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REVIEW ARTICLE

Recent Progress in Allergen Immunotherapy

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ABSTRACT

The efficacy of allergen immunotherapy for the treatment of allergic rhinoconjunctivitis with or without seasonal bronchial asthma and anaphylaxis caused by the sting of the hymenoptera class of insects has been clearly demonstrated in numerous well-designed, placebo-controlled trials. Immunotherapy whether by subcutaneous injection of allergen extract or by oral/sublingual routes modifies peripheral and mucosal T_H2 responses in favour of T_H1 responses and augments IL-10 synthesis by T_{Regs} both locally and by peripheral T cells. Recent researches into the cellular and molecular basis of allergic reactions have advanced our understanding of the mechanisms involved in allergic diseases. They have also helped the development of innovative approaches that are likely to further improve the control of allergic responses in the future. Novel approaches to immunotherapy that are currently being explored include the use of peptide-based allergen preparations, which do not bind IgE and therefore do not activate mast cells, but reduce both T_H1 and T_H2-cytokine synthesis, while increasing levels of IL-10. Alternative strategies include the use of adjuvants, such as nucleotide immunostimulatory sequences derived from bacteria CpG or monophosphoryl lipid A that potentiate TH1 responses. Blocking the effects of IgE using anti-IgE such as omalizumab, a recombinant humanized monoclonal antibody that selectively binds to IgE, has been shown to be a useful strategy in the treatment of allergic asthma and rhinitis. The combination of anti-IgE-monoclonal antibody omalizumab with allergen immunotherapy has proved beneficial for the treatment of allergic diseases, offering improved efficacy, limited adverse effects, and potential immune-modifying effects. This combination may also accelerate the rapidity by which immunotherapy induces T_{Reg} cells. If allergic diseases are due to a lack of allergen-specific T_{Reg} cells, then effective therapies should target the induction and the development of T_{Reg} cells producing cytokines such as IL-10.

Keywords: Allergy, IgE, Rhinitis, Immunotherapy

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List of abbreviations

RANTES	Regulated upon activation normal T cell express and secreted	**SIT**	Specific immunotherapy
TARC	Thymus and activation regulated chemokine	**VCAM-1**	Vascular cell adhesion molecule -1
MCP-4	Monocyte chemoattractant protein-4	**LAR**	Late asthmatic reactions
MDC	Macrophage derived chemokine	**T_{Reg}**	Regulatory T cells
MPL	Monophosphoryl lipid A	***Bet v***	*Betula Verrucosa*
SLIT	Sublingual immunotherapy	***rBet v***	Recombinant *Bet v*
PBMC	Peripheral blood mononuclear cells	***Fel d 1***	*Felis domesticus*
PLA	Phospholipase A	***Amb a***	*Ambrosia artemisiifolia*
AIC	*Amb a 1* immunostimulatory conjugate	***Der p***	*Dermatophagoides Pteronyssinus*
TGF-β	Transforming growth factor -β	***Ph l p***	*Phleum Pratense*

INTRODUCTION

Rhinitis is a common condition causing widespread morbidity, with substantial socio-economic burden, reduced work productivity and loss of school and work days. About 50% of patients suffering from rhinitis have allergic rhinitis while the other 50% do not have a defined allergic aetiology. The allergic rhinitis is an IgE-mediated inflammatory disease affecting the nasal mucous membranes. It can affect people of all ages, sexes, social and ethnic groups and has an impact on their quality of life as well as healthcare utilisation. An increasing prevalence of allergic rhinitis over the last decades has been recognized, and it is often associated with asthma (1-3).

Allergic rhinitis is characterized by an inflammatory infiltrate composed of different cells. This cellular response includes selective recruitment and transendothelial migration of cells and localization of cells within the different compartments of the nasal mucosa.

Activation and differentiation of infiltrating cells, release of mediators by these cells, and regulation of local and systemic IgE synthesis are amongst immunological events occuring in the nasal mucosa (4, 5).

IMMUNE MECHANISMS OF ALLERGIC RHINITIS

Early Phase Response. Following inhalation and deposition in the nasal mucosal layer, allergens are taken up by antigen presenting cells, processed and presented to helper T lymphocytes. Activated helper T lymphocytes release cytokines like IL-4, IL-5 and IL-13 and interact with B lymphocytes to induce the synthesis of allergen-specific IgE which then binds to the high-affinity receptor for IgE on the surface of mast cells (6). An allergen can induce both an immediate type I and a delayed type IV hypersensitivity reactions (7). The early or immediate response occurs in sensitized individuals within minutes of allergen exposure. One of the cardinal features of the early phase response is the degranulation of mast cells present in the epithelial compartment of the nasal mucosa (8, 9) and release of a variety of mediators such as histamine, leukotrienes and prostaglandins resulting in the symptoms of the early phase response (10). This can lead to acute clinical symptoms such as rhinorrhea, sneezing, itching, nasal blockage and conjunctivitis.

Late Phase Response. The early responses are usually followed by the late responses which occur 4–6 h after antigen stimulation. The late responses are characterized by a

prolongation of symptoms which lasts for about 18–24 h. It is predominantly inflammatory in nature and is characterized by an inflammatory cellular influx composed of T lymphocytes, basophils and eosinophils. The key to the orchestration of the late-phase response lies in the production and release of a variety of cytokines and chemokines like IL-4 and IL-13 from T cells, eosinophils and mast cells (11-13) resulting in the upregulation of adhesion molecules like vascular cell adhesion molecule-1 (VCAM-1) on the endothelial cells facilitating the infiltration of eosinophils, T cells and basophils into the nasal mucosa. Chemokines like RANTES (Regulated upon activation normal T cell express and secreted), eotaxin, MCP-4 (monocyte chemoattractant protein-4), TARC (thymus and activation regulated chemokine) and MDC (macrophage derived chemokine) released from epithelial cells serve as chemoattractants for eosinophils, basophils and T lymphocytes (14-16). In addition, eosinophils that are major players in the late phase response release an array of proinflammatory mediators, including cysteinyl leukotrienes, cationic proteins, eosinophil peroxidase, and major basic protein, and may serve as a major source of IL-3, IL-4, IL-5, GM-CSF, and IL-13 (11, 16).

The initial event responsible for the development of allergic diseases is the generation of allergen-specific $CD4^+$ T helper cells. The current view is that under the influence of IL-4, naïve T cells activated by antigen presenting cells differentiate into Th2 cells. Once generated, effector T_H2 cells produce IL-4, IL-5 and IL-13 and mediate several regulatory and effector functions. These cytokines induce the production of allergen-specific IgE by B cells, development and recruitment of eosinophils, production of mucus and contraction of smooth muscles. Furthermore, the degranulation of basophils and mast cells by IgE-mediated cross-linking of receptors is the key event in type I hypersensitivity, which may lead to chronic allergic inflammation. Importantly, although T_H2 cells are responsible for the development of allergic diseases, T_H1 cells may contribute to chronicity and effector phase in allergic diseases. Distinct T_H1 and T_H2 subpopulations of T cells counter-regulate each other and play a role in distinct diseases (17).

MANAGEMENT

The symptoms of IgE-mediated allergic reactions, such as rhinitis, conjunctivitis and asthma, can be ameliorated by temporary suppression of mediators and immune cells (by anti-histamines, anti-leukotrienes, β2 adrenergic receptor anatgonists and corticosteroids) together with measures to avoid allergens (18-20). Some patients on medication may experience side effects and allergy avoidance can be costly as well as impractical. However, a more long term solution could be *Specific Allergen Immunotherapy* that specially restores a normal immunity against allergens.

Specific Allergen Immunotherapy (SIT) provides an alternative treatment, in patients with immunoglobulin E (IgE)-mediated disease particularly those with severe seasonal pollinosis. SIT, or allergy vaccination was first described almost 100 years ago (21), and is based on the application of small but increasing doses of allergen to which the patient is sensitized. Historically, relatively crude allergen extracts have been used for SIT, but for certain allergens, such as peanut and latex, these are associated with a high risk of life-threatening anaphylaxis.

Allergen immunotherapy involves administration of allergen using subcutaneous, sublingual or intranasal route. SIT is highly effective and the only treatment to date that can affect the natural course of allergic rhinitis/conjunctivitis, allergic reactions to stinging

insects and allergic asthma according to many double blind randomized studies (22-25). Conventional allergen-desensitization immunotherapy is believed to prevent the development of asthma in patients with allergic rhinitis as well as further allergen sensitisations (26). In children, 3 years of house dust mite extract immunotherapy prevented the onset of new allergen sensitivities and resulted in a 2- to 3-fold reduction in the risk of developing asthma (27, 28).

Mechanisms of Allergen Immunotherapy. Despite its usage in clinical practice for nearly a century, the underlying immunological mechanisms of allergen-SIT are slowly elucidated. The mechanisms associated with injection allergen immunotherapy are thought to involve both cellular and humoral immune responses (29).

Mast Cells and Basophils. Mast cell numbers in skin biopsy specimens from sites of allergen provocation before and after immunotherapy for grass pollen allergy were examined by Durham et al (30). 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 ($r = 0.61$, $P = 0.001$; $r = 0.75$, $p = 0\ .0001$, respectively), however the underlying mechanism of which is not understood (30). In another study, specific markers for basophils, eosinophils, and mast cells were analysed 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, mast cells, and eosinophils. In immunotherapy-treated patients smaller but still significant increases in basophils and eosinophils were observed, but not in mast cells. In the epithelium, on the other hand, mast cells and eosinophils showed seasonal increases in both groups. Basophils were present in the epitheliums of 6 of 17 in the placebo-treated group and 1 of 20 in the immunotherapy-treated group. A significant correlation was observed between eosinophils and IL-5 expression ($r = 0.5$; $p < 0.05$). Both eosinophils ($r = 0.6$; $p < 0.02$) and IL-5 ($r = 0.6$; $p < 0.02$) correlated with symptoms after immunotherapy. These findings support the hypothesis that the anti-inflammatory effects of immunotherapy extend to both basophils and eosinophils (31,32).

Cytokine Production by PBMC. Specific allergen IT has been found to be associated with a decrease in local IL-4 and IL-5 production by $CD4^+$ T cells and a shift from T_H2 cytokine pattern towards increased IFN-γ production in variety of allergic conditions such as grass pollen, birch and house dust mite (HDM), allergy to bee venom and wasp venom (33,34).

Cytokine expression in PBMCs before and after birch pollen immunotherapy was studied by Söderlund et al (35). 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 and IL-5 was increased during the pollen season in both groups and presumably not influenced by immunotherapy (35).

Van Bever et al compared SIT treated, house dust mite sensitive asthmatics with untreated control subjects. PBMCs from treated patients secreted less IL-4 and IL-5 after immunotherapy (36). Klimek et al studied immunotherapy with birch pollen allergoid and found an increase in IFN-γ concentration, a decrease in the amounts of IL-5 and no

measurable levels of IL-4 were detected in the nasal secretion. In contrast, in allergen-stimulated cultures of T cells, no changes were found in cytokine expression for IL-4, IL-5, IL-10, or IFN-γ (37). Wachholz et al reported 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 (38).

IL-10 production by PBMCs from patients who had undergone a year of grass pollen immunotherapy was significantly greater than untreated allergic rhinitics. PBMCs from immunotherapy-treated patients stimulated with *Phleum pratense* (grass pollen extract) produced significantly more IL-10 than atopic control subjects. The number of $CD4^+CD25^+$ cells identified after allergen stimulation was also greater in the immunotherapy group. $CD4^+CD25^+$ T cells from immunotherapy-treated patients were almost exclusively positive for intracellular IL-10. In contrast, the levels of Th2 cytokines production (IL-4, IL-5, and IL-13) were similarly greater in both the immunotherapy and atopic groups than in the nonatopic control group (39).

In studies of immunotherapy for insect venom anaphylaxis, 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 have been reported (40). The authors attributed the latter effects to the ability of IL-10 to block the costimulatory CD28-B7.1 interaction and subsequent signalling pathways in T cells. A further study (41) 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 house dust mite, *Dermatophagoides pteronyssinus (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 (41).

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 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 (42), graft rejection (43), inflammatory arthritis (44), experimental autoimmune encephalomyelitis (an animal model of multiple sclerosis) (45), colitis (46) and allergic inflammation (47, 48). IL-10 has a number of documented anti-allergic properties that might be important to immunotherapy (49). These include modulation of IL-4–induced B-cell IgE production in favor of IgG4 (50), inhibition of IgE-dependent mast cell activation (51), and inhibition of human eosinophil cytokine production and survival (52). In human T cells IL-10 suppresses production of pro-allergic cytokines, such as IL-5 (53), and is able to induce a state of antigen-specific hyporesponsiveness or anergy (54). This might occur as a result of IL-10 receptor–dependent blockade of CD28 T-cell costimulation because CD28 tyrosine phosphorylation and subsequent signalling in T cells in response to ligation by B7 molecules on antigen-presenting cells are inhibited by IL-10 (55).

It appears however, that the induction of a tolerance state in peripheral T cells represents an essential step in allergen-SIT. Peripheral T cell tolerance is characterized mainly by suppressed proliferative and cytokine responses against the major allergens and its T cell recognition sites. Furthermore, IL-10 down regulates eosinophil function and activity and suppresses IL-5 production by human resting T_H0 and T_H2 cells. More-

over, IL-10 inhibits endogenous GM-CSF production and CD40 expression by activated eosinophils and enhances eosinophil cell death (56, 57).

Local Nasal Cytokine Expression. Grass pollen immunotherapy inhibits allergen-induced infiltration of $CD4^+$ T lymphocytes, eosinophils and IL-4, IL-5 cytokine mRNA expressing cells in the nasal mucosa and increases the number of cells expressing IFN-γ mRNA (58, 59). The role of IL-10 in the induction of clinical, cellular, and humoral tolerance during immunotherapy for local mucosal allergy was studied in subjects with seasonal pollinosis (60). Local and systemic IL-10 responses and serum Ab concentrations were measured before/after a double-blind trial of grass pollen (*Phleum pratense, Phl p*) immunotherapy. Local increases in IL-10 mRNA-positive cells in the nasal mucosa were observed after 2 years of immunotherapy, but only during the pollen season (Figure 1). IL-10 protein-positive cells were also increased and correlated with IL-10 $mRNA^+$ cells. These changes were not seen in placebo-treated subjects or in healthy controls. Fifteen and 35% of IL-10 mRNA signals were colocalized to $CD3^+$ T cells and $CD68^+$ macrophages, respectively, whereas only 1–2% of total $CD3^+$ cells and 4% of macrophages expressed IL-10 (Figure 2). Following 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. Post-immunotherapy serum exhibited inhibitory activity, which co-eluted with IgG4, and blocked IgE-facilitated binding of allergen-IgE complexes to B cells. Both the increases in IgG and the IgG "blocking" activity correlated with the patients' overall assessment of improvement. Thus, grass pollen immunotherapy may induce allergen-specific, IL-10-dependent "protective" IgG4 responses (60).

In 44 patients with seasonal rhinitis/asthma serum IgA1, IgA2 and polymeric (J chain containing) antibodies to the major allergen *Phl p 5* were determined by ELISA before and after a 2-years double-blind trial of grass pollen (*Phleum pratense, Phl p*)-injection IT. Sera from five IT patients were fractionated for functional analysis of the effects of IgA and IgG antibodies on IL-10 production by blood monocytes and allergen-IgE binding to B cells. Serum *Phl p 5*-specific IgA2 antibodies increased after 2 year-treatment (~8-fold increase, $p=0.002$), in contrast to IgA1. Increases in polymeric antibodies to *Phl p 5* (~2-fold increase, $p=0.02$) and in nasal TGF-β mRNA ($p=0.05$) were also observed, and TGF-β mRNA correlated with serum *Phl p 5* IgA2 ($r=0.61$, $p=0.009$) (Figure 3). Post-IT IgA fractions triggered IL-10 secretion by monocytes, while not inhibiting allergen-IgE binding to B cells as observed with IgG fractions. This study shows for the first time that the IgA response to IT is selective for IgA2, correlates with increased local mucosal TGF-β expression and induces monocyte IL-10 expression, and suggests that IgA antibodies could thereby contribute to the tolerance developed in IT-treated allergic patients (61).

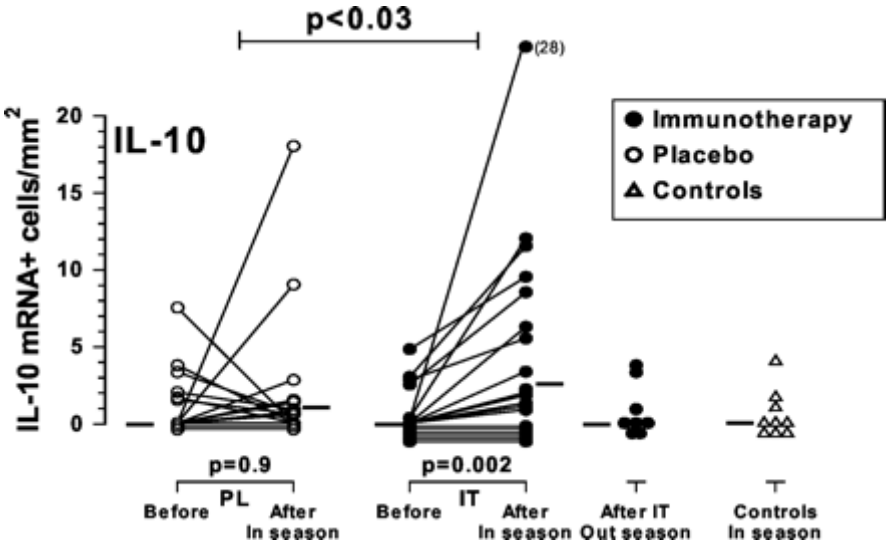

Figure 1. IL-10 mRNA$^+$ cells in the nasal mucosa of IT-treated patients (•), placebo-treated (PL) patients (○), and normal non-atopic control subjects (△). Results are expressed as the number of cells per square millimeter. Pretreatment biopsies were taken outside the pollen season and posttreatment biopsies were taken during the peak pollen season after 2 years of treatment. Biopsies were also taken from IT patients after treatment, outside the pollen season. (adapted from J Immunol 2004;172:3252–3259).

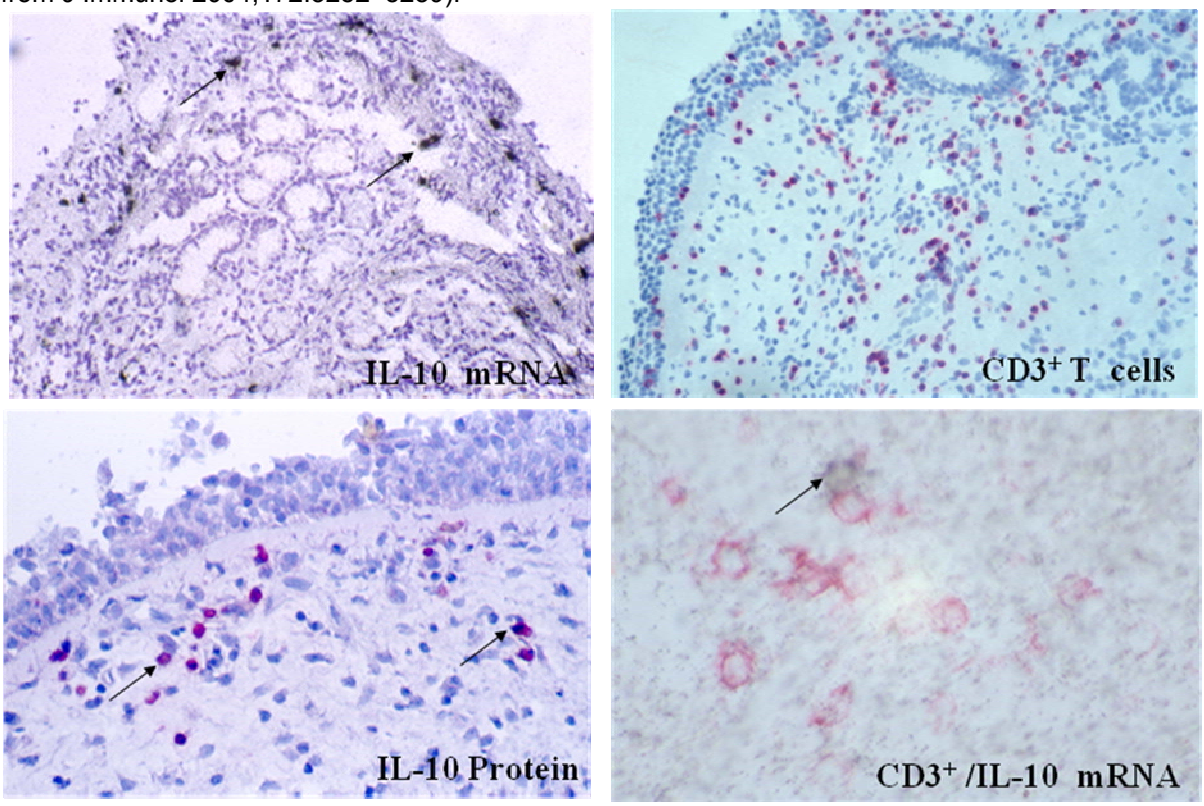

Figure 2. IL-10 mRNA$^+$ cells detected by in situ hybridization (^{35}S-labeled riboprobe; magnification, x40). CD3$^+$ T lymphocytes detected by immunohistochemistry (alkaline phosphatase anti-alkaline phosphatase technique; magnification, x40). IL-10 protein-positive cells detected by immunohistochemistry (avidin-biotin technique; magnification, x40). Colocalization of IL-10 mRNA to CD3$^+$ T cells by sequential immunohistochemistry followed by in situ hybridization (magnification, x100). Arrows show individual positive cells. (Adapted from J Immunol 2004;172:3252–3259).

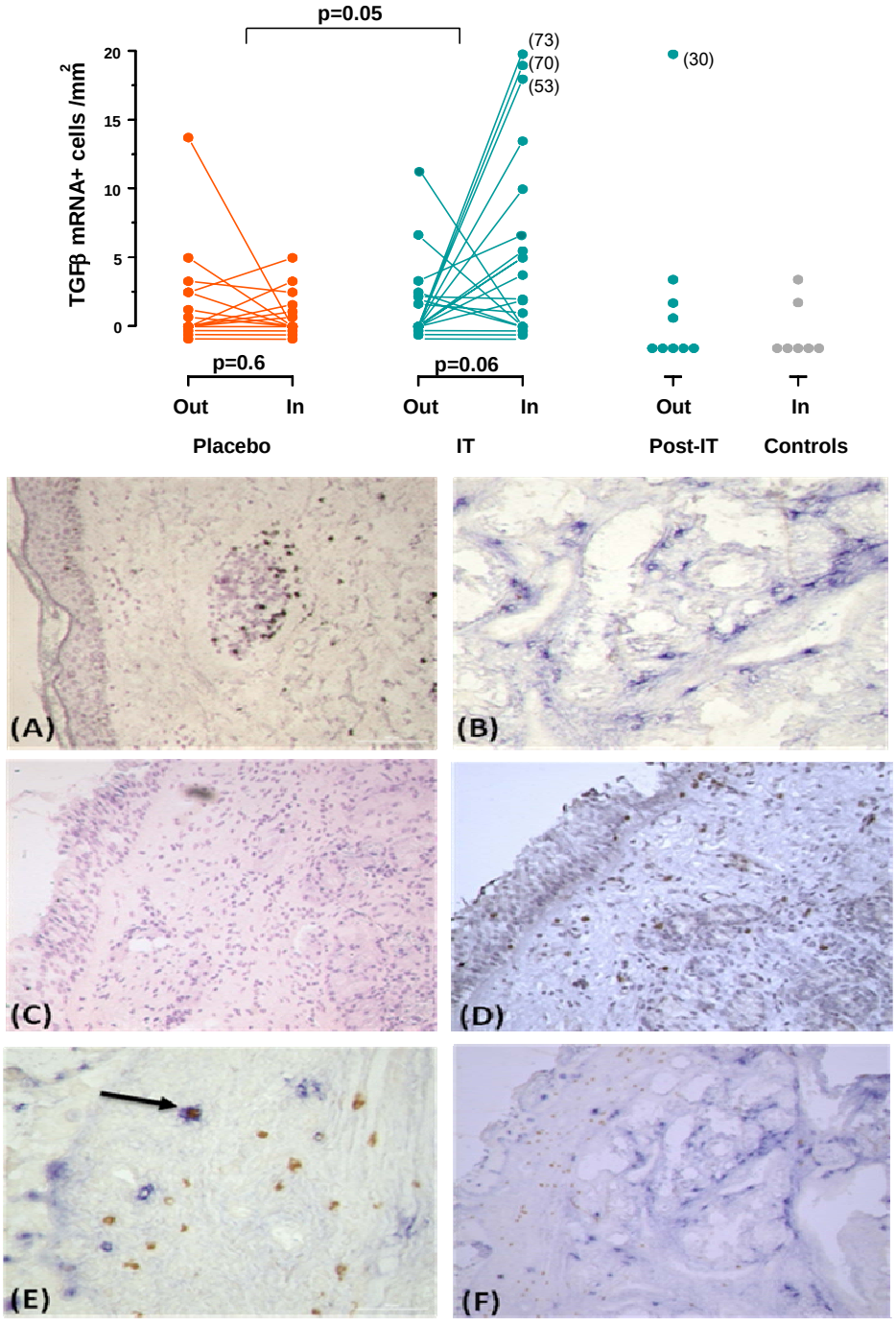

Figure 3. Nasal TGF-β mRNA expression after immunotherapy and colocalization of nasalIg A1/2-expressing cells to *Phl p5*. TGF-β expression was assessed by in situ hybridization in nasal biopsies obtained from grass pollen allergic patients before/after IT, during the peak (In) and outside (Out) the pollen season. Biopsies were also obtained 2 years after completion of IT (Post-IT) as well as in healthy nonatopic controls. Bars represent median values. Pictures (x200 original magnification) show a representative TGF-β mRNA in situ hybridization signal (*A*) and staining of IgA^+ (blue) and/or *Phl* $p5^+$ cells (brown) cells in nasal biopsy sections from a IT-treated patient: IgA2 (*B*), isotypic control (*C*), *Phl p5* (*D*), *Phl p5*/IgA2 dual staining (*E*, arrow), and *Phl p5*/IgA1 dual staining (*F*) (adapted from J Immunol 2007;178:4658-4666).

Recent studies have demonstrated that peripheral T cell tolerance is crucial for a healthy immune response and successful treatment of allergic disorders (62). A further subtype of T cells with immunosuppressive function and cytokine profiles distinct from either T_H1 and T_H2 cells, termed regulatory/suppressor T cells (T_{Regs}) has been described and evidence for their existence in humans has been demonstrated. Regulatory T cells are defined by the expression of $CD4^+CD25^+Foxp3^+$ T cells and high levels of IL-10 production.

The majority of $CD4^+CD25^+$ T_{Regs} emerge from the thymus and constitute 5–10% of peripheral $CD4^+$ T cells in healthy mice and humans. The suppressive mechanism of $CD25^+$Tregs is unclear at present but is believed to be mainly cell-contact dependant *in vitro* although suppressive cytokines like IL-10 and TGF-β have been reported to play a role, particularly *in vivo*. $CD25^+$ T_{Regs} are best recognized by expression of the transcriptional regulator Foxp3 (FOXP3 in humans), which appears to serve as a master switch gene for Treg development and function. Foxp3 expression closely correlates with $CD4^+CD25^+$ T cells in mice and to that of $CD4^+CD25^{high}$ cells in humans although $Foxp3^+CD25^-/CD25^{low}$ cells with suppressive activity also exist. Other commonly used $CD25^+$ T_{Regs} markers are CTLA-4 (CD152) and GITR (glucocorticoid induced TNF family-related gene/protein), but neither these nor any other so far described surface markers are exclusively expressed by $CD4^+CD25^+$Tregs, making it difficult to differentiate T_{Regs} from other T cells, especially after activation. The elevated frequency of $CD4^+CD25^+Foxp3^+$ T cells in a variety of allergic conditions have been demonstrated post allergen-specific immunotherapy and were able to inhibit the development of allergic T_H2 responses. Thus, successful allergen immunotherapy is associated with a decrease in an allergen-specific T_H2 response and the induction of allergen-induced IL-10 secreting, TGF-β producing $CD4^+CD25^+$ T_{Regs} (63- 66).

Humoral Immune Responses. The serum levels of specific IgE and IgG4 antibodies delineate allergic and normal immunity to an allergen. Although peripheral tolerance was demonstrated in allergen specific T cells following SIT, the capacity of B cells to produce specific IgE and IgG4 antibodies was not eliminated. In fact, specific serum levels of both isotypes increased during the early phase of treatment. However, the increase in grass pollen specific IgG4 was more pronounced and the ratio of specific IgE to IgG4 decreased by 10 to 100 fold. A similar change in specific isotype ratio was observed in SIT of various allergies including grass pollen and bee venom. In one study, the in vitro production of PLA-specific IgE and IgG4 antibodies by PBMC paralleled the changes in serum levels of specific isotypes (67). IL-10 which is induced and increasingly secreted by regulatory T cells during and after SIT, appears to counter-regulate antigen-specific IgE and IgG4 antibody synthesis (68). IL-10 is a potent suppressor of both total and allergen-specific IgE, while it simultaneously increases IgG4 production. Thus IL-10 not only generates tolerance in T cells, it also regulates specific isotype formation and skews the specific response from an IgE to an IgG4 dominated phenotype. The healthy immune response to *Der p 1* demonstrated increased specific IgA and IgG4, small amounts of IgG1 and almost undetectable IgE antibodies in serum (41, 69). House dust mite (HDM)-SIT did not significantly change specific IgE levels after 70 days of treatment; however, a significant increase in specific IgA, IgG1 and IgG4 was observed. The increase of specific IgA and IgG4 in serum coincides with increased TGF-β and IL-10 respectively. This may account for the role of IgA and TGF-β as well as IgG4 and IL-10 in peripheral mucosal immune responses to allergens in healthy individuals (70, 71).

Wachholz et al hypothesized that allergen-specific IgG antibodies "blocking IgG" induced during the course of immunotherapy can disrupt formation of allergen-IgE complexes that bind to FcεRII expressed APC and facilitate allergen presentation to T lymphocytes. In 10 patients who received active grass pollen immunotherapy, there was induction of a serum activity that inhibited allergen-IgE binding to B cells as well as subsequent allergen presentation to T cells (72). This serum fraction was co-purified to IgG and demonstrated to be allergen specific since sera from grass pollen immunotherapy treated patients who were also birch pollen sensitive did not inhibit IgE–birch pollen allergen binding to B cells. These observations were further supported by another study 2 years after immunotherapy (60).

A rise in allergen-blocking IgG antibodies, particularly of IgG4 class, and inhibition of IgE facilitated antigen presentation, and the generation of IgE-modulating $CD8^+$ T cells have been shown to be associated with successful allergen-SIT (73).

Long-Term Effects of Immunotherapy. The first study on the long term benefit of SIT was reported by Durham et al. Relief of grass pollen hay fever continued 3 to 4 years after discontinuation of 3 years of grass pollen immunotherapy. Sixteen patients received maintenance injections for an additional 3 years, 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 (74, 75). This study confirmed that immunotherapy for respiratory allergies offers long-term improvement just as insect venom immunotherapy protects against anaphylaxis long after it has been discontinued. Similar observations on the long term effects of allergen immunotherapy have been reported for other seasonal allergic pollinoses and house dust mite-allergic children with asthma (76, 77).

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

Currently, allergy diagnosis and specific allergen immunotherapy have been performed with crude allergen extracts, which consist of a mixture of various amounts of allergenic and non-allergenic components. The composition and the allergen content of such extracts is unpredictable and depends on various factors (e.g. protein degradation, heterogeneity of allergen sources). Several recent studies revealed that despite efforts to standardize commercial allergen extracts, there is great heterogeneity of these extracts and even the contents of major allergens may vary considerably between different batches and products (78, 79).

The development of therapeutic strategies that avoid activation of mast cells and basophils has been a recurrent theme in immunotherapy for the last 50 years. Alternative approaches to conventional allergen immunotherapy are: a) the use of purer allergen preparations, including recombinant allergen proteins, which may increase the safety and specificity of immunotherapy, as well as improve the diagnosis of specific allergy (80). This strategy is dependent on a clear understanding of the important allergenic epitopes that induce T_{Reg} cells, b) to limit the allergenicity of whole allergens, investigators

have examined peptide-based allergen preparations, which do not bind IgE and therefore do not activate mast cells, but reduce both T_H1 and T_H2-cytokine synthesis, while increasing levels of IL-10 (81, 83), c) a third strategy, which may improve the safety of allergen immunotherapy, is to administer anti-IgE-monoclonal antibody omalizumab with allergen immunotherapy (84, 85), d) the use of adjuvants such as aluminium hydroxide or immunostimulatory sequences (CpG), either mixed or conjugated with allergens or monophosphoryl lipid A (MPL) enhances immune responses in favour of T_H1 cells (86-88). Such a combination may improve the rapidity by which immunotherapy induces T_{Reg} cells. Administration of allergens by sublingual route rather than conventional subcutaneous injection immunotherapy has also proved successful.

SUBLINGUAL IMMUNOTHERAPY

Although, it was regarded as an ineffective route for immunotherapy for years, sublingual (with or without swallowing) administration of allergen extracts is now supported by many studies in Europe with a number of allergens. Sublingual immunotherapy (SLIT) has now been widely proposed as an alternative to subcutaneous injection immunotherapy or continued medication. SLIT is a desensitisation method approved for clinical use in respiratory allergies and has been developed to make immunotherapy available to a broader group of allergic patients.

Locally applied immunotherapy, whether oral (swallowed), sublingual (with or without swallowing), nasal, or bronchial, was extensively reviewed in 2003 by Canonica and Passalacqua (89), whose clinic has carried out many investigations on sublingual immunotherapy (SLIT). However, the nasal and bronchial routes have been abandoned because of local side effects (90). Straight swallow (i.e, no retention in the mouth) is also less favoured than SLIT because the dose required is larger and more likely to induce gastrointestinal side effects. Twenty two double-blind, placebo-controlled trials of SLIT were conducted with adequate methods and analysis that have been reviewed. All but 3 studies confirmed clinical efficacy in rhinitis induced by common allergens, such as grass, mites, birch, and *Parietaria* species. The magnitude of the clinical efficacy ranged between 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 optimum dose in those 22 studies is unknown. In the studies cited, effective doses ranged from approximately 3 to 5 times to 375 times the doses used in subcutaneous immunotherapy (89).

Few direct comparisons of SLIT with subcutaneous immunotherapy are available. The two routes in a placebo controlled trial of dust mite–induced rhinitis and asthma were compared by Mungan et al (91). The cumulative dose of SLIT was about 86 times greater in subcutaneous IT. Subcutaneous immunotherapy for both rhinitis and asthma was clinically effective. Patients treated with SLIT had decreased rhinitis symptoms, but no changes in asthma scores were reported. Medication scores were significantly decreased in both actively treated groups 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,* ($p < 0.01$), *D farinae* ($p < 0.05$), and histamine ($p < 0.05$), whereas the SLIT and placebo groups showed no difference. Side effects were minimal in both treated groups.

In a double-blind placebo controlled birch pollen allergen immunotherapy, the cumulative dose of allergen after the first treatment season was 4717mcg of Bet v 1 in the SLIT group and 27mcg of Bet v 1 in the subcutaneous group, implying that SLIT-treated patients on average received 175 times more allergen than the subcutaneous group (92). Although both treatment groups scored significantly better during the two following birch pollen seasons than the placebo groups, on the basis different methods of estimating symptoms, there was not a significant difference between the two active treatments. However, 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 oedema in the mouth, throat, or both associated with drop intakes (92).

A recent Cochrane meta-analysis on the efficacy and safety of SLIT has confirmed that this is a safe treatment which significantly reduces symptoms and medication requirements in allergic rhinitis when compared to placebo with minimal side effects. However, the studies assessed in this meta-analysis were, in general, small, and there was considerable heterogeneity among them (93). For this reason, there was a need to establish a well documented efficacy profile for the sublingual route in large and well designed clinical trials. Recently, in the largest clinical programme ever conducted within allergen specific immunotherapy, a new fast-dissolving, once-daily, immunotherapy grass allergen tablet for home administration has been studied. These studies have shown that for pre-seasonal/seasonal grass pollen SLIT, there is a clear dose-response relationship and that more than 8 weeks pre-seasonal treatment is highly efficacious. Efficacy analysis results using 75,000 SQ-T/tablet daily (equivalent to 15 mcg of *Phleum pratense* major allergen protein) showed a reduction of 30% in rhinoconjunctivitis medication scores ($p<0.0001$) and reduction of 38% in rhinoconjunctivitis medication scores ($p<0.0001$) compared with placebo group. The most frequently reported adverse events were oral pruritus, mouth oedema, and ear pruritus and throat irritation. The majority of the adverse events were mild to moderate with transient local allergic reactions. No systemic reactions with hypotension and the need of intramuscular adrenaline were observed. This excellent safety profile makes this sublingual treatment suitable for home use (94).

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 (95, 96). Fanta et al (97) 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 mcg of major allergen).

SLIT has been shown to have a long term effect after discontinuation with a significant reduction in the skin test reactivity and inhibition of progression from rhinitis to asthma in some studies (76,98), but more data from better designed studies is necessary to support this concept. 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.

PEPTIDE IMMUNOTHERAPY (PIT)

Although specific immunotherapy is a highly effective form of treatment for allergic diseases, one major drawback of this treatment is the observed adverse reactions to relatively high doses of allergen that can sometimes lead to anaphylaxis. Therefore, other approaches to allergy vaccination have been investigated that aim to avoid the cross-linking of IgE. Peptides have the potential to inhibit T-cell function but not induce anaphylaxis, because of the loss of three-dimensional conformational determinants, and therefore provide a suitable form of treatment with reduced capacity to induce anaphylaxis (99).

PIT is an attractive approach for treatment of autoimmune conditions and allergic diseases, based on the identification immunodominant epitopes using overlapping T cell peptides in humans (100, 101). Short allergen peptides, either native sequences or altered peptide ligands, with amino acids substitutions not containing epitopes for IgE cross-linking, do not induce anaphylaxis. There is considerable rationale for targeting T cells with synthetic peptides based on such T cell epitopes. Peptide-based IT has been developed in animal models and has been evaluated in different pathological conditions in man (102). Dominant T cell epitopes have been identified in both murine and human systems. Peptides IT based on T cell epitopes have been shown to prevent the induction of disease and to modulate ongoing disease in murine models following subcutaneous, oral, intranasal and intravenous administration (103). Peptide-induced tolerance has been demonstrated in models of experimental autoimmune encephalomyelitis (104), collagen-induced arthritis (105), diabetes (IDDM) (106), myasthenia gravis (107) and more recently, in models of allergic diseases. Mice primed with the cat allergen *Fel d 1* peptide demonstrated the ability to inhibit T cell cytokine secretion and antibody synthesis in a subsequent allergen exposure (108). The ability of peptides IT from the house dust mite allergen *Der p 2* revealed down regulation of T cell responses and antibody production to intact protein (109).

In multiple sclerosis (MS) where clear associations between human leukocyte antigen (HLA) haplotype and disease are seen, identification of immunodominant T cell peptide epitope restricted by HLA-DR2 has led to a peptide from myelin basic protein (MBP) being administered to MS patients in phase I clinical trail (110). The polymorphism displayed by both the human major histocompatibility complex (MHC) and many allergen genes has led to the opinion that peptide immunotherapy for allergic diseases in humans will be impractical as it will not be possible to accommodate the large number of potential epitope-MHC combinations involved in disease pathogenesis. The problem of polymorphism is particularly pertinent to the allergic diseases since, unlike many autoimmune diseases, there are few strong (HLA) disease associations. Altered peptide ligands based on the same epitope have also been evaluated with mixed results.

To date, clinical trials of PIT have been performed in two allergic conditions. In the first trial, relatively long peptides of 27 and 35 amino acids of the major cat allergen *Fel d 1* containing the T cell epitopes or mixture of peptides spanning the whole protein sequence were used to treat allergy to cats and resulted in the induction of tolerance in IL-4-producing cells (111). The other trial, PIT of bee venom allergy was performed with a mixture of short peptides that directly represent the T cell epitopes of the bee venom major allergen, phospholipase A2 (PLA2). The study showed modulation of the immune response against the whole allergen, inducing specific T cell tolerance and a decrease in the specific IgE: IgG4 ratio (112). Single amino acid alteration in T cell epi-

topes can modify specific T cell activation and cytokine production. Recent studies suggest that, under highly controlled experimental conditions, allergic diseases can be inhibited by altered peptide ligand administration. Whether this is due to T_H2 to T_H1 immune deviation or the induction of T_{Reg} cells remains to be elucidated (99-100). A potential barrier to PIT of allergy is the apparent complexity of the allergen specific T cell response in terms of epitope usage and dominant epitopes in humans.

Mechanisms of PIT. Blaser and colleagues identified three T cell peptide epitopes in the bee venom phospholiapase (PLA2) molecule and have used these peptides to desensitize five allergic subjects (112). Peptides were well tolerated and despite the differing MHC backgrounds of the subjects, T cell responses to all three peptides were observed suggesting that the problems of using peptide immunotherapy in an outbred population such as man, may not present as much of a problem as has been envisaged in the past. Fellrath and colleagues treated bee venom allergic subjects with long peptides from PLA2. This phase I study was associated with increased IFN-γ and IL-10 responses and increased IgG4 levels (113). Tarzi and colleagues treated subjects with mild bee venom allergy using four peptides selected from the sequence of PLA2 on the basis of their MHC binding characteristics (114). Significant reductions in the magnitude of the cutaneous late phase reaction to intradermal allergen challenge and PBMC responses to allergen (proliferation, production of T_H1 and T_H2 cytokines) were accompanied by an increase in IL-10 production by PBMC in response to culture with allergen (114).

Broad patterns of peptide reactivity have also been reported by Haselden and colleagues investigating responses to three peptides derived from the cat allergen *Fel d 1*. In that study, peptides were administered intradermally to cat allergic asthmatic subjects resulting in the induction of late asthmatic reactions (LAR) in a proportion of individuals (115). Each of the three peptides was capable of inducing peripheral blood mononuclear cell proliferation in a percentage of the subjects. The ability to induce isolated LAR did not correlate with peptide-induced proliferative responses since the latter may be dose dependent and the dose administered in the study was the lowest dose demonstrated to induce LAR. T cell responses to two or the three peptides were shown to be MHC-restricted and subjects experiencing LAR were shown to express HLA-DR molecules associated with peptide restriction (116). Interestingly, promiscuous binding of peptides to more than one DR microvariant and in the case of one peptide, to more than one DR specificity was observed. The ability of a certain peptide epitope to bind to many HLA molecules has become increasingly well documented and has led to the designation of HLA supertypes to which certain peptide sequences bind promiscuously. These observations together with the findings in human studies suggest that the initial concerns about MHC-restricted T-cell recognition of peptides in outbred human populations may be unfounded.

Using a mixture of overlapping peptides (16-17 mers) spanning the majority of the *Fel d 1* molecule, Oldfield and colleagues demonstrated significant reduction in the magnitude of the cutaneous late-phase reaction to intradermal challenge with whole cat dander allergen extract (117). Following a single injection of a mixture of 12 peptides (5mcg of each), an approximate 50% reduction in the 6 hour cutaneous late phase response was observed. More recently, using the same mixture of peptides in a randomised, double-blind, placebo-controlled study, Oldfiled and colleagues demonstrated that following an incremental series of peptide injections, both the late-phase and the early-phase skin response to whole allergen challenge was significantly reduced (117). Reductions in cutaneous reactions were accompanied by reduced proliferative responses of PBMC. Fur-

thermore, treatment with peptide was associated with decreased levels of pro-inflammatory cytokines of both the T_H1 and T_H2 class and increased production of the regulatory cytokine IL-10 (118). Although subjective outcome measures were not analysed extensively in this study, subjects on active treatment did not report a significant improvement in their ability to tolerate exposure to cats following treatment. Alexander and colleagues demonstrated increased recruitment of $CD25^+$ T cells and $CD4^+$ IFN-γ^+ T cells to the cutaneous site of allergen challenge following peptide therapy (119,120). No elevation of IL-10 was noted in the skin although a significant increase in TGF-β was reported. In the same open label study, a reduction in bronchial hypersensitivity and cutaneous late phase reaction to allergen challenge was also observed. In related studies employing higher peptide doses, improved nasal symptom scores were recorded together with a significant reduction in the allergen-induced late asthmatic reactions following inhaled allergen challenge. Most recently, peptide immunotherapy has been associated with the induction of a $CD4^+$ population of T cells that have regulatory activity in vitro. $CD4^+$ T cells were isolated from PBMC taken before and after peptide immunotherapy and their ability to suppress allergen-driven T cell proliferation of the PBMC $CD4^-$ fraction of cells was measured. CD4 cells isolated after therapy were able to significantly suppress the response of pre-treatment PBMC supporting the concept that peptide immunotherapy induces a population of active regulatory T cells (120,121).

Immunostimulatory Sequences. Although IT for allergic diseases is widely practiced, many efforts have been made to improve its efficacy and safety, since its use became common about a century ago (21). Since the dose in which the allergen induces systemic reactions is limited, efforts have largely been directed to decrease the allergenicity of the antigens while maintaining or increasing their immunogenicity. Chemical modifications of the allergens have resulted in reduction in both allergenicity and immunogenicity.

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 (86,122). The immunostimulatory bacterial DNA sequences (i.e. higher frequency of CpG motifs and the absence of cytosine methylation in bacteria, as opposed to vertebrate DNA) are capable of enhancement of T_H1 responses by producing a potent IL-12 activation and IFN-γ secretion and inhibition of T_H2 cell activation and IL-4 and IL-5 production (123,124).

A variety of animal studies identified first bacterial DNA and then specific palindrome DNA motifs (CpG) found in many bacteria 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 (125). 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 (126). 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 (127,128**).** 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, (126) and the biology of toll-like receptors has been reviewed by Zuany-Amorin et al. (127). Both deal with the potential use of CpG in the treatment of asthma.

An initial clinical trial with ragweed allergen *Amb a 1*- immunostimualtory oligonucleotide (AIC) utilised the method of quantitative intradermal skin titration to assess the relative potency of AIC vs licensed ragweed in six ragweed allergic volunteers. A subsequent blinded study used a 6-injection regimen with a target dose of conjugate equivalent to 12mcg of *Amb a 1*. An increase of IgG antibodies to *Amb a 1* was again observed. (128-130). The skin test study provided initial safety data in a group of patients with a wide range of ragweed sensitivities. The ragweed AIC product was approximately 200 fold less reactive than licensed ragweed extract when injected intra-cutaneously into the skin (131,132). A comparison on the basis of basophil histamine release confirmed a 10 - 100-fold reduction in allergenicity. These in vivo observations were also correlated with in vitro studies of human basophils that demonstrated diminished histamine release to AIC in comparison to *Amb a 1*. A subsequent subcutaneous injection study with the AIC product has explored the safety and immunologic response to AIC in a dose escalation trial. In this study ragweed allergic subjects immunized with AIC were observed to have an IgG *anti-Amb a 1* antibody response similar to conventional immunotherapy (129). Furthermore, in contrast to conventional immunotherapy, AIC did not result in a boost in IgE antibody. No serious adverse events have been observed in these initial clinical trials in humans. These studies provide initial evidence that AIC exhibits reduced allergenicity and yet maintains its immunogenicity.

Tulic et al reported 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 (133). The first post immunotherapy 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 $mRNA^+$ cells and an increased number of IFN-γ $mRNA^+$ cells compared with placebo-treated patients (134,135). 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 (134). These results provide evidence for long-lasting effects from a single short course of this DNA conjugate.

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 both and inhibit airway inflammation in murine models of asthma (123). Immunostimulatory sequences appear to be even more effective as an adjuvant for murine and human T_H1 responses when directly conjugated to allergen (129). An immunostimulatory sequence–ragweed allergen (*Amb a 1*) conjugate suppressed murine airway eosinophilia and hyperresponsiveness (134,135). 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 (136).

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, and has been successfully used as an adjuvant in viral vaccines

(137). In a double-blind placebo-controlled trial tyrosine-absorbed glutaraldehyde-modified grass pollen extract containing MPL reduced hay fever symptoms and medication requirements and increased allergen-specific IgG levels (138). 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.

Recombinant and Engineered Allergens. One approach in eliminating the risk of anaphylaxis has been to develop recombinant genetically modified allergen proteins that show reduced IgE binding while still containing the relevant T-cell epitopes. A compelling case for the further development of recombinant allergens for diagnosis and immunotherapy was presented by Valenta and Niederberger (139-141). This approach overcomes the major obstacle of standardization of natural allergen extracts and allows the production in unlimited amounts of allergens of defined and consistent composition. Other advantages outlined include the avoidance of contaminants, the potential to adjust allergen potencies and ratios precisely for tailor-made therapy, and the availability of pure molecules for mechanistic studies and for development of bioassays for clinical monitoring. Options include use of mixtures of recombinant allergens or recombinant hybrids to substitute natural allergens and/or the development of recombinant hypoallergenic variants. There may be potential disadvantages of the recombinant approach such as issues surrounding the level of glycosylation or the accuracy of refolding of recombinant allergens that may unpredictably alter their biological properties (142-143). Furthermore, it is possible that contaminants in natural allergen products may potentially have an adjuvant effect that may be important for clinical efficacy. The clinical evidence base for use of recombinant allergens, although encouraging is very limited, and further controlled trials are urgently needed.

Chapman et al (80) 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.

Further 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 were made by Valenta and Kraft (144). From the availability of the first allergen-encoding complementary DNAs and the first production of recombinant allergens at the end of the 1980s, recombinant allergens have made their way progressively from the bench to the clinics. The usefulness of recombinant allergens for the in-vitro diagnosis of allergy was demonstrated from 1991 onwards. Skin prick tests with recombinant allergens were performed from 1994 on and from 1995 on strategies have been developed to engineer allergy vaccines based on recombinant DNA and synthetic peptide chemistry using the sequences and structures of allergens as templates. After the successful evaluation of genetically engineered hypoallergenic allergen derivatives in patients by provocation testing, the first clinical trials with the new vaccines have been initiated. Right now we see the results of the first immunotherapy trial that was con-

ducted with genetically engineered allergens (145,146) and anticipate results from several ongoing trials in the near future.

Genetically Modified Allergens Target B Cells As Well As T cells. Genetically modified recombinant allergen derivatives offer several advantages (139,143). These molecules preserve the repertoire of allergen-specific T cell epitopes and hence can be utilized for the targeting of T cells. Furthermore, genetically engineered hypoallergenic allergen-derivatives induce blocking antibodies that inhibit the binding of allergic patients' IgE antibodies to allergens and hence also represent a B cell-based approach. Furthermore, these molecules can be engineered to reduce their allergenic activity and even to change their immunological properties. In this context, it was noted that certain genetically engineered allergens could even alter the type of immune response towards a T_H1 phenotype (147).

The hypoallergenic derivatives of the major birch pollen allergen *Bet v 1*, two recombinant *Bet v 1* fragments, a *Bet v 1* trimer and r*Bet v 1* variants, have been extensively evaluated regarding safety by provocation testing in patients and their profoundly reduced allergenic activity could be confirmed (148, 149). In animals r*Bet v 1* fragments and trimer induced blocking IgG antibodies.

The first immunotherapy study evaluating genetically modified allergen-derivatives was in fact performed with the *Bet v 1*-trimer and the *Bet v 1*-fragments in a double-blind, placebo-controlled multicentre immunotherapy trial in 124 birch pollen allergic patients and was recently published (150-152). Treatment was performed with aluminium-hydroxide adsorbed molecules giving increasing doses (1-80 μg) in one to two-weekly intervals as one pre-seasonal treatment course. Treatment with the hypoallergenic derivatives induced strong IgG responses against the *Bet v 1* wild-type allergens (153). A reduction of cutaneous reactivity and improvement of symptoms was found in the actively treated patients. In addition, the rise of allergen-specific IgE production induced by seasonal allergen contact was inhibited in the vaccinated patients suggesting that this treatment also blocks the IgE memory response (154).

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 slightly, 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. Kuehr et al (155) 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 scores when compared with patients who had immunotherapy alone ($p = 0.003$ for birch and $p = 0.001$ 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.

Omalizumab, a recombinant humanized monoclonal antibody against immunoglobulin (IgE), represents a unique therapeutic approach for the treatment of allergic diseases. This agent acts as a neutralizing antibody by binding IgE at the same site as the high-affinity receptor. Subsequently, IgE is prevented from sensitizing cells bearing high-affinity receptors. Inhibition of the biological effects of IgE targets an early phase of the allergic cascade before the generation of allergic symptoms. Currently, omalizumab has been approved for the treatment of persistent allergic asthma in patients who are poorly controlled with inhaled corticosteroids (85). However, other allergic disorders may be

amenable to treatment with omalizumab because of its ability to inhibit effector functions of IgE. Studies of omalizumab in the treatment of allergic rhinitis comprise the greater part of the literature pertaining to the use of this agent for clinical indications other than asthma. The article summarizes clinical trials of omalizumab in allergic rhinitis and examines the evidence regarding the effects of omalizumab on the pathophysiological mechanisms underlying allergic rhinitis. Additionally, the author consider the role of this novel therapeutic agent in combination with specific allergen immunotherapy and discuss other potential indications for omalizumab in IgE-mediated disorders, including food allergy, latex allergy, atopic dermatitis, and chronic urticaria (85).
In another study, ragweed allergen immunotherapy with and without omalizumab therapy was tested in a 4-arm, double-blind, placebo-controlled study. Flow cytometry was used to detect serum inhibitory activity for IgE-facilitated CD23-dependent allergen binding to B cells as a surrogate marker for facilitated antigen presentation. Serum ragweed-specific IgG4 was measured by means of ELISA. Immunotherapy alone resulted in partial inhibition of allergen-IgE binding after 5 to 19 weeks of treatment compared with baseline ($p < 0.01$). Complete inhibition of allergen-specific IgE binding was observed in both treatment groups receiving omalizumab ($p < 0.001$). Allergen-specific IgG4 levels were only increased after immunotherapy ($p < 0.05$), both in the presence and absence of anti-IgE treatment. Combined treatment resulted in the induction of long-lasting inhibitory antibody function for up to 42 weeks compared with either treatment alone. These observations revealed that ragweed immunotherapy induced serum regulatory antibodies that partially blocked binding of allergen-IgE complexes to B cells. Additional treatment with anti-IgE, by directly blocking IgE binding to CD23, completely inhibited allergen-IgE binding. The combination of ragweed immunotherapy and anti-IgE resulted in prolonged inhibition of allergen-IgE binding compared with either treatment alone, events that might contribute to enhanced efficacy (156). Although the cost of the combination of immunotherapy with anti-IgE treatment is high, this should be considered in view of the enhanced benefit/risk ratio and the known long-term benefits of allergen immunotherapy. Whether the prolonged inhibition of allergen-IgE binding that was seen after discontinuation of the combination compared with either treatment alone could result in a more prolonged duration of efficacy remains to be determined.

CONCLUSIONS

Specific allergen immunotherapy (SIT) is highly effective in selected patients with IgE-mediated disease who are monoallergic or have a limited number of allergen sensitivities. SIT is the only antigen-specific immunomodulatory treatment in routine use with long-term benefits which also modifies the natural history of allergic disease for at least several years after discontinuation. SIT inhibits allergen-induced late responses in the skin, nose, and lung and is associated with increases in serum allergen-specific IgG levels, particularly IgG4 (157). The blocking antibodies compete with IgE in allergen binding *in vitro*, although the clinical importance of these effects remains to be evaluated. Immunotherapy alters the T_H2/T_Hl balance in favour of T_H1 responses and induces IL-10 and TGF-β production by activated regulatory T cells. The elevated IL-10 has been detected in the peripheral blood and in the target organ, nasal mucosa, after immunotherapy. IL-10 has numerous potential anti-allergic properties against mast cells, T cells, and eosinophils and also promotes IgG4 production by B cells (158). The clinical effi-

cacy and safety of immunotherapy might be improved by novel strategies that directly target the T-cell response and/or the route of administration. 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. New knowledge of the mechanisms of IT is necessary for the development of immunoassys to predict the efficacy of IT, when to stop treatment and possibly to predict relapse and the need for further IT, etc (159). Understanding mechanisms are also important for development of novel approaches, including adjuvants (CpG, MPL) and use of alternative routes, the most promising currently being sublingual IT.

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