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Pharmacia Diagnostics AB,
 Research and Development,
 SE-751 82 Uppsala, Sweden
 Tel.: +46 18 163 753
 Fax: +46 18 166 330
robert.moverare@pharmacia.com

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Immunological mechanisms of specific immunotherapy with pollen vaccines: implications for diagnostics and the development of improved vaccination strategies

Robert Moverare

This review deals with specific immunotherapy performed with pollen vaccines. Pollen allergens, the immunological mechanism of allergic inflammation and the mechanisms behind successful specific immunotherapy treatment of seasonal pollen allergy shall be discussed. The different approaches to develop improved regimes for specific immunotherapy based on new diagnostic tools, improved pollen vaccines and alternative routes for administration of the vaccines will be highlighted. Diagnostic tools to select patients for specific immunotherapy and for monitoring the treatment are quantitative measurements of allergen-specific immunoglobulin E, G and G4 antibodies and component-resolved diagnostics based on immunoglobulin E binding to individual allergenic components. Future pollen vaccines may consist of hypoallergenic recombinant allergens and T-helper cell type 1-inducing immunostimulatory adjuvants. Both sublingual and nasal administration of pollen vaccines for specific immunotherapy may be used more in future.

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The number of individuals that suffer from immunoglobulin (Ig)E-mediated allergic diseases in developed countries has increased epidemically during the last decades [1]. The European Community Respiratory Health Survey (ECRHS) reported a prevalence of 9.5–40.9% for nasal allergy and hayfever in different countries [2]. The corresponding prevalence for current asthma was 1.2–13.0% [2]. The estimated total cost of asthma in the USA in 1994 was US\$10.7 billion [1]. Even if the subgroup of seasonal pollen-allergic patients represents only a part of the total asthma costs, it is a large group consisting of patients mainly with hayfever symptoms rather than asthma. IgE-mediated inflammatory reactions are directed towards foreign molecules called allergens. Individuals that develop IgE antibodies against allergens are described as sensitized to the allergens in question. The prevalence for

sensitization is higher in individuals having an atopic predisposition, that is, having allergic diseases in the family. In eastern Europe the prevalence of allergic sensitization is lower than in west, possibly due to differences in living standard and exposure to various bacteria and viruses [3]. Allergic sensitivity is diagnosed by measurement of allergen-specific IgE antibodies *in vitro* in blood tests or by performing skin-prick testing *in vivo* with allergen extracts.

IgE-mediated allergy can be manifested as several different diseases, such as rhinitis, conjunctivitis, asthma and eczema (atopic dermatitis). The allergic inflammation process has two phases: the immediate early reaction and the late-phase reaction. The early symptoms often start within minutes after an allergen challenge and continue for up to a couple of hours. The late-phase reaction (showing similar symptoms) starts some hours after the

allergen exposure and lasts for several hours. It may even be chronic. In the clinical situation, the immediate early reaction is often nondistinguishable from the late-phase reaction.

Birch and grass pollen allergy are two of the most common allergies in northern and central Europe [4]. A prevalence of 5–54% for sensitization to birch pollen allergens and 17–90% for sensitization to grass pollen allergens have been reported for seasonal pollen-allergic patients from different European countries [4]. The major symptoms appear in the respiratory airways and the eyes. Common hayfever symptoms are sneezing, nasal obstruction, running nose, itching eyes, tearing and in the case of pollen, asthma and bronchoconstriction. Several pollen-allergic patients also have oral symptoms when eating plant-derived food [5]. Common conditions among patients allergic to birch pollen are hypersensitivity to apple and/or nuts [5,6].

Pollen allergens are generally small, water-soluble proteins or glycoproteins with a molecular weight between 10 and 60 kDa. The allergens are named according to a nomenclature based on abbreviations of their Latin names followed by an identification number. For example, the major birch pollen allergen is called Bet v 1 (*Betula verrucosa* allergen 1). IgE immunoblots have shown heterogenic IgE-binding patterns in sera from pollen-allergic patients to the proteins in the pollen extracts [7]. However, it was not until the introduction of cDNA cloning techniques that the molecular and biochemical characterization of allergenic proteins made considerable progress. Currently we know the complete nucleotide sequences and deduced amino acid sequences of numerous cDNA clones coding for pollen allergens.

Certain pollen allergens belong to families of genetically-related proteins that are highly conserved regarding both amino acid sequence and 3D structure. These proteins might be expressed in different plant tissues as highly cross-reactive allergens, such that IgE antibodies induced against one particular allergen from a protein family generally cross-reacts with other proteins within the same family. Some homologous allergens are exclusively expressed in pollen, for example the two EF-hand Ca^{2+} -binding allergens, Aln g 4 (alder pollen), Bet v 4 (birch pollen), Cyn d 7 (Bermuda grass pollen), Fra e 3 (ash pollen), Ole e 3 (olive pollen) and Phl p 7 (timothy grass pollen) [8]. Other pollen allergens are expressed in various plant tissues. The Bet v 1-related allergens are found in pollen of trees belonging to the order of *Fagales* and related trees but not in grass or weed pollen. They are also found in plant-derived foods, for example, fruits, vegetables, spices and certain nuts. Besides Bet v 1 (birch pollen), other allergens in this group are: Aln g 1 (alder pollen), Api g 1 (celery), Car b 1 (hornbeam pollen), Cor a 1 (hazel pollen and hazel nut), Dau c 1 (carrot), Mal d 1 (apple), Pru ar 1 (apricot), Pru av 1 (cherry) and Pyr c 1 (pear) [8–10]. Another group of allergens belongs to the profilin family. Profilins are highly cross-reactive actin-binding proteins and have been found in virtually all eukaryotic cells [11]. Among the profilins known to be allergens are: Api g 4 (celery), Ara h 5 (peanut), Bet v 2 (birch pollen), Cyn d 12 (Bermuda grass pollen), Gly m 3 (soybean), Hel a 2 (sunflower pollen), Hev b 8 (latex), Ole e 2 (olive pollen), Phl p 12 (timothy grass pollen), Pru av 4

(cherry) and Pyr c 4 (pear) [8,10,12]. The picture is complicated by the fact that pollen allergens often exist in several isoforms that differ by a few amino acids. The major allergen in birch pollen, Bet v 1, exists in more than 20 isoforms as indicated by 2D gel electrophoresis [13].

Seven birch pollen allergens have so far been identified and described (TABLE 1). Among the most important are Bet v 1, Bet v 2 and Bet v 4. The major birch pollen allergen Bet v 1 at 17 kDa is recognized by serum IgE of a majority of birch pollen-allergic patients [7]. Bet v 2 and Bet v 4 are minor allergens recognized by IgE of up to 20% of birch pollen-allergic patients [14–16]. Studies have shown that the IgE-reactivity pattern to individual birch and grass pollen allergens differs between populations of pollen-allergic patients [17,18]. The prevalence of IgE-reactivity to Bet v 1 in different European populations of birch pollen-sensitive patients differ between 62 and 100% [17]. The reason might be differences in the primary sensitization pathways. The exposure to various pollens from trees, grasses or weeds varies greatly between different European regions due to their individual climate and flora [4].

Polysensitivity to many different pollen allergens measured with *in vitro* tests or skin-prick tests can be caused by a true polysensitization to allergenic components in each individual allergen source (e.g., tree, grass and weed pollen). It can also be due to cross-reactive IgE antibodies in originally monosensitized patients. The clinical significance of cross-reactive IgE antibodies is under debate [10]. Cross-reactive IgE antibodies to pollen allergens and allergens from fruits and vegetables, such as apple, peach, carrot and celery, as well as from certain nuts are common among pollen-allergic patients [19,20]. These cross-reactive IgE antibodies are believed to be the cause of the food-related

Table 1. Birch pollen allergens.

Allergen	Prevalence of IgE reactivity [§]	Molecular weight (kDa)	Comment
Bet v 1	>95%	17	Major allergen
Bet v 2	10–20%	13–14.4	Profilin, actin-binding protein
Bet v 3	10%	24	Ca^{2+} -binding protein
Bet v 4	20%	9.3	Two EF-hand Ca^{2+} -binding protein
Bet v 6	12%	35	Phenylcoumaran benzylic ether and isoflavonoid reductase (former Bet v 5)
Bet v 7	<21%	18	Cyclophilin
(Bet v 8)	unknown	65	Plant pectin esterase

[§] Concerning birch pollen allergic patients. Ca: Calcium; Ig: Immunoglobulin.

oral symptoms observed among pollen-allergic patients. Clinical oral symptoms to plant-derived foods in birch pollen-allergic patients have been shown to be associated with sensitization primarily to Bet v 1 rather than to Bet v 2 [10,14], but some patients that mainly suffer from allergy to grass pollen might express oral symptoms when eating fruits caused by IgE reactivity to profilins [21,22]. Today, research tests for measurement of IgE antibodies to several allergenic components in pollen are available. Most of these tests are based on recombinant allergens.

Mechanism of allergy

The initiation of allergen-specific IgE production by B-cells is called the sensitization process. Interleukin (IL)-4 and most likely IL-13 have important roles in the B-cell switch from IgG to IgE production [23,24] and correlation between the concentration of birch pollen-specific serum IgE antibodies and the birch pollen-specific IL-4 and IL-13 production *in vitro* have been reported [25–28]. The most important source of IL-4, IL-5 and IL-13 are the T-helper (Th) cells of type 2 (Th2). Allergen-specific Th2 cells are overrepresented in allergic individuals on the expense of Th1 cells that produce high levels of interferon (IFN)- γ , tumor necrosis factor (TNF)- β and IL-2, cytokines that protect against allergy development [25,27–30]. Th0 cells produce a combination of both Th1-type and Th2-type cytokines. A dominating Th0-type response to pollen allergens can be observed in nonallergic individuals leading to the production of IgG antibodies [31]. Allergen-specific IgE antibodies can be measured in sera of sensitized individuals months or years after the initial sensitization. However, the major role of IgE in the allergic reaction takes place after the antibodies have been bound to high affinity receptors on mast cells and basophils.

Mast cells are found in various tissues, including nasal and bronchial mucosa and are highly involved in the onset of the allergic inflammation process [32]. When allergic patients are exposed to allergens to which they are sensitized, the allergens cross-link receptor-bound IgE on the surface of mast cells, which triggers the degranulation of prestored inflammatory mediators, including histamine. The inflammatory cascade then continues with the synthesis of leukotriene (LT) B_4 and LTC $_4$ (with its metabolic derivatives LTD $_4$ and LTE $_4$), prostaglandin D $_2$ and other inflammatory-promoting substances that act on vasculature, smooth muscle, connective tissue, mucous glands and inflammatory cells [33]. Additionally, the mast cell also produces TNF- β , IL-4, IL-5, IL-13 and other cytokines with various functions in the continuation and enhancement of the inflammation [32]. They act by inducing sequential upregulation of adhesion molecules on endothelial cells in the blood vessels and by attracting leukocytes involved in the late-phase reaction, such as Th2 cells and eosinophils [32,34]. The number of mast cells is also increased at the site of the late-phase response [34]. The Th2 cell has a central role in maintaining the allergic late-phase inflammation. When memory Th cells of Th2 type are reactivated by allergens presented by local antigen-presenting cells (APCs) in the airways, it is believed that

high amounts of inflammatory-promoting cytokines including IL-4, IL-5 and IL-13 are synthesized.

Eosinophils are supposed to have an important role in the allergic late-phase reaction, to which they are recruited in high numbers [35]. A characteristic phenomenon of allergic asthma is activated eosinophils in endobronchial biopsies [34,36]. Local eosinophilia is also a feature of allergic rhinitis [37]. The IL-5 production by Th cells located in the airway mucosa stimulates migration of eosinophils from the blood [35,38]. IL-5 also activates the eosinophils, such that it primes them for degranulation and increases their survival by inhibiting apoptosis (programmed cell death) [39]. The eosinophils express a number of Ig receptors including receptors for IgE [40]. Receptors that are probably involved in the activation and release of prestored granular basic mediators, such as major basic protein (MBP) and eosinophil cationic protein (ECP). These proteins are toxic and are believed to be involved in the destruction of the epithelial cell layer that is characteristic for allergic asthma [35,40].

Specific immunotherapy

Although many new and improved pharmacological drugs to reduce the symptoms of allergic diseases have been introduced, for instance antihistamines and steroids, only specific immunotherapy (SIT) targets the natural cause of allergic diseases. SIT was invented by Noon and Freeman to treat hayfever in 1911 [41]. Even if many variants of SIT have been tested, the common way of performing SIT treatment today is in principal the same as for almost a century ago. SIT has mainly been used for treatment of rhinoconjunctivitis and asthma. The treatment has also shown good clinical effect on severe stinging insect allergy [42]. However, SIT has not yet been found useful in allergic eczema (atopic dermatitis), urticaria, or food intolerance. The focus of this review is on SIT in pollen allergy and asthma. SIT is normally managed by subcutaneous injections of allergen vaccines, for example, pollen vaccines [42]. The pollen vaccines are delivered in gradually increased doses during the up dosing phase until the maintenance dose is reached. When following a conventional SIT regime the up dosing phase can involve weekly injections during 2–3 months. The increasing doses of pollen vaccine can also be delivered more frequently, especially at the lower concentrations. During rush SIT, the up dosing phase can be finished during the initial week. The maintenance dose is normally administered every 3–6 weeks and continued for about 3–5 years. Variations in the treatment regimes are common and local administrations of pollen vaccines have been tested [42]. Both sublingual SIT and nasal SIT with pollen extracts have been investigated in double-blind, placebo-controlled clinical trials with overall positive results [43,44]. However, the immunological mechanisms of local SIT are not clear and long-term clinical effects have not yet been proven [43,44].

The pollen vaccines today are based on rather crude soluble extracts of pollen and therefore consist of both allergens and nonallergenic proteins. An important improvement during the last decades is the use of standardized allergen extracts. The introduction of standardized extracts was forced by a number of

fatal anaphylactic reactions during SIT treatment partly due to variations in strength between allergen vaccines. Standards for a number of allergen extracts, including birch and timothy pollen extracts, have been developed in the allergen standardization program of World Health Organization (WHO), International Union of Immunological Societies (IUIS) and International Academy of Allergy and Clinical Immunology (IAACI) [45,46]. Regulations for production and release of allergen vaccines have been published by European medical agencies and the Food and Drug Administration (FDA) in the USA [47–49]. Methods to measure the potency of the extracts have been described to minimize lot-to-lot variations and variations between pollen vaccines [47,48]. However, the extracts contain a heterogeneous mixture of allergens and other molecules and in many cases only a minority of them are characterized.

Attempts to modify allergens to reduce their allergenic properties and hence the risk of side effects have been somewhat disappointing. Allergoids are obtained by chemical modification of allergens with formaldehyde or glutaraldehyde to reduce IgE binding without destroying the immunostimulating capacity [50]. Allergoids have been used for more than 10 years but their superiority over natural allergens is uncertain [51,52].

Pollen vaccines can be delivered as aqueous extracts or as depot preparations in mixture with aluminum hydroxide as adjuvant. Aqueous extracts of allergens are, in contrast to the depot preparations, rapidly cleared from the site of injection [53]. They are often used when the increment of allergen doses follows a rush regime with several injections each day (i.e., rush SIT). Aluminum hydroxide is a well-known inducer of high levels of IgE antibodies in mice, probably due to high IL-4 production [53]. Whether aluminum hydroxide has a similar effect in humans when used as adjuvant is not known but the function of aluminum salts as potent Th2 inducers in mice has raised questions about their suitability in vaccine formulations [53].

The efficacy of SIT has been proven in a number of studies involving patients allergic to tree, grass and weed pollen [42,54]. Clinical improvements are usually observed already during the first year of SIT [55–57] and increase during continuous treatment [56]. Clinical benefits remain for at least several years after discontinuation of pollen SIT, which indicates that persistent immunological changes have occurred during treatment [58,59]. Allergic patients with asthma can also be treated with SIT with good clinical efficacy [42,55,60]. However, in a clinical trial of asthmatic children polysensitized to various aeroallergens, no clinical efficacy of SIT was observed in the actively-treated group compared with placebo [61]. The discrepancy between the studies indicates that the selection of patients for SIT is of great importance.

SIT has been shown to suppress the development of allergic disease. In an open controlled study, it was found that SIT had a protective effect on patients suffering from allergic rhinoconjunctivitis for the development of asthma [62]. Furthermore, different studies have shown that SIT treatment of house-dust mite-allergic children can reduce the development of new sensitizations to other unrelated allergens and therefore it may protect against a spread of the allergy [63,64].

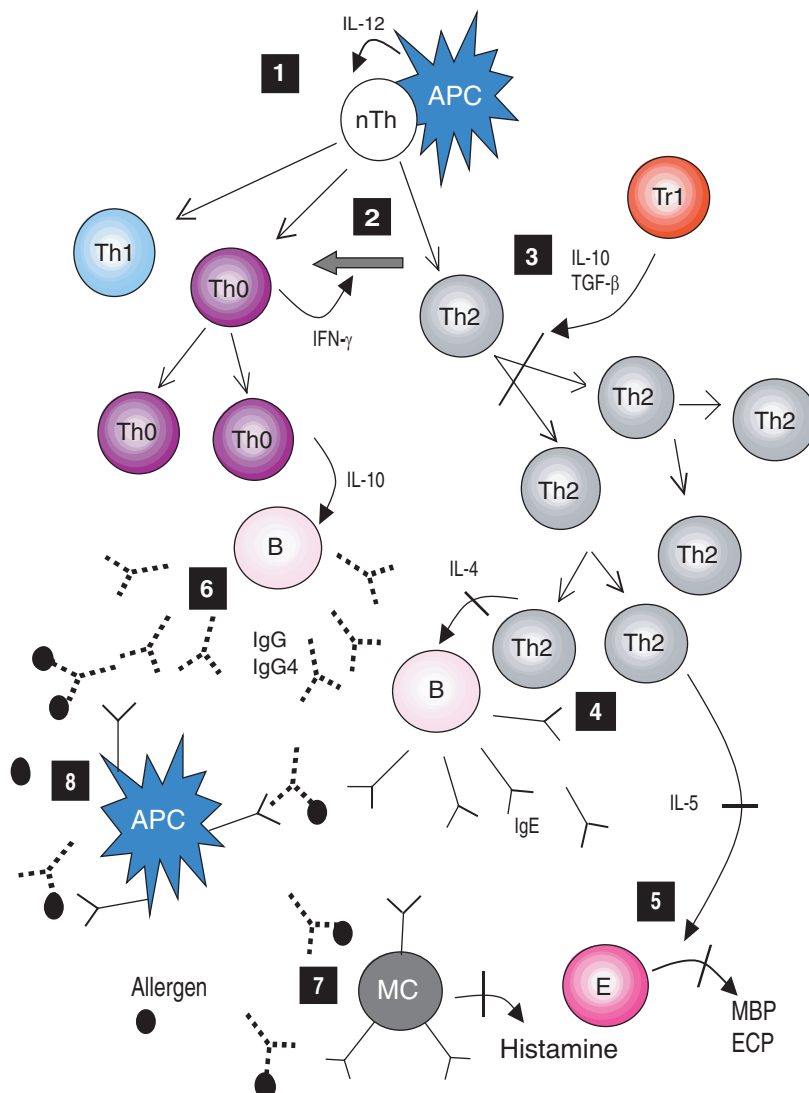
Side effects of SIT are common but they are normally mild and easy to manage [42]. The side effects are normally itching wheals at the site of vaccine injection within 15–20 min. Mild late-phase skin reactions are also frequently seen. Systemic reactions are unusual. Fatalities from anaphylactic shock reactions during SIT have been reported from earlier years but today SIT is regarded as a relatively safe treatment [42]. If symptoms of severe side effects are observed, which generally happens during the first hour after the injection, the need of immediate care to control the systemic reactions is vital.

Mechanisms of pollen SIT

The mechanisms underlying successful SIT with injected pollen vaccines are not fully understood but a lot of knowledge has been collected over the years (FIGURE 1). SIT has been shown to influence the function and the migration of effector cells into the site of inflammation. Decreased allergen-induced migration of eosinophils into nasal mucosa after SIT has been observed [65,66]. Furthermore, the seasonal increase of eosinophils in bronchoalveolar lavage fluid during birch-pollen season is blunted in SIT-treated patients with seasonal asthma [67]. This is probably partly due to decreased adhesion of eosinophils to vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 on endothelial cells [68]. In line with these observations, the seasonal increase in serum ECP, an indication of systemic priming of eosinophils, is abolished after SIT [69]. Studies have shown that both basophils and mast cells are affected by SIT. Ragweed-pollen SIT has been reported to be associated with a defective function of the degranulation of basophils [70,71]. Studies of wasp-venom rush SIT indicate that this imperfection is induced by IL-10 and IFN- γ [72]. The number of mast cells in nasal mucosa during allergen provocation is reduced after SIT [73] and it was shown that grass-pollen SIT decreased the immediate cutaneous allergen sensitivity together with a sevenfold reduction in the number of mast cells [74]. Furthermore, the seasonal increase in levels of both ECP and tryptase in nasal secretion are significantly lower in SIT-treated patients than in control patients [75].

Many studies have shown that the serum concentration of allergen-specific IgE increases during the early phase of SIT, followed by a gradual fall to normal or decreased levels [51,76,77]. During rush SIT, an increase in the allergen-specific serum IgE concentrations can already be observed after a few days of treatment, indicating a rapid immunological response to aqueous pollen extracts [77]. For conventional SIT treatment with depot preparations of pollen vaccines, the kinetics of the IgE increase are slower [28]. The temporary increase in specific IgE might be associated with an early strengthened Th2 response and increased IL-4 synthesis due to the allergen injections [28,78,79]. Conventional SIT starts with injections of very low allergen doses, which may lead to an immune response of Th2 type [80,81]. Furthermore, the aluminum hydroxide in the depot preparations might support a Th2-like immune response by influencing the cytokine production by APCs [53].

Figure 1. Current knowledge of the mechanisms of conventional pollen SIT may be described as follows. High doses of injected allergens induce elevated IL-12 synthesis by antigen-presenting cells (APC), for example, dendritic cells, which preferentially promote the development of naive T-helper cells (nTh) towards Th cells of Th1 or Th0 type (1). This leads to an immune deviation from an allergic Th2-type response to a Th1/Th0-type response and is supported by high IFN- γ production (2). The immune deviation is strengthened by induction of T-cell tolerance (anergy or clonal elimination) of the memory Th2 cells under influence of regulatory T-cells (Tr1) producing the immunosuppressive cytokines IL-10 and TGF- β (3). Decreased activation and proliferation of Th2 cells also means less IL-4 synthesis and decreased IgE production by B-cells (B) (4). Decreased infiltration and activation of Th2 cells in the nasal and bronchial mucosa results in reduced IL-5 synthesis, leading to reduced infiltration of eosinophils (E) into the airways and greatly diminished activation and release of prestored inflammatory mediators from eosinophils like major basic protein (MBP) and eosinophil cationic protein (ECP) (5). The new allergen-specific Th cell clones of predominately Th0 phenotype produce a mixture of both Th1- and Th2-type cytokines promoting B-cell production of high amounts of allergen-specific IgG antibodies (6). High levels of IL-10 induce elevated synthesis of allergen-specific IgG4. The allergen-specific IgG/IgG4 antibodies may act as blocking-antibodies. They prevent allergens from cross-linking IgE antibodies bound to receptors on mast cells and thereby inhibit degranulation and histamine release (7). They also block IgE-mediated allergen uptake and processing by antigen-presenting cells (8), which will suppress the immune reaction to the allergens.



Although some of the beneficial effects of SIT might be explained by decreased levels of allergen-specific IgE in the long-term, the diminished IgE production might actually be a consequence of an immune deviation of the allergen-specific Th cells from a Th2-like to a more Th0-like phenotype. Decreased synthesis of several Th2-type cytokines promoting the allergic inflammation would have beneficial effects on the associated symptoms. It has been shown that allergen-induced histamine release by basophils is decreased while specific serum IgE levels are increased early after initiation of SIT, which indicates that the IgE level might not always be associated with clinical improvements [82]. A temporary increase in the serum IgE levels during or after the pollen season is often observed in seasonal pollen-allergic patients but the increase is commonly blunted in patients treated with SIT [71]. This is probably a consequence of reduced seasonal activation of B-cells [83].

IgG antibodies and especially IgG4 antibodies specific for proteins in the extract-based grass or tree pollen vaccines increase significantly during SIT [51,52,56,57,60,76,77]. It is known

that high levels of IL-4 and/or IL-10 induce elevated synthesis of allergen-specific IgG4 [84,85]. An attractive theory states that allergen-specific IgG acts as 'blocking-antibodies' – they compete with IgE for allergen binding and inhibit IgE-mediated granular release of mast cells and basophils. However, there are few data supporting this theory, although *in vitro* experiments have shown that allergen-specific IgG antibodies can block allergen-induced histamine release [70,86]. Allergen-specific IgG/IgG4 might also have a suppressive effect on the allergic late-phase response by inhibiting the IgE-mediated allergen uptake by APCs via their IgE receptors and subsequent presentation for Th cells [87]. The increases in specific IgG and IgG4 have so far not shown good correlation with clinical efficacy. This suggests that allergen-specific IgG/IgG4 measurements might be used to monitor the ongoing immune reaction during pollen SIT rather than to predict clinical improvements.

The fact that Th cells are believed to have an important role in the development of allergy and sustained allergic inflammation has put them in focus when studying mechanisms of

pollen SIT. Analysis of the proliferative response to birch pollen allergens in cultures of peripheral blood mononuclear cells (PBMC) during the pollen season often reveals allergen-specific T-cells in both allergic and nonallergic individuals, although the proliferative response by PBMCs isolated from the allergic subjects is higher [27,88,89]. Peripheral T-cell tolerance is probably a significant mechanism of pollen SIT, which has been indicated by several groups [88–92], even if contradictory results have also been reported [93,94]. The impeded T-cell proliferation *in vitro* could be due to anergy induction or clonal elimination of allergen-specific T-cells. It was recently shown that bee venom SIT induces T-cell anergy associated with enhanced IL-10 synthesis [95]. Neutralization of IL-10 with specific antibodies restored proliferation and cytokine synthesis. However, the role of IL-10 in conventional tree, grass or weed pollen SIT remains to be evaluated.

Immune deviation of the allergen-specific Th cell response from a Th2- to Th0/Th1-like immune response, promoted by high IFN- γ levels, is a possible mechanism of successful SIT. It is well known that high antigen concentrations preferentially induce Th1-like Th cell responses, whereas low concentrations lead to Th2-like responses [8,81]. The maintenance dose of the major birch pollen allergen Bet v 1 given during birch pollen SIT can be more than 10 μ g. However, not only IgE but also IgG4 production is induced by the Th2-type cytokine IL-4 [84]. The large increases in specific IgG4 levels together with unaffected or only slightly decreased specific IgE levels observed during SIT might therefore also be referred to as a modified Th2 response [96].

Seasonal pollen allergy is characterized by periodical exposure of pollen allergens, with long symptom-free periods between the pollen seasons. Consequently, the Th2-type cytokine profile seems to be less pronounced in Th-cells isolated from patients suffering from seasonal pollen allergy than patients with more severe allergy, for example patients with perennial allergy and severe asthma [29,97–99]. Immune deviation of peripheral blood Th cells has been shown after venom SIT and SIT with perennial respiratory allergens (e.g., in cat allergy and house-dust mite allergy). It has also been shown after successful pollen SIT [90,100]. However, in several independent studies of pollen SIT no systemic changes in the cytokine profile of allergen-specific Th cells were observed [28,78,89,93,94]. In one of the studies no decrease was observed in the IL-5 production by peripheral T-cells from grass pollen-allergic patients treated with conventional SIT for as long as 6–7 years [93]. It is reasonable to believe that the major immunological effect of pollen SIT is to be found at the site of the allergic inflammation rather than in the pool of circulating T-cells [94,101]. T-cell responses have been studied in skin and nasal mucosa. These studies revealed a decreased recruitment of CD4+ T-cells after local allergen provocation in patients treated with grass pollen SIT compared with placebo [65,102]. A significant increase in IFN- γ and IL-2 mRNA-positive cells was also observed, while the number of IL-4 and IL-5 mRNA-positive cells were not significantly affected [65,102].

The reduced late cutaneous response to allergen provocation after grass pollen SIT is associated with increased numbers of IL-12 mRNA-expressing cells [103]. IL-12 produced by APCs might be crucial in shifting the T-cell response from Th2- to Th0/Th1-type during SIT. Whether this influence of IL-12 is located mainly in the target organs for allergic diseases or in the follicles of lymph nodes is not known. Changed synthesis of IL-12 by APCs during the early phase of SIT should also explain findings of nonspecific changes in cytokine production by PBMC *in vitro* from patients during the updosing phase of SIT [28,79].

There has been somewhat of a revival in the role of regulatory T-cells in recent years, in attempts to explain the mechanism of SIT. Although the presented data is to some extent contradictory, so-called Tr1 cells are described as a subset of CD4+CD25+ T-cells with suppressive ability on T-cell-mediated immune response of Th2-type. The suppression is mediated by the production of the immunoinhibitory cytokines IL-10 and transforming growth factor (TGF)- β [104].

Ongoing research & development

Knowledge has been accumulated during the years about the importance of a correct diagnosis of the symptom-inducing allergens for an effective treatment strategy for allergy and asthma. It is known that mono- or oligosensitized patients have a better outcome of SIT than polysensitized patients [42,61]. Increasing knowledge about structural and antigenic relationships between inhalant and food allergens and the possibility to study IgE reactivity to panels of individual allergenic components, sometimes called component-resolved diagnostics [105], might be helpful in the selection of patients for pollen SIT [8]. For example, it is believed that birch pollen SIT is well suited to severe birch pollen-allergic patients with IgE antibodies to the major birch pollen allergen Bet v 1 [8]. Thus far, there are no studies proving that statement but it is known that Bet v 1 is a major component in birch pollen extracts and birch pollen vaccines are generally standardized regarding their contents of Bet v 1. Not only the presence but also the concentration of allergen-specific IgE antibodies may help the clinician to decide suitable treatment regimes for the individual patients [106].

Even if reduced induction of new sensitizations have been reported after successful house-dust mite SIT [63,64], studies of pollen SIT have shown the presence of new IgE specificities to allergens in the extracts that were used for vaccination [76,107–109]. The new IgE reactivities seem to evolve late during SIT and the IgE concentrations are often low [107]. If new IgE reactivities are developed against highly cross-reacting allergens they might lead to new allergic reactions to additional pollens or when eating plant-derived foods. Studies have indicated that SIT with house-dust mite extract can lead to the development of new cross-reacting IgE antibodies to snail and shrimp and in rare cases to aggravated clinical symptoms when eating these foods [110,111]. However, no spread in allergy symptoms has yet been reported during SIT with pollen vaccines. Hence, it is most likely not a major problem, even if up to 65% of the patients treated with birch pollen

SIT appeared to have developed new IgE reactivities to allergens in the extract in one recent study [107].

Why should an allergic patient that has IgE reactivity to only one or a few allergens in the pollen extract receive SIT based on the whole crude mixture of allergens and nonallergenic proteins that are present in the extract? The SIT based on pollen extract may be less effective due to low amounts of some important allergens that are not standardized in the extract. More than 30 years ago it was shown that SIT with the single purified major ragweed allergen Amb a 1 (antigen E) was clinically effective in ragweed allergic patients [112]. Achievements in cloning genes coding for allergens might provide vaccines of recombinant allergens in the future. Large-scale production of recombinant allergens can provide component-resolved SIT with vaccines tailor-made for each patient and based on *in vitro* measurements of the individual IgE reactivity pattern [105]. Some degree of caution is appropriate however, since the IgE reactivity does not necessarily correspond to the Th cell reactivity to the pollen allergens [113]. Studies have proven that recombinant pollen allergens behave similarly *in vivo* and in basophil histamine release assays and T-cell stimulation assays as their natural counterparts [14,114,115]. A careful selection between the isoforms is important, since the reactivity to different isoforms of pollen allergens may be very heterogeneous among Th cell clones due to differences in epitope recognition [98,115,116]. Thus, a combination of more than one isoform may be required for optimal effect of SIT.

An advantage with recombinant technology is the possibility to perform genetic engineering on allergen genes. Several attempts to reduce the allergenicity of recombinant allergens without diminishing their immunostimulating capacity have been presented. One possible way is to destroy the IgE-recognizing epitopes on the allergens by site-directed mutagenesis [117]. For many pollen allergens IgE antibodies have been shown to bind to structural rather than linear epitopes. The IgE binding is therefore sensitive for disturbances in the three-dimensional structure of the allergens. The T-cell epitopes are always linear. Consequently, the Th-cell-stimulating activity is not dependent upon the 3D structure of the allergens. Clinical SIT studies have been started using a recombinant oligomer (trimer) of Bet v 1 with reduced capacity to induce allergic reactions. Another approach has been to produce two recombinant Bet v 1 fragments where the 3D structures are very different from the wild type form of the allergen. Nasal challenges with both the recombinant Bet v 1 trimer and the two Bet v 1 fragments in humans have shown promising results [118]. The Bet v 1 derivatives induced decreased nasal symptoms and were accompanied by decreased synthesis of tryptase, ECP and eosinophil peroxidase as compared with challenges with wild type Bet v 1. Recombinant hybrid molecules consisting of several important timothy pollen allergens for use in grass pollen SIT are also considered [119]. The use of T-cell epitope-containing peptides for SIT treatment of pollen allergy has also been considered. However, clinical SIT studies using peptides from the major cat allergen Fel d 1 have not been convincing regarding the clinical efficacy of

peptide-based vaccines [120]. Furthermore, side effects (late asthmatic reactions) were frequently observed.

Since the conventional adjuvant aluminum hydroxide is a potent inducer of Th2 responses and IgE production, alternative adjuvants for SIT have been proposed. Liposomes are potent immunological adjuvants with pronounced depot effect and Th1-promoting activity. Liposome-encapsulated extracts of house dust mites have been tested in SIT trials with good clinical efficacy [121]. Another new Th1-inducing immunostimulatory adjuvant is monophosphoryl lipid A (MPL[®]) [122]. Other options are immunostimulatory sequence oligodeoxynucleotides (ISS-ODNs) that have shown promising results in asthma animal models where it induced pronounced Th1 responses [123] and allergen-conjugated carbohydrate-based particles that in a very recent paper was shown to induce a strong Th0-type response in mice [124].

Summary & conclusion

The prevalence of IgE-mediated allergy and asthma are increasing. The only treatment that affects the immunological disorder behind allergy is SIT. Conventional SIT for treatment of pollen allergy is administered by subcutaneous injections of gradually increasing doses of pollen extracts (pollen vaccines). The extracts contain both allergens and nonallergenic proteins. Pollen SIT is clinically effective since it reduces allergic symptoms, protects for the development of asthma and decreases the development of new allergies. However, SIT may also induce new IgE specificities to components in the extract. Accurate diagnosis of the patients is important for successful pollen SIT. Increasing knowledge about structural and antigenic relationships between allergenic components and the development of assays based on recombinant allergens (component-resolved diagnostics) might lead to new possibilities to select suitable treatments for pollen allergic patients.

The mechanism of pollen SIT is not clear. Several mechanisms have been suggested. A decrease in specific IgE antibodies together with increased levels of IgG antibodies (especially of the IgG4 subclass) with allergen-blocking capacity has been suggested as a possible explanation of the clinical efficacy of SIT but it is a controversial theory. The hypothesis of an immune deviation from a Th2-type cytokine response to a Th1- or Th0-type response together with induction of T-cell tolerance among the allergen-specific Th cells has more supporters and many studies have confirmed the theory. The involvement of IL-10 and TGF- β -producing regulatory T-cells (Tr1 cells) is more debatable but Tr1 cells could explain the immunosuppressive effect of SIT.

Several projects have been started with the aim to improve the vaccines used for pollen SIT. They include developments of new Th1-inducing immunostimulatory adjuvants and hypoallergenic allergens with reduced IgE-binding capacity but retained ability to stimulate a T-cell response. The hypoallergenic allergens can be created using recombinant technology. A panel of recombinant allergens for SIT together with component-resolved diagnostics will enable tailor-made SIT treatment for

individual patients. Also various routes for administration of the pollen vaccines are studied. Both nasal and sublingual administrations of pollen extracts have shown promising results.

Expert opinion

SIT for the treatment of pollen allergy will probably have an increasing importance in the future. Even if improved pharmacological drugs have been introduced, only SIT can affect the natural course of allergy. New exciting projects aiming to improve SIT regimes have commenced. They focus not only on the vaccine formulation but also on alternative routes for the administration of the vaccines and on improvements in the diagnosis of the patients and in the monitoring of SIT.

An accurate diagnosis of the patients that are intended to be treated with pollen SIT is in my opinion critical for successful treatment. Either *in vitro* testing or skin-prick testing based on allergen extracts is essential. Quantitative specific IgE measurement using modern *in vitro* systems, such as Pharmacia CAP System™ and UniCAP® (Pharmacia Diagnostics, Uppsala, Sweden) can give additional information regarding the allergic status of the patient. Furthermore, pollen-allergic patients that have been considered for SIT with pollen vaccines can be further studied regarding their specific IgE reactivity to major pollen allergens. Component-resolved diagnostics based on recombinant pollen allergens give important information about the pollen source responsible for the original IgE sensitization and distinguish that from positive results in allergy tests due to cross-reactive IgE antibodies. IgE reactivity to major pollen allergens is generally an incentive for pollen SIT with the extract in question [8]. Therefore, it has been suggested that birch pollen-sensitive patients that lack Bet v 1-specific IgE antibodies should not be treated with birch pollen SIT [8]. Their test results based on birch pollen extracts may be due to IgE antibodies recognizing minor allergens, such as the highly cross-reactive allergens Bet v 2 (profilin) or Bet v 4 [17]. Similarly, grass pollen SIT with vaccines based on timothy grass extract are probably best suited for truly grass-sensitized patients revealed by their IgE reactivity to the major timothy pollen allergens Phl p 1 or Phl p 5 [8]. Parietaria is a weed common in coastal Mediterranean areas [4]. The presence of IgE antibodies to the major allergen Par j 2 in sera from patients allergic to Parietaria pollen is a strong indicator for SIT with Parietaria pollen extract. Pollen SIT can also be monitored for immunological responses to the injected allergens by measurement of the allergen-specific IgG (especially IgG4) antibodies. The induction of new IgE reactivities to components in the extract-based vaccines can also be monitored using component-resolved diagnostics and actions can be taken if needed (e.g., to stop the treatment due to new symptoms after new IgE specificities have appeared).

Regarding the development of new improved pollen vaccines, the following are probably important:

- The use of recombinant hypoallergenic allergens with improved safety profiles
- The use of new adjuvants with Th1-promoting activity (for injection SIT)
- Alternative ways of vaccine administration, either by the sublingual or nasal route

Combinations of recombinant pollen allergens and alternative routes of vaccine administration are under development and the author is sure that recombinant hypoallergenic allergens will also be tested together with new Th1-inducing adjuvants, hopefully with promising results. The big issue is, however, whether the new vaccines for pollen SIT will be superior to the conventional pollen vaccines based on allergen extracts which are currently used, both regarding their clinical effect on reducing allergic symptoms and their safety profiles. The author is not convinced that the achievements to develop new improved pollen vaccines will result in much better clinical efficacy of SIT. The mechanism behind allergy development and the mechanism of pollen SIT is not yet clear enough to allow reliable predictions of the method for perfect SIT treatment of pollen allergy. We have to wait and see the results of the ongoing studies. However, conventional pollen SIT is already an attractive alternative for treatment of seasonal pollen allergy and asthma. With the use of accurate diagnostics for the selection of patients, the use of pollen SIT will probably increase. SIT is still yet the only treatment that results in a long-term suppression of the underlying immunological mechanism behind seasonal pollen allergy.

The recently developed anti-IgE treatment, for which its effect on allergic symptoms have been proven in several clinical trials [125], may improve the clinical efficacy and safety profile of pollen SIT when coadministered. Anti-IgE treatment reduces the serum IgE levels and makes mast cells and basophils more resistant to allergen-induced degranulation. A combination of the two treatments may reduce the risk of side effects of pollen SIT due to the decreased IgE levels and thereby allow higher doses of the pollen vaccine to be administered. It is an exciting possibility that should be investigated.

Five-year view

Increasing knowledge of the mechanisms behind successful pollen SIT will probably lead to improvements in pollen vaccines and treatment regimes. Also of importance is the ongoing research regarding pollen allergens and how results from quantitative *in vitro* tests for antibody measurements can be used to select patients for SIT, as well as for monitoring the immunological response of SIT and the development of new IgE reactivities. Component-resolved diagnostics based on recombinant pollen allergens is now a reality for research use but the clinical relevance for selecting patients for pollen SIT based on their IgE reactivity profile remains to be demonstrated. This can hopefully be achieved during the coming 5 years. Planned, or just started, clinical studies using recombinant hypoallergenic allergens in SIT will soon result in valuable information about their possible superiority over conventional extract-based pollen vaccines. If they are tolerated to a greater extent even when administered at higher doses than conventional pollen vaccines, with maintained or improved clinical efficacy, they probably will substitute the conventional extract-based vaccines. The launch of vaccines for SIT based on recombinant allergens will, however, probably not be a reality until the end of the coming 5-year period, or later.

What we might be able to see during the coming 5 years is an increasing use of sublingual SIT and nasal SIT for treatment of seasonal pollen allergy. A leading European producer of pollen vaccines for injections has recently made an agreement with a large international pharmaceutical company for collaboration in a clinical SIT trial using a grass pollen allergy tablet for sublingual delivery. The vaccine tablet is believed to have an improved safety profile. Different variants of adjuvants for SIT vaccines, as alternative to the Th2-promoting aluminum salts, will probably also be tested and some may be launched the coming years. Indeed, a pollen vaccine based on glutaraldehyde-modified pollen extracts and the Th1-inducing adjuvant MPL has recently been introduced for preseasonal SIT.

Information resources

Review article

- Bousquet J, Lockey RE, Malling HJ. Allergen immunotherapy – therapeutic vaccines for allergic diseases. *Allergy*

53(Suppl. 44), 4–42 (1998).

- A WHO position paper concerning SIT.

Websites

- World Allergy Organization, www.worldallergy.org
- European Academy of Allergology and Clinical Immunology, www.eaaci.org
- American Academy of Allergy, Asthma & Immunology, www.aaaai.org
- International Union of Immunological Societies, Allergen Nomenclature Sub-Committee, www.allergen.org

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Key issues

- Conventional specific immunotherapy (SIT) for pollen allergy involves subcutaneous injections of gradually increasing doses of pollen extracts (vaccine) and 3–5 years of regular maintenance injections with high allergen doses. SIT influences the underlying mechanism of allergy and leads to persistent improvements in symptoms.
- Seasonal pollen allergy is characterized by allergen-specific immunoglobulin (IgE) antibodies and a T-helper (Th) cell response skewed towards the Th2 phenotype, in other words high production of interleukin (IL)-4, IL-5 and IL-13. The proposed ways of action of conventional pollen SIT are synthesis of allergen-blocking IgG/IgG4 antibodies, immune deviation from a Th2-type response to Th0- or Th1-type response and induction of Th cell tolerance.
- An accurate diagnosis of the patients intended to be treated with pollen SIT is crucial. Quantitative measurements of specific serum IgE in standardized allergy *in vitro* testing or skin-prick testing with allergen extracts are the conventional methods. The new concept component-resolved diagnostics based on recombinant allergens may be used (yet only for research purposes) to select patients for pollen SIT.
- Hypoallergenic recombinant allergens will provide new vaccines for SIT for pollen allergy. These vaccines might be given at higher doses due to the impaired IgE-binding capacity of the hypoallergenic allergens and thus, would have a stronger influence on the immune system than conventional extract-based pollen vaccines.
- New forms of Th1-inducing immunostimulatory adjuvants are under development. One of them, MPL has shown good clinical and immunological performance in clinical studies.
- Sublingual or nasal administration of pollen vaccines will probably be safer than conventional SIT and thereby provide new forms of treatment. Their clinical efficacy compared with conventional SIT is however not clear.
- Clinical studies should be started to investigate if coadministration of pollen SIT and anti-IgE treatment may improve the clinical efficacy and safety profile of pollen SIT.

References

Papers of special note have been highlighted as:

- of interest

- 1 Beasley R. The burden of asthma with specific reference to the United States. *J. Allergy Clin. Immunol.* 109(5 Suppl), S482–S489 (2002).
- 2 Janson C, Anto J, Burney P *et al.* The European Community Respiratory Health Survey: what are the main results so far? *Eur. Respir. J.* 18(3), 598–611 (2001).
- 3 Vartiainen E, Petays T, Haahtela T, Jousilahti P, Pekkanen J. Allergic diseases, skin prick test responses and IgE levels in North Karelia, Finland and the Republic of Karelia, Russia. *J. Allergy Clin. Immunol.* 109(4), 643–648 (2002).
- 4 D'Amato G, Spiekma FTM, Liccardi G *et al.* Pollen-related allergy in Europe. *Allergy* 53(6), 567–578 (1998).
- 5 Vieths S, Scheurer S, Ballmer-Weber B. Current understanding of cross-reactivity of food allergens and pollen. *Ann. NY Acad. Sci.* 964, 47–68 (2002).
- 6 Fogle-Hansson M, Bende M. The significance of hypersensitivity to nuts in patients with birch pollen allergy. *Allergy* 48(4), 282–284 (1993).
- 7 Jarolim E, Rumpold H, Endler AT, Ebner H, Breitenbach M, Scheiner O, Kraft D.

- IgE and IgG antibodies of patients with allergy to birch pollen as tools to define the allergen profile of *Betula verrucosa*. *Allergy* 44(6), 385–395 (1989).
- 8 Kazemi-Shirazi L, Niederberger V, Linhart B, Lidholm J, Kraft D, Valenta R. Recombinant marker allergens: diagnostic gatekeepers for the treatment of allergy. *Int. Arch. Allergy Immunol.* 127(4), 259–268 (2002).
 - Discusses cross-reactivity between pollen and food allergens and how component-resolved diagnostics may be used to select patients for pollen SIT.
 - 9 Luttkopf D, Muller U, Skov PS *et al.* Comparison of four variants of a major allergen in hazelnut (*Corylus avellana*) Cor a 1.04 with the major hazel pollen allergen Cor a 1.01. *Mol. Immunol.* 38(7), 515–525 (2002).
 - 10 Wensing M, Akkerdaas JH, van Leeuwen WA *et al.* IgE to Bet v 1 and profilin: cross-reactivity patterns and clinical relevance. *J. Allergy Clin. Immunol.* 110(3), 435–442 (2002).
 - 11 Valenta R, Duchene M, Ebner C *et al.* Profilins constitute a novel family of functional plant pan-allergens. *J. Exp. Med.* 175(2), 377–385 (1992).
 - 12 Scheurer S, Wangorsch A, Nerkamp J *et al.* Cross-reactivity within the profilin panallergen family investigated by comparison of recombinant profilins from pear (Pyr c 4), cherry (Pru av 4) and celery (Api g 4) with birch pollen profilin Bet v 2. *J. Chromatogr. Biomed. Sci. Appl.* 756(1–2), 315–325 (2001).
 - 13 Swoboda I, Jilek A, Ferreira F *et al.* Isoforms of Bet v 1, the major birch pollen allergen, analyzed by liquid chromatography, mass spectrometry and cDNA cloning. *J. Biol. Chem.* 270(6), 2607–2613 (1995).
 - 14 Pauli G, Oster JP, Deviller P *et al.* Skin testing with recombinant allergens rBet v 1 and birch profilin, rBet v 2: diagnostic value for birch pollen and associated allergies. *J. Allergy Clin. Immunol.* 97(5), 1100–1109 (1996).
 - 15 Valenta R, Duchene M, Vrtala S *et al.* Recombinant allergens for immunoblot diagnosis of tree-pollen allergy. *J. Allergy Clin. Immunol.* 88(6), 889–894 (1991).
 - 16 Twardosz A, Hayek B, Seiberler S *et al.* Molecular characterization, expression in *Escherichia coli* and epitope analysis of a two EF-hand calcium-binding birch pollen allergen, Bet v 4. *Biochem. Biophys. Res. Commun.* 239(1), 197–204 (1997).
 - 17 Moverare R, Westritschnig K, Svensson M *et al.* Different IgE reactivity profiles in birch pollen-sensitive patients from six European populations revealed by recombinant allergens. An imprint of local sensitization. *Int. Arch. Allergy Immunol.* 128(4), 325–335 (2002).
 - 18 Laffer S, Spitzauer S, Susani M *et al.* Comparison of recombinant timothy grass pollen allergens with natural extract for diagnosis of grass pollen allergy in different populations. *J. Allergy Clin. Immunol.* 98(3), 652–658 (1996).
 - 19 Hirschwehr R, Valenta R, Ebner C *et al.* Identification of common allergenic structures in hazel pollen and hazelnuts: a possible explanation for sensitivity to hazelnuts in patients allergic to tree pollen. *J. Allergy Clin. Immunol.* 90(6 Pt 1), 927–936 (1992).
 - 20 Kazemi-Shirazi L, Pauli G, Purohit A *et al.* Quantitative IgE inhibition experiments with purified recombinant allergens indicate pollen-derived allergens as the sensitizing agents responsible for many forms of plant food allergy. *J. Allergy Clin. Immunol.* 105(1 Pt 1), 116–125 (2000).
 - Demonstrates that pollen-related food allergy in most cases is due to IgE sensitization to pollen allergens and not the vice versa.
 - 21 van Ree R, Fernandez Rivas M, Cuevas M, van Wijngaarden M, Aalberse RC. Pollen-related allergy to peach and apple: an important role for profilin. *J. Allergy Clin. Immunol.* 95(3), 726–734 (1995).
 - 22 Diez-Gomez ML, Quirce S, Cuevas M *et al.* Fruit-pollen-latex cross-reactivity: implication of profilin (Bet v 2). *Allergy* 54(9), 951–961 (1999).
 - 23 Punnonen J, Aversa G, Cocks BG *et al.* Interleukin-13 induces interleukin-4-independent IgG4 and IgE synthesis and CD23 expression by human B-cells. *Proc. Natl Acad. Sci. USA* 90(8), 3730–3734 (1993).
 - 24 Pene J, Rousset F, Briere F, Chretien I *et al.* IgE production by normal human lymphocytes is induced by interleukin-4 and suppressed by interferons gamma and alpha and prostaglandin E2. *Proc. Natl Acad. Sci. USA* 85(18), 6880–6884 (1988).
 - 25 Gabrielsson S, Soderlund A, Paulie S, Rak S, van der Pouw Kraan TC, Troye-Blomberg M. Increased frequencies of allergen-induced interleukin-13-producing cells in atopic individuals during the pollen season. *Scand. J. Immunol.* 48, 429–435 (1998).
 - 26 Kimura M, Tsuruta S, Yoshida T. Unique profile of IL-4 and IFN-gamma production by peripheral blood mononuclear cells in infants with atopic dermatitis. *J. Allergy Clin. Immunol.* 102(2), 238–244 (1998).
 - 27 Moverare R, Elfman L, Stalenheim G, Bjornsson E. Study of the Th1/Th2 balance, including the IL-10 production, in cultures of peripheral blood mononuclear cells from birch-pollen-allergic patients. *Allergy* 55(2), 171–175 (2000).
 - 28 Moverare R, Elfman L, Bjornsson E, Stalenheim G. Changes in cytokine production *in vitro* during the early phase of birch-pollen immunotherapy. *Scand. J. Immunol.* 52, 200–206 (2000).
 - 29 Ebner C, Schenk S, Szepefalusi Z *et al.* Multiple T-cell specificities for Bet v 1, the major birch pollen allergen, within single individuals. Studies using specific T-cell clones and overlapping peptides. *Eur. J. Immunol.* 23(7), 1523–1527 (1993).
 - 30 Gabrielsson S, Paulie S, Rak S *et al.* Specific induction of interleukin-4-producing cells in response to *in vitro* allergen stimulation in atopic individuals. *Clin. Exp. Allergy* 27(7), 808–815 (1997).
 - 31 Ebner C, Siemann U, Najafian N, Scheiner O, Kraft D. Characterization of allergen (Bet v 1)-specific T-cell lines and clones from non-allergic individuals. *Int. Arch. Allergy Immunol.* 107(1–3), 183–185 (1995).
 - 32 Church MK, Bradding P, Walls AF, Okayama K. Human mast cells and basophils. In: *Allergy and allergic diseases (Volume 1)*. Kay AB (Ed.). Blackwell Science, Oxford, UK, 149–170 (1997).
 - 33 Borish L, Joseph BZ. Inflammation and the allergic response. *Med. Clin. North Am.* 76(4), 765–787 (1992).
 - 34 Montefort S, Gratziau C, Goulding D *et al.* Bronchial biopsy evidence for leukocyte infiltration and upregulation of leukocyte-endothelial cell adhesion molecules 6 h after local allergen challenge of sensitized asthmatic airways. *J. Clin. Invest.* 93(4), 1411–1421 (1994).
 - 35 Gleich GJ. Mechanisms of eosinophil-associated inflammation. *J. Allergy Clin. Immunol.* 105(4), 651–663 (2000).
 - 36 Amin K, Ludviksdottir D, Janson C *et al.* Inflammation and structural changes in the airways of patients with atopic and nonatopic asthma. *Am. J. Respir. Crit. Care Med.* 162(6), 2295–2301 (2000).

- 37 Varney VA, Jacobson MR, Sudderick RM *et al.* Immunohistology of the nasal mucosa following allergen-induced rhinitis. Identification of activated T-lymphocytes, eosinophils and neutrophils. *Am. Rev. Respir. Dis.* 146(1), 170–176 (1992).
- 38 Hamelmann E, Gelfand EW. Role of IL-5 in the development of allergen-induced airway hyperresponsiveness. *Int. Arch. Allergy Immunol.* 120(1), 8–16 (1999).
- 39 Yamaguchi Y, Hayashi Y, Sugama Y *et al.* Highly purified murine interleukin-5 (IL-5) stimulates eosinophil function and prolongs *in vitro* survival. IL-5 as an eosinophil chemotactic factor. *J. Exp. Med.* 167(5), 1737–1742 (1988).
- 40 Desreumaux P, Capron M. Eosinophils in allergic reactions. *Curr. Opin. Immunol.* 8(6), 790–795 (1996).
- 41 Noon L, Cantab BC, Eng FRCS. Prophylactic inoculation against hay fever. *Lancet* i, 1572–1573 (1911).
 - First publication concerning pollen SIT.
- 42 Bousquet J, Lockey RF, Malling HJ. Allergen immunotherapy – therapeutic vaccines for allergic diseases. *Allergy* 53(Suppl. 44), 4–42 (1998).
 - Comprehensive review and WHO position paper for SIT with allergen vaccines.
- 43 Malling HJ, Abreu-Nogueira J, Alvarez-Cuesta E *et al.* Local immunotherapy. *Allergy* 53(10), 933–944 (1998).
 - Comprehensive review of local SIT including sublingual and nasal administration (position paper).
- 44 Kagi MK, Wuthrich B. Different methods of local allergen-specific immunotherapy. *Allergy* 57(5), 379–388 (2002).
 - Updated review of sublingual SIT and nasal SIT.
- 45 Gjesing B, Jager L, Marsh DG, Lowenstein H. The international collaborative study establishing the first international standard for timothy (*Phleum pratense*) grass pollen allergenic extract. *J. Allergy Clin. Immunol.* 75(2), 258–267 (1985).
- 46 Arntzen FC, Wilhelmsen TW, Lowenstein H *et al.* The international collaborative study on the first international standard of birch (*Betula verrucosa*)-pollen extract. *J. Allergy Clin. Immunol.* 83(1), 66–82 (1989).
- 47 Dreborg S. Skin testing in allergen standardization and research. *Immunol. Allergy Clin. North Am.* 21(2), 329–354 (2001).
- 48 Nordic Council on Medicines. Registration of allergen preparations. Nordic guidelines. MLN Publication No 23, Uppsala, Sweden. (1989).
- 49 US Department of Health and Human Services, FDA. Guidance for reviewers. Potency limits for standardized dust mite and grass allergen vaccines: A revised protocol (2000).
- 50 Platts-Mills TA, Mueller GA, Wheatley LM. Future directions for allergen immunotherapy. *J. Allergy Clin. Immunol.* 102(3), 335–343 (1998).
- 51 Pastorello EA, Pravettoni V, Incorvaia C *et al.* Clinical and immunological effects of immunotherapy with alum-absorbed grass allergoid in grass-pollen-induced hay fever. *Allergy* 47(4 Pt 1), 281–290 (1992).
- 52 Bousquet J, Hejjaoui A, Skassa-Brociek W *et al.* Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. I. Rush immunotherapy with allergoids and standardized orchard grass-pollen extract. *J. Allergy Clin. Immunol.* 80(4), 591–598 (1987).
- 53 Gupta RK. Aluminum compounds as vaccine adjuvants. *Adv. Drug Deliv. Rev.* 32(3), 155–172 (1998).
- 54 Arvidsson MB, Lowhagen O, Rak S. Effect of 2-year placebo-controlled immunotherapy on airway symptoms and medication in patients with birch pollen allergy. *J. Allergy Clin. Immunol.* 109(5), 777–783 (2002).
- 55 Reid MJ, Moss RB, Hsu YP, Kwasnicki JM, Commerford TM, Nelson BL. Seasonal asthma in northern California: allergic causes and efficacy of immunotherapy. *J. Allergy Clin. Immunol.* 78(4 Pt 1), 590–600 (1986).
- 56 Dolz I, Martinez-Cocera C, Bartolome JM, Cimarra M. A double-blind, placebo-controlled study of immunotherapy with grass-pollen extract Alutard SQ during a 3-year period with initial rush immunotherapy. *Allergy* 51(7), 489–500 (1996).
- 57 Balda BR, Wolf H, Baumgarten C *et al.* Tree-pollen allergy is efficiently treated by short-term immunotherapy (STI) with seven preseasonal injections of molecular standardized allergens. *Allergy* 53(8), 740–748 (1998).
- 58 Jacobsen L, Nuchel Petersen B, Wihl JA, Lowenstein H, Ipsen H. Immunotherapy with partially purified and standardized tree pollen extracts. IV. Results from long-term (6-year) follow-up. *Allergy* 52(9), 914–920 (1997).
- 59 Durham SR, Walker SM, Varga EM *et al.* Long-term clinical efficacy of grass-pollen immunotherapy. *N. Engl. J. Med.* 341(7), 468–475 (1999).
 - Provides evidence for persistent clinical efficacy after discontinued pollen SIT.
- 60 Ortolani C, Pastorello E, Moss RB *et al.* Grass pollen immunotherapy: a single year double-blind, placebo-controlled study in patients with grass pollen-induced asthma and rhinitis. *J. Allergy Clin. Immunol.* 73(2), 283–290 (1984).
- 61 Adkinson NF Jr, Eggleston PA, Eney D *et al.* A controlled trial of immunotherapy for asthma in allergic children. *N. Engl. J. Med.* 336(5), 324–331 (1997).
- 62 Moller C, Dreborg S, Ferdousi HA *et al.* Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). *J. Allergy Clin. Immunol.* 109(2), 251–256 (2002).
- 63 Pajno GB, Barberio G, De Luca F, Morabito L, Parmiani S. Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. *Clin. Exp. Allergy* 31(9), 1392–1397 (2001).
- 64 Des Roches A, Paradis L, Menardo JL, Bouges S, Daures JP, Bousquet J. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. VI. Specific immunotherapy prevents the onset of new sensitizations in children. *J. Allergy Clin. Immunol.* 99(4), 450–453 (1997).
- 65 Durham SR, Ying S, Varney VA *et al.* Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4⁺ T-lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon-gamma. *J. Allergy Clin. Immunol.* 97(6), 1356–1365 (1996).
- 66 Furin MJ, Norman PS, Creticos PS, Proud D, Kagey Sobotka A, Lichtenstein LM, Naclerio RM. Immunotherapy decreases antigen-induced eosinophil cell migration into the nasal cavity. *J. Allergy Clin. Immunol.* 88(1), 27–32 (1991).
- 67 Rak S, Bjornson A, Hakanson L, Sorenson S, Venge P. The effect of immunotherapy on eosinophil accumulation and production of eosinophil chemotactic activity in the lung of subjects with asthma during natural pollen exposure. *J. Allergy*

- Clin. Immunol.* 88(6), 878–888 (1991).
- 68 Hakansson L, Heinrich C, Rak S, Venge P. Priming of eosinophil adhesion in patients with birch pollen allergy during pollen season: effect of immunotherapy. *J. Allergy Clin. Immunol.* 99(4), 551–562 (1997).
- 69 Rak S, Lowhagen O, Venge P. The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen-allergic patients. *J. Allergy Clin. Immunol.* 82(3 Pt 1), 470–480 (1988).
- 70 Levy DA, Lichtenstein LM, Goldstein EO, Ishizaka K. Immunologic and cellular changes accompanying the therapy of pollen allergy. *J. Clin. Invest.* 50(2), 360–369 (1971).
- 71 Brunet C, Bedard PM, Lavoie A, Jobin M, Hebert J. Allergic rhinitis to ragweed pollen. I. Reassessment of the effects of immunotherapy on cellular and humoral responses. *J. Allergy Clin. Immunol.* 89(1 Pt 1), 76–86 (1992).
- 72 Pierkes M, Bellinghausen I, Hultsch T, Metz G, Knop J, Saloga J. Decreased release of histamine and sulfidoleukotrienes by human peripheral blood leukocytes after wasp venom immunotherapy is partially due to induction of IL-10 and IFN- γ production of T-cells. *J. Allergy Clin. Immunol.* 103(2 Part 1), 326–332 (1999).
- 73 Otsuka H, Mezawa A, Ohnishi M, Okubo K, Seki H, Okuda M. Changes in nasal metachromatic cells during allergen immunotherapy. *Clin. Exp. Allergy* 21(1), 115–119 (1991).
- 74 Durham SR, Varney VA, Gaga M *et al.* Grass pollen immunotherapy decreases the number of mast cells in the skin. *Clin. Exp. Allergy* 29(11), 1490–1496 (1999).
- 75 Klimek L, Wolf H, Mewes T, Dormann D *et al.* The effect of short-term immunotherapy with molecular standardized grass and rye allergens on eosinophil cationic protein and tryptase in nasal secretions. *J. Allergy Clin. Immunol.* 103(1 Pt 1), 47–53 (1999).
- 76 Moller C, Dreborg S, Einarsson R. Immunotherapy to deciduous tree pollens: specific IgE and IgG antibody patterns. *Clin. Allergy* 17(6), 551–562 (1987).
- 77 Moverare R, Sorva R, Metso T, Vesterinen E, Elfman L, Haahtela T. Pollen-specific rush immunotherapy: clinical efficacy and effects on antibody concentrations. *Ann. Allergy Asthma Immunol.* 86(3), 337–342 (2001).
- 78 Moverare R, Rak S, Elfman L. Allergen-specific increase in interleukin (IL)-4 and IL-5 secretion from peripheral blood mononuclear cells during birch-pollen immunotherapy. *Allergy* 53(3), 275–281 (1998).
- 79 Benjaponpitak S, Oro A, Maguire P, Marinkovich V, DeKruyff RH, Umetsu DT. The kinetics of change in cytokine production by CD4 T-cells during conventional allergen immunotherapy. *J. Allergy Clin. Immunol.* 103(3 Pt 1), 468–475 (1999).
- 80 Secrist H, DeKruyff RH, Umetsu DT. Interleukin-4 production by CD4+ T-cells from allergic individuals is modulated by antigen concentration and antigen-presenting cell type. *J. Exp. Med.* 181(3), 1081–1089 (1995).
- 81 Constant S, Pfeiffer C, Woodard A, Pasqualini T, Bottomly K. Extent of T-cell receptor ligation can determine the functional differentiation of naive CD4+ T-cells. *J. Exp. Med.* 182(5), 1591–1596 (1995).
- 82 Malling HJ, Skov PS, Permin H, Norn S, Weeke B. Basophil histamine release and humoral changes during immunotherapy. Dissociation between basophil-bound specific IgE, serum value and cell sensitivity. *Allergy* 37(3), 187–190 (1982).
- 83 Hakansson L, Heinrich C, Rak S, Venge P. Activation of B-lymphocytes during pollen season. Effect of immunotherapy. *Clin. Exp. Allergy* 28(7), 791–798 (1998).
- 84 Lundgren M, Persson U, Larsson P *et al.* Interleukin-4 induces synthesis of IgE and IgG4 in human B-cells. *Eur. J. Immunol.* 19(7), 1311–1315 (1989).
- 85 Jeannin P, Lecoanet S, Delneste Y, Gauchat JF, Bonnefoy JY. IgE versus IgG4 production can be differentially regulated by IL-10. *J. Immunol.* 160(7), 3555–3561 (1998).
- 86 Vrtala S, Ball T, Spitzauer S, Pandjaitan B *et al.* Immunization with purified natural and recombinant allergens induces mouse IgG1 antibodies that recognize similar epitopes as human IgE and inhibit the human IgE-allergen interaction and allergen-induced basophil degranulation. *J. Immunol.* 160(12), 6137–6144 (1998).
- 87 van Neerven RJ, Wikborg T, Lund G *et al.* Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4(+) T-cells by inhibiting serum-IgE-facilitated allergen presentation. *J. Immunol.* 163(5), 2944–2952 (1999).
- 88 Baskar S, Hamilton RG, Norman PS, Ansari AA. Grass immunotherapy induces inhibition of allergen-specific human peripheral blood mononuclear cell proliferation. *Int. Arch. Allergy Immunol.* 112(2), 184–190 (1997).
- 89 Moverare R, Elfman L, Bjornsson E, Stalenheim G. Cytokine production by peripheral blood mononuclear cells following birch-pollen immunotherapy. *Immunol. Lett.* 73, 51–56 (2000).
- 90 Ebner C, Siemann U, Bohle *et al.* Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from Th2 to Th1 in T-cell clones specific for Phl p 1, a major grass pollen allergen. *Clin. Exp. Allergy* 27(9), 1007–1015 (1997).
- Indicates that Th cell tolerance and immune deviation from Th2–Th1-type responses are significant mechanisms of pollen SIT.
- 91 Evans R, Pence H, Kaplan H, Rocklin RE. The effect of immunotherapy on humoral and cellular responses in ragweed hayfever. *J. Clin. Invest.* 57(5), 1378–1385 (1976).
- 92 Lack G, Nelson HS, Amran D *et al.* Rush immunotherapy results in allergen-specific alterations in lymphocyte function and interferon- γ production in CD4+ T-cells. *J. Allergy Clin. Immunol.* 99(4), 530–538 (1997).
- 93 Till S, Walker S, Dickason R *et al.* IL-5 production by allergen-stimulated T-cells following grass pollen immunotherapy for seasonal allergic rhinitis. *Clin. Exp. Immunol.* 110(1), 114–121 (1997).
- 94 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* 29, 1326–1335 (1999).
- 95 Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin-10 in specific immunotherapy. *J. Clin. Invest.* 102(1), 98–106 (1998).
- 96 Platts-Mills T, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitisation, asthma and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet* 357(9258), 752–756 (2001).
- 97 Leonard C, Tormey V, Burke C, Poulter LW. Allergen-induced cytokine production in atopic disease and its relationship to disease severity. *Am. J. Respir. Cell Mol. Biol.* 17(3), 368–375 (1997).
- 98 Wurtzen PA, Wissenbach M, Ipsen H, Bufe A, Arnved J, van Neerven RJ. Highly

- heterogeneous Phl p 5-specific T-cells from patients with allergic rhinitis differentially recognize recombinant Phl p 5 isoallergens. *J. Allergy Clin. Immunol.* 104(1), 115–122 (1999).
- 99 van Neerven RJ, van de Pol MM, van Milligen FJ *et al.* Characterization of cat dander-specific T-lymphocytes from atopic patients. *J. Immunol.* 152(8), 4203–4210 (1994).
- 100 Secrist H, Chelen CJ, Wen Y, Marshall JD, Umetsu DT. Allergen immunotherapy decreases interleukin-4 production in CD4+ T-cells from allergic individuals. *J. Exp. Med.* 178(6), 2123–2130 (1993).
- 101 Wachholz PA, Nouri-Aria KT, Wilson DR *et al.* Grass pollen immunotherapy for hayfever is associated with increases in local nasal but not peripheral Th1:Th2 cytokine ratios. *Immunology* 105(1), 56–62 (2002).
- Indicates an immune deviation from a Th2–Th1-type response in nasal mucosa but not in peripheral T-cells after pollen SIT.
- 102 Varney VA, Hamid QA, Gaga M *et al.* Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J. Clin. Invest.* 92, 644–651 (1993).
- 103 Hamid QA, Schotman E, Jacobson MR, Walker SM, Durham SR. Increases in IL-12 messenger RNA+ cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. *J. Allergy Clin. Immunol.* 99(2), 254–260 (1997).
- Demonstrates an increased IL-12 mRNA expression by APCs after pollen SIT which might be an important mechanism of SIT.
- 104 Cottrez F, Hurst SD, Coffman RL, Groux H. T regulatory cells 1 inhibit a Th2-specific response *in vivo*. *J. Immunol.* 165(9), 4848–4853 (2000).
- 105 Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft D, Gronlund H. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin. Exp. Allergy* 29(7), 896–904 (1999).
- Explains the concept of component-resolved diagnostics and immunotherapy.
- 106 Ahlstedt S. Understanding the usefulness of specific IgE blood tests in allergy. *Clin. Exp. Allergy* 32(1), 11–16 (2002).
- 107 Moverare R, Elfman L, Vesterinen E, Metso T, Haahtela T. Development of new IgE specificities to allergenic components in birch pollen extract during specific immunotherapy studied with immunoblotting and Pharmacia CAP System. *Allergy* 57(5), 423–430 (2002).
- 108 Richter M, Sehon AH, Gordon J, Gregoire C, Rose B. Demonstration of reagent of a new specificity in sera of treated ragweed-sensitive persons. *J. Allergy* 29, 287–292 (1958).
- 109 Birkner T, Rumpold H, Jarolim E *et al.* Evaluation of immunotherapy-induced changes in specific IgE, IgG and IgG subclasses in birch pollen allergic patients by means of immunoblotting. Correlation with clinical response. *Allergy* 45(6), 418–426 (1990).
- 110 van Ree R, Antonicevic L, Akkerdaas JH, Garritani MS, Aalberse RC, Bonifazi F. Possible induction of food allergy during mite immunotherapy. *Allergy* 51(2), 108–113 (1996).
- 111 Pajno GB, La Grutta S, Barberio G, Canonica GW, Passalacqua G. Harmful effect of immunotherapy in children with combined snail and mite allergy. *J. Allergy Clin. Immunol.* 109(4), 627–629 (2002).
- 112 Norman PS, Winkenwerder WL, Lichtenstein LM. Immunotherapy of hay fever with ragweed antigen E: comparisons with whole pollen extract and placebos. *J. Allergy* 42(2), 93–108 (1968).
- 113 van Neerven RJ, Arned J, Ipsen H. *Phleum pratense*-specific T-cells of allergic rhinitis patients display a broader recognition pattern than *phleum pratense*-specific serum immunoglobulin E. *Clin. Exp. Allergy* 30(2), 242–254 (2000).
- 114 Valenta R, Sperr WR, Ferreira F *et al.* Induction of specific histamine release from basophils with purified natural and recombinant birch pollen allergens. *J. Allergy Clin. Immunol.* 91(1 Pt 1), 88–97 (1993).
- 115 Ferreira F, Hirtenlehner K, Jilek A *et al.* Dissection of immunoglobulin E and T-lymphocyte reactivity of isoforms of the major birch pollen allergen Bet v 1: potential use of hypoallergenic isoforms for immunotherapy. *J. Exp. Med.* 183(2), 599–609 (1996).
- 116 Sparholt SH, Larsen JN, Ipsen H, Schou C, van Neerven RJ. Crossreactivity and T-cell epitope specificity of Bet v 1-specific T-cells suggest the involvement of multiple isoallergens in sensitization to birch pollen. *Clin. Exp. Allergy* 27(8), 932–941 (1997).
- 117 Swoboda I, De Weerd N, Bhalla PL *et al.* Mutants of the major ryegrass pollen allergen, Lol p 5, with reduced IgE-binding capacity: candidates for grass pollen-specific immunotherapy. *Eur. J. Immunol.* 32(1), 270–280 (2002).
- 118 van Hage-Hamsten M, Johansson E, Roquet A *et al.* Nasal challenges with recombinant derivatives of the major birch pollen allergen Bet v 1 induce fewer symptoms and lower mediator release than rBet v 1 wild type in patients with allergic rhinitis. *Clin. Exp. Allergy* 32(10), 1448–1453 (2002).
- 119 Linhart B, Jahn-Schmid B, Verdino P *et al.* Combination vaccines for the treatment of grass pollen allergy consisting of genetically engineered hybrid molecules with increased immunogenicity. *FASEB J.* 16(10), 1301–1303 (2002).
- 120 Haselden BM, Kay AB, Larche M. Peptide-mediated immune responses in specific immunotherapy. *Int. Arch. Allergy Immunol.* 122(4), 229–237 (2000).
- 121 Basomba A, Tabar AI, de Rojas DH *et al.* Allergen vaccination with a liposome-encapsulated extract of *Dermatophagoides pteronyssinus*: a randomized, double-blind, placebo-controlled trial in asthmatic patients. *J. Allergy Clin. Immunol.* 109(6), 943–948 (2002).
- 122 Wheeler AW, Marshall JS, Ulrich JT. A Th1-inducing adjuvant, MPL, enhances antibody profiles in experimental animals suggesting it has the potential to improve the efficacy of allergy vaccines. *Int. Arch. Allergy Immunol.* 126(2), 135–139 (2001).
- 123 Santeliz JV, Van Nest G, Traquina P, Larsen E, Wills-Karp M. Amb a 1-linked CpG oligodeoxynucleotides reverse established airway hyperresponsiveness in a murine model of asthma. *J. Allergy Clin. Immunol.* 109(3), 455–462 (2002).
- 124 Gronlund H, Vitala S, Wiedermann U *et al.* Carbohydrate-based particles: a new adjuvant for allergen-specific immunotherapy. *Immunology* 107(4), 523–529 (2002).
- 125 Hamelmann E, Rolinck-Werninghaus C, Wahn U. From IgE to anti-IgE: where do we stand? *Allergy* 57(11), 983–994 (2002).

Affiliation

- Robert Moverare, PhD, Pharmacia Diagnostics AB and Department of Medical Sciences, Respiratory Medicine and Allergology, Uppsala University, Uppsala, Sweden.
robert.moverare@pharmacia.com