunotherapy safer (see below).

NEW METHODS

Peptides

In human lymphocyte cultures and whole sensitized animals, synthetic peptides representing T-cell epitopes, if presented without costimulation, can induce anergy to subsequent challenges with the peptide or whole protein (see review by Haselden et al⁴⁶). Peptides are not expected to strengthen T_H1-mediated immunity but rather to induce a state of tolerance specific to an allergen. Peptides for treatment are selected from candidate peptides on the basis of their ability to induce proliferation in lymphocyte lines from a panel of patients with a specific allergy.

An initial clinical trial with a combination of two 27-amino-acid peptides based on the cat allergen Fel d 1 used 4 subcutaneous injections. It showed amelioration of symptoms when subjects were exposed to cats.⁴⁷ Another study with 3 similarly sized peptides based on ragweed Amb a 1 showed a modest reduction in symptoms during the ragweed season.⁴⁸ With both sets of peptides, mild allergic symptoms (often asthmatic) occurred 1 to 3 hours after the initial injection in about one third of patients, and there were infrequent systemic reactions interpreted as anaphylaxis. Despite proof of principle, further commercial development of these materials was abandoned after the US Food and Drug Administration put additional trials on clinical hold.

A group headed by Kay and Larché has taken up tolerogenic peptide development. The study by Haselden et al⁴⁹ showed that an intracutaneous injection of Fel d 1 peptides (not the same as reported above) resulted in a late asthmatic reaction (LAR) in 9 of 40 cat-sensitive subjects, followed by a period of tolerance to repeat injections. Cell-culture studies indicated that responses to specific peptides

were major histocompatibility complex restricted. A further study of this phenomenon carried out in asthmatic patients with cat allergy used a mix of 12 peptides of short length (16-17 residues) that overlapped and covered most of the Amb a 1 molecule. Doses from 1 to 5 µg administered intracutaneously resulted in an LAR in 7 subjects. Subsequent peptide injections showed a state of pulmonary hyporesponsiveness that lasted as long as 40 weeks. The pretreatment state, however, eventually returned. Late-phase skin responses to cat hair extract were also inhibited, even in subjects who did not exhibit an initial LAR. The treatment also decreased peptide, and whole allergen induced proliferation of PBMCs and production of IL-4, IL-13, and IFN-γ in cultures.⁵⁰

A subsequent placebo-controlled clinical trial by Oldfield et al⁵¹ used progressively larger doses in 5 injections for a total dose of 90 µg of peptide. There were 16 subjects treated with peptides and 8 subjects treated with placebo, and all evaluations were completed in 14 and 6 patients, respectively. Two patients had LARs to the early injections sufficient to warrant stretching the series to 9 and 10 doses. They became desensitized and had no LAR to the later doses. Treated patients had a significant reduction in the size of both the early and late cutaneous reactions to cat dander extract and Fel d 1. Furthermore, PBMC cultures showed reductions in proliferation and IL-4, IL-13, and IFN-γ production in the presence of antigen, along with an increase in IL-10. Clinically, the treated patients reported less severe responses to the presence of cats than in the placebo-treated patients, but the difference was not significant. The duration of the observed changes was not reported beyond the second follow-up examination, which varied between 3 and 9 months.⁵¹ Few patients in a study make significant clinical results unlikely, but the changes in objective parameters encourage further investigation.

CpG-enhanced immunotherapy

The use of adjuvants (aluminum hydroxide salts, lipopolysaccharides, and Freund adjuvant) has long been used to enhance immune responses without any detailed understanding of their mode of action. A variety of animal studies identified first bacterial DNA and then specific palindrome DNA motifs (CpG) found in many bacteria but rarely in eukaryotes as potent adjuvants for T_H1 responses. These sequences appear to account for the adjuvant action of mycobacteria. They likely act through toll-like receptor 9 on dendritic cells because mice that lack toll-like receptor 9 have no adjuvant response. Although a number of sequences have adjuvant effects, hexamers based on the general formula of 5'purine-purine-CG-pyrimidine-pyrimidine-3' are considered optimal. Bacterial DNA and synthetic oligonucleotides of this structure induce B-cell proliferation and immunoglobulin production, as well as macrophage and dendritic cell secretion of IFN-α, IFN-β, IL-12, and IL-18, cytokines that drive differentiation of T_H0 to T_H1 cells. These mechanisms appear to be one of the actions of the innate immune system to drive the phylogenetically more recent

adaptive immune system. The biology of CpG motifs has been reviewed by Krieg and Wagner,⁵² and the biology of toll-like receptors has been reviewed by Zuany-Amorin et al.⁵³ Both deal with potential use of CpG in the treatment of asthma.

One goal of immunotherapy for allergies is to activate or enhance T_H1 responses to specific allergens. Hence adding CpG sequences to an allergen might potentiate the desired response. Synthesized CpG motifs might be either mixed with an allergen vaccine or covalently conjugated to an allergen. First trials of this concept have used a conjugated product developed by Dynavax Technologies Corporation that has an average ratio of 6 CpG residues per ragweed Amb a 1 molecule. In mice such conjugates promote allergen-specific IgG2a antibody production, a marker for T_H1 responses. Such responses occur not only in naive animals but also in animals previously sensitized with allergen. The conjugate was stimulatory for T_H1 responses in rabbits and monkeys as well.⁵⁴

The first safety trial used skin tests to show that the allergenicity of the conjugate was 185-fold less reactive than a licensed ragweed extract. A comparison on the basis of basophil histamine release confirmed a 10- to 100-fold reduction in allergenicity. Subcutaneous administration induced IgG antibody production.^{55,56} A subsequent blinded study used a 6-injection regimen with a target dose of conjugate equivalent to 12 µg of Amb a 1. An increase of IgG antibodies to Amb a 1 was again observed.⁵⁷ A detailed report of clinical results during the first and second ragweed seasons after immunization is in preparation by Creticos et al.

Tulic et al⁵⁸ report clinical and immunologic results comparing this regimen (n = 28) with placebo (n = 29) in patients with ragweed-induced hay fever. A subset of patients had nasal biopsies 24 hours after ragweed challenges. The first postimmunotherapy ragweed season started 3 weeks after the last injection. Symptom reporting was not different between treated patients and patients receiving placebo. However, after the end of the season, biopsy specimens after challenge in treated patients showed a significantly reduced increase in eosinophils and IL-4 mRNA—positive cells and an increased number of IFN-γ mRNA—positive cells compared with placebo-treated patients. Without further treatment, during the next ragweed season, there was a significant decrease in chest symptoms and a trend toward reduced nasal symptoms in the treated group.⁵⁸ These results provide evidence for long-lasting effects from a single short course of this DNA conjugate. Additional study is projected.

Anti-IgE and immunotherapy

A combination of anti-IgE (omalizumab) and allergen immunotherapy might offer advantages that neither method provides separately. Immunotherapy reduces serum IgE levels but little, whereas anti-IgE is not expected to alter lymphocyte physiology. Furthermore, anti-IgE administered during the induction phase of immunotherapy might reduce the risk of IgE-mediated anaphylaxis. A cooperative study by Kuehr et al⁵⁹

administered preseasonal immunotherapy for both birch- and grass pollen-induced hay fever and followed with omalizumab or placebo during the season while maintenance immunotherapy continued. In both conditions, the patients who received omalizumab had about a 50% reduction in symptom load when compared with patients who had immunotherapy alone ($P = .003$ for birch and $P = .001$ for grass). Although this study showed additive effects for the 2 methods, it did not test whether omalizumab protected against anaphylactic reactions during the build-up phase of immunotherapy. A prospective study testing this hypothesis is now underway.

Recombinant and engineered allergens

A review by Chapman et al⁶⁰ in 2000 listed 19 recombinant allergens from cat, mite, cockroach, grass, ragweed, birch, and peanut that show allergenic activity appropriate for their use in diagnostics, such as skin tests and *in vitro* tests. The authors propose that the use of proteins of defined structure prepared in appropriate vectors would provide a more rational basis for diagnosis and treatment. Single proteins used for *in vitro* diagnostic tests should be less subject to interference by irrelevant proteins in crude extracts. Testing for allergy to single proteins would allow the preparation of combinations of proteins specific for an individual's allergies. Such cocktails would not be subject to degradation by unwanted enzymes in crude extracts. Dosage could more accurately be measured than is possible with crude combinations.

The European Union has funded a study called CREATE (Development of certified reference materials for allergenic products and the validation of methods for their quantification; cocordinator, R. van Ree, Amsterdam, The Netherlands). The study will evaluate the use of recombinant allergens as a basis for standardization of allergenic products.⁶¹ A review by Valenta and Kraft⁶² further makes the case for the use of recombinant allergens for immunotherapy either in forms that reproduce natural allergens or as proteins that have been genetically engineered to reduce allergenicity.

OVERVIEW

The last 5 years has seen a better (although still incomplete) understanding of mechanism, convincing evidence of long-standing clinical improvement in respiratory allergy, and the demonstration that adequate doses applied sublingually can alter rhinitis and asthma. In the absence of extracts approved for sublingual use, these findings have not materially changed the recommendations for allergy practice in the United States. Critics continue to point to the long-term commitment, expense in money and time, and risk of anaphylaxis as drawbacks that limit the use of immunotherapy. Nevertheless, as opposed to drug therapy, immunotherapy offers the chance of prolonged modification of the abnormal immunologic responses that underlie allergic diseases. Several improvements in the materials used for immunotherapy are under investigation that offer promise

in reducing the risk of anaphylaxis and thereby the commitment to multiple injections under office supervision. How long it might take for any one of these to be ready for general use is conjectural.

REFERENCES

- Norman PS. Immunotherapy: past and present. *J Allergy Clin Immunol* 1998;102:1-10.
- Durham SR, Till SJ. Immunologic changes associated with allergen immunotherapy. *J Allergy Clin Immunol* 1998;102:157-64.
- Söderlund A, Gabrielsson S, Paulie S, Hammarström ML, Rak S, Troye-Blomberg M. Allergen induced cytokine profiles in type I allergic individuals before and after immunotherapy. *Immunol Lett* 1997;57:177-81.
- Van Bever HP, Vereecke IF, Bridts CH, De Clerck LS, Stevens WJ. Comparison between the in vitro cytokine production of mononuclear cells of young asthmatics with and without immunotherapy (IT). *Clin Exp Allergy* 1998;28:943-9.
- Klimek L, Dormann D, Jarman ER, Cromwell O, Riechelmann H, Reske-Kunz AB. Short-term preseasonal birch pollen allergoid immunotherapy influences symptoms, specific nasal provocation and cytokine levels in nasal secretions, but not peripheral T-cell responses, in patients with allergic rhinitis. *Clin Exp Allergy* 1999;29:1326-35.
- Wachholz PA, Nouri-Aria KT, Wilson DR, Walker SM, Verhoef A, Till SJ, et al. Grass pollen immunotherapy for hayfever is associated with increases in local nasal but not peripheral Th1:Th2 cytokine ratios. *Immunology* 2002;105:56-62.
- Moverare R, Elfman L, Björnsson E, Stalenheim G. Changes in cytokine production in vitro during the early phase of birch-pollen immunotherapy. *Scand J Immunol* 2000;52:200-6.
- Majori M, Caminati A, Corradi M, Brianti E, Scarpa S, Pesci A. T-cell cytokine pattern at three time points during specific immunotherapy for mite-sensitive asthma. *Clin Exp Allergy* 2000;30:341-7.
- Oda N, Yamashita N, Minoguchi K, Takeno M, Kaneko S, Sakane T, et al. Long-term analysis of allergen-specific T cell clones from patients with asthma treated with allergen rush immunotherapy. *Cell Immunol* 1998;190:43-50.
- Akdis CA, Blaser K. Role of IL-10 in allergen-specific immunotherapy and normal response to allergens. *Microbes Infect* 2001;3:891-8.
- Jutel M, Akdis M, Budak F, Aebischer-Casaulta C, Wrzyszc M, Blaser K, et al. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 2003;33:1205-14.
- Francis JN, Till SJ, Durham SR. Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. *J Allergy Clin Immunol* 2003;111:1255-61.
- Nouri-Aria KT, Kayhan A, Wachholz PA, Francis JN, Jacobson MR, Walker SM, et al. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. *J Immunol* 2004;172:3252-9.
- Guerra F, Carracedo J, Solana-Lara R, Sanchez-Guijo P, Ramirez R. TH2 lymphocytes from atopic patients treated with immunotherapy undergo rapid apoptosis after culture with specific allergens. *J Allergy Clin Immunol* 2001;107:647-53.
- Laaksonen K, Junikka M, Laheesmaa R, Terho EO, Savolainen J. In vitro allergen-induced mRNA expression of signaling lymphocytic activation molecule by PBMC of patients with allergic rhinitis is increased during specific pollen immunotherapy. *J Allergy Clin Immunol* 2003;112:1171-7.
- Sade K, Kivity S, Levy A, Fireman E. The effect of specific immunotherapy on T-cell receptor repertoire in patients with allergy to house-dust mite. *Allergy* 2003;58:430-4.
- Håkansson L, Heinrich C, Rak S, Venge P. Activation of B-lymphocytes during pollen season. Effect of immunotherapy. *Clin Exp Allergy* 1998;28:791-8.
- Wachholz PA, Kristensen Soni N, Till SJ, Durham SR. Inhibition of allergen-IgE binding to B cells by IgG antibodies after grass pollen immunotherapy. *J Allergy Clin Immunol* 2003;112:915-22.
- Meissner N, Kochs S, Coutelle J, Kussebi F, Baumgarten C, Lowenstein H, et al. Modified T-cell activation pattern during specific immunotherapy (SIT) in cat-allergic patients. *Clin Exp Allergy* 1999;29:618-25.
- Monteseirín J, Bonilla I, Camacho J, Conde J, Sobrino F. Elevated secretion of myeloperoxidase by neutrophils from asthmatic patients: the effect of immunotherapy. *J Allergy Clin Immunol* 2001;107:623-6.
- Wilson DR, Nouri-Aria KT, Walker SM, Pajno GB, O'Brien F, Jacobson MR, et al. Grass pollen immunotherapy: symptomatic improvement correlates with reductions in eosinophils and IL-5 mRNA expression in the nasal mucosa during the pollen season. *J Allergy Clin Immunol* 2001;107:971-6.
- De Amici M, Puggioni F, Casali L, Alesina R. Variations in serum levels of interleukin (IL)-1 β , IL-2, IL-6, and tumor necrosis factor- α ; during specific immunotherapy. *Ann Allergy* 2001;86:311-3.
- Durham SR, Varney VA, Gaga M, Jacobson MR, Varga EM, Frew AJ, et al. Grass pollen immunotherapy decreases the number of mast cells in the skin. *Clin Exp Allergy* 1999;29:1490-6.
- Wilson DR, Irani A-M, Walker SM, Jacobson MR, Mackay IS, Schwartz LB, et al. Grass pollen immunotherapy inhibits seasonal increases in basophils and eosinophils in the nasal epithelium. *Clin Exp Allergy* 2001;31:1705-13.
- Pierson-Mullany LK, Jackola D, Blumenthal M, Rosenberg A. Altered allergen binding capacities of Amb a 1-specific IgE and IgG4 from ragweed-sensitive patients receiving immunotherapy. *Ann Allergy* 2000;84:241-3.
- Durham SR, Walker SM, Varga EM, Jacobson MR, O'Brien F, Noble W, et al. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 1999;341:468-75.
- Canonica GW, Passalacqua G. Noninjection routes for immunotherapy. *J Allergy Clin Immunol* 2003;111:437-49.
- Mungan D, Misirligil Z, Gürbüz L. Comparison of the efficacy of subcutaneous and sublingual immunotherapy in mite-sensitive patients with rhinitis and asthma-a placebo controlled study. *Ann Allergy* 1999;82:485-90.
- Khinchi MS, Poulsen LK, Carat F, Andre C, Hansen AB, Malling H-J. Clinical efficacy of sublingual and subcutaneous birch pollen allergen-specific immunotherapy: a randomized, placebo-controlled, double-blind, double-dummy study. *Allergy* 2004;59:45-53.
- Bagnasco M, Mariani G, Passalacqua G, Motta C, Bartolomei M, Falagiani P, et al. Absorption and distribution kinetics of the major *Parietaria judaica* allergen (Par j 1) administered by noninjectable routes in healthy human beings. *J Allergy Clin Immunol* 1997;100:122-9.
- Bagnasco M, Passalacqua G, Villa G, Augeri C, Flamigni G, Borini E, et al. Pharmacokinetics of an allergen and a monomeric allergoid for oromucosal immunotherapy in allergic volunteers. *Clin Exp Allergy* 2001;31:54-60.
- Fanta C, Hirt W, Siemann U, Horak F, Kraft D, Ebner H, et al. Systemic immunological changes induced by administration of grass pollen allergens via the oral mucosa during sublingual immunotherapy. *Int Arch Allergy Immunol* 1999;120:218-24.
- Moller C, Dreborg S, Ferdousi HA, Halken S, Host A, Jacobsen L, et al. Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). *J Allergy Clin Immunol* 2002;109:251-6.
- Cools M, Van Bever HP, Weyler JJ, Stevens WJ. Long term effects of specific immunotherapy, administered during childhood, in asthmatic patients allergic to either house-dust mite or to both house-dust mite and grass pollen. *Allergy* 2000;55:69-73.
- Abramson M, Puy R, Weiner J. Allergen immunotherapy for asthma. *Cochrane Database Syst Rev* 2003;4:CD001186.
- Adkinson NF, Jr. Con: immunotherapy is not clinically indicated in the management of allergic asthma. *Am J Respir Crit Care Med* 2001;164:2140-2.
- Bousquet J. Pro: immunotherapy is clinically indicated in the management of allergic asthma. *Am J Respir Crit Care Med* 2001;164:2139-42.
- Park H-S, Nahm D-H, Kim H-Y, Suh Y-J, Cho J-W, Kim S-S, et al. Clinical and immunologic changes after allergen immunotherapy with Hop Japanese pollen. *Ann Allergy* 2001;86:444-8.
- Pereira C, Rico P, Lourenco M, Lombardero M, Pinto-Mendes J, Chieira C. Specific immunotherapy for occupational latex allergy. *Allergy* 1999;54:291-3.

40. Pereira C, Pedro E, Tavares B, Ferreira MB, Carrapatoso I, Rico P, et al. Specific immunotherapy for severe latex allergy. *Allerg Immunol (Paris)* 2003;35:217-25.
41. Leynadier F, Herman D, Vervloet D, Andre C. Specific immunotherapy with a standardized latex extract versus placebo in allergic healthcare workers. *J Allergy Clin Immunol* 2000;106:585-90.
42. Sastre J, Fernandez-Nieto M, Rico P, Martin S, Barber D, Cuesta J, et al. Specific immunotherapy with a standardized latex extract in allergic workers: a double-blind, placebo-controlled study. *J Allergy Clin Immunol* 2003;111:985-94.
43. Oppenheimer JJ, Nelson HS, Bock SA, Christensen F, Leung DY. Treatment of peanut allergy with rush immunotherapy. *J Allergy Clin Immunol* 1992;90:256-62.
44. Nelson HS, Lahr J, Rule R, Bock A, Leung D. Treatment of anaphylactic sensitivity to peanuts by immunotherapy with injections of aqueous peanut extract. *J Allergy Clin Immunol* 1997;99:744-51.
45. Bannon GA, Cockrell G, Connaughton C, West CM, Helm R, Stanley JS, et al. Engineering, characterization and in vitro efficacy of the major peanut allergens for use in immunotherapy. *Int Arch Allergy Immunol* 2001;124:70-2.
46. Haselden BM, Kay AB, Larché M. Peptide-Mediated Immune Responses in Specific Immunotherapy. *Int Arch Allergy Immunol* 2000;122:229-37.
47. Norman PS, Ohman JL, Jr, Long AA, Creticos PS, Gefter MS, Shaked Z, et al. Treatment of cat allergy with T cell reactive peptides. *Am J Respir Crit Care Med* 1996;154:1623-8.
48. Creticos PS, Hebert J, Philip G, Group ARS. Efficacy of Allervax® ragweed peptides in the treatment of ragweed-induced allergy. *J Allergy Clin Immunol* 1997;99(suppl):S401.
49. Haselden BM, Barry Kay A, Larche M. Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reactions. *J Exp Med* 1999;189:1885-94.
50. Oldfield WL, Kay AB, Larche M. Allergen-derived T cell peptide-induced late asthmatic reactions precede the induction of antigen-specific hyporesponsiveness in atopic allergic asthmatic subjects. *J Immunol* 2001;167:1734-9.
51. Oldfield WL, Larche M, Kay AB. Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. *Lancet* 2002;360:47-53.
52. Krieg AM, Wagner H. Causing a commotion in the blood: immunotherapy progresses from bacteria to bacterial DNA. *Immunol Today* 2000;21:521-6.
53. Zuany-Amorim C, Hastewell J, Walker C. Toll-like receptors as potential therapeutic targets for multiple diseases. *Nat Rev Drug Discov* 2002;1:797-807.
54. Tighe H, Takabayashi K, Schwartz D, Van Nest G, Tuck S, Eiden JJ, et al. Conjugation of immunostimulatory DNA to the short ragweed allergen Amb a 1 enhances its immunogenicity and reduces its allergenicity. *J Allergy Clin Immunol* 2000;106:124-34.
55. Creticos PS, Eiden JJ, Balcer SL, Van Nest G, Kagey-Sabotka A, Tuck SF, et al. Immunostimulatory oligonucleotides conjugated to Amb a 1: safety, skin test reactivity, and basophil histamine release. *J Allergy Clin Immunol* 2000;105(suppl):S70.
56. Creticos PS, Balcer SL, Schroeder JT, Hamilton RG, Chung B, Norman PS, et al. Initial immunotherapy trial to explore the safety, tolerability and immunogenicity of subcutaneous injection of an Amb a 1 immunostimulatory oligonucleotide conjugate (Aic) in ragweed allergic adults. *J Allergy Clin Immunol* 2001;107(suppl):S216.
57. Creticos PS, Eiden JJ, Broide D, Balcer-Whaley SL, Schroeder JT, Khattignavong A, et al. Immunotherapy with immunostimulatory oligonucleotides linked to purified ragweed Amb a 1 allergen: effects on antibody production, nasal allergen provocation, and ragweed seasonal rhinitis. *J Allergy Clin Immunol* 2002;109:743.
58. Tulic MK, Fiset PO, Christodoulouopoulos P, Vaillancourt P, Desrosiers M, Lavigne F, et al. Amb a 1-immunostimulatory oligodeoxynucleotide conjugate immunotherapy decreases the nasal inflammatory response. *J Allergy Clin Immunol* 2004;113:235-41.
59. Kuehr J, Brauburger J, Zielen S, Schauer U, Kamin W, Von Berg A, et al. Efficacy of combination treatment with anti-IgE plus specific immunotherapy in polysensitized children and adolescents with seasonal allergic rhinitis. *J Allergy Clin Immunol* 2002;109:274-80.
60. Chapman MD, Smith AM, Vailes LD, Arruda LK, Dhanaraj V, Pomes A. Recombinant allergens for diagnosis and therapy of allergic disease. *J Allergy Clin Immunol* 2000;106:409-18.
61. Bousquet J, Ansotegui JJ, van Ree R, Burney PG, Zuberbier T, van Cauwenberge P. European Union meets the challenge of the growing importance of allergy and asthma in Europe. *Allergy* 2004;59:1-4.
62. Valenta R, Kraft D. From allergen structure to new forms of allergen-specific immunotherapy. *Curr Opin Immunol* 2002;14:718-27.