

OPINIONS IN ALLERGY

Developments in allergen-specific immunotherapy: from allergen extracts to allergy vaccines bypassing allergen-specific immunoglobulin E and T cell reactivity

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Summary

Allergen-specific immunotherapy (SIT) is the only specific and disease-modifying approach for the treatment of allergy but several disadvantages have limited its broad applicability. We argue that the majority of the possible disadvantages of SIT such as unwanted effects, poor efficacy and specificity as well as inconvenient application are related to the poor quality of natural allergen extracts, which are the active ingredients of all currently available allergy vaccines. Because of the progress made in the field of molecular allergen characterization, new allergy vaccines based on recombinant allergens, recombinant hypoallergenic allergen derivatives and allergen-derived T cell peptides have entered clinical testing and hold promise to reduce the side-effects and to increase the specificity as well as the efficacy of SIT. Here, we present a refined immunotherapy concept, which is based on the use of peptides derived from allergen surfaces that exhibit reduced, allergen-specific IgE as well as T cell reactivity. These peptides when fused to non-allergenic carriers give rise to allergen-specific protective IgG responses with T cell help from a non-allergenic carrier molecule. We summarize the experimental data demonstrating that such peptide vaccines can bypass allergen-specific IgE as well as T cell activation and may be administered at high doses without IgE- and T cell-mediated side-effects. Should these peptide vaccines prove efficacious and safe in clinical trials, it may become possible to develop convenient, safe and broadly applicable forms of SIT as true alternatives to symptomatic, drug-based allergy treatment.

Background

Type I allergy, an IgE-mediated hypersensitivity disease, affects almost 30% of the population in industrialized countries [1, 2]. During allergic sensitization, which seems to generally occur shortly after birth, allergen exposure triggers B cells to produce specific IgE antibodies via Th2-mediated class-switching in genetically predisposed atopic individuals [3, 4]. Allergen-specific IgE antibodies bind to Fc epsilon receptor 1 (FcεRI) on basophils or mast cells and after cross-linking by consecutive allergen contact induce a signalling cascade that leads to the release of inflammatory mediators, cytokines and proteases. This process is responsible for the immediate-type symptoms of allergic diseases and also paves the way for late-phase inflammatory responses caused by basophils, eosinophils and T cells [2, 5–7]. Several studies

performed with synthetic allergen-derived peptides and recombinant hypoallergenic allergen derivatives containing allergen-derived T cell epitopes without IgE reactivity have demonstrated that there are also IgE-independent mechanisms for the activation of T cells which can contribute to late-phase and chronic allergic inflammation [8–10].

In allergic patients, allergen contact can induce a variety of different allergic symptoms, which, as mentioned above, can be caused by IgE-dependent and/or by IgE-independent mechanisms. These manifestations include allergic rhinitis, conjunctivitis, asthma, urticaria, food allergy, atopic dermatitis and systemic life-threatening anaphylaxis.

Currently, the great majority of allergic patients are treated by symptomatic medication using drugs that, for example, prevent the degranulation of mast cells, neutralize certain mast cell mediators, suppress

T cell activation, exhibit general anti-inflammatory activity and/or reverse bronchoconstriction or vasodilatation [11]. However, symptomatic medication fails to target the mechanisms underlying allergic disease, often has only short activity, may exhibit several side-effects and often needs to be administered in combination with other drugs to address the various pathways of allergic inflammation. Yet, the pharmacotherapy of allergy dominates over causative treatment options because symptomatic medication is inexpensive, easy to administer, can also be prescribed by non-specialists and does not require extensive diagnostic skills or special knowledge.

At present, allergen-specific immunotherapy (SIT) is the only allergen-specific, causative and disease-modifying treatment of allergy [12–17]. SIT is based on the repeated administration of the disease-eliciting allergens with the aim of reducing the sensitivity to the administered allergens through various immunological mechanisms. It has been demonstrated that SIT modifies the responses of antigen-presenting cells (APCs), T cells, B cells and effector cells towards allergens. In the course of injection immunotherapy, allergen-specific IgG antibodies (i.e. blocking antibodies) are induced, which compete with IgE for allergen binding and thereby prevent an allergen-induced activation of effector cells as well as an IgE-mediated presentation of allergens to T cells (reviewed in Valenta *et al.* [17] and van Neerven *et al.* [18, 19], Wachholz *et al.* [20]). Furthermore, therapy-induced allergen-specific IgG antibodies reduce the boosting of the allergen-specific IgE responses by allergen exposure, and thus may down-regulate allergen-specific IgE production [21–23]. SIT is also associated with the modification of T cell responses, which may be due to the induction of regulatory T cells, increases in the ratio of Th1 to Th2 cytokines and/or the production of cytokines with regulatory activity (IL-10, TGF- β) [24]. The inhibition of IgE-facilitated allergen presentation to T cells by SIT-induced blocking antibodies may also contribute to the down-modulation of both T cell activation and the release of pro-inflammatory cytokines [19, 20]. In summary, the advantages of SIT over symptomatic pharmacotherapy include the high specificity of SIT, the objective and proven reduction of allergen-specific sensitivity in the target organs of allergy (i.e. nose, eyes, lung, gut, skin), the reduction of IgE and effector cell responses, the prevention of the progression of the disease from mild to severe manifestations and the long-term efficacy of the treatment [13, 15].

Factors limiting the broad application of allergen-specific immunotherapy

We believe that the majority of disadvantages that limit the broad applicability of SIT are related to the poor

quality of allergen extracts [25–32]. SIT seems to be less effective for certain clinical manifestations of allergy such as asthma, food allergy and atopic dermatitis and for certain allergen sources such as house dust mites, moulds, cat, dog and food allergens [12]. In this context, it is well known that natural allergen extracts from the latter allergen sources are of particularly low quality.

Until now, allergen extracts represent the active ingredients in all forms of immunotherapy that are currently approved, such as injection immunotherapy without or with adjuvants and mucosal forms of SIT such as sublingual immunotherapy (SLIT). Natural allergen extracts are prepared directly from the raw allergen sources and, if not modified, contain allergenic material. The injection or mucosal administration (SLIT) of allergen extracts can therefore induce a variety of side-effects ranging from local side-effects to severe systemic side-effects, which in the worst case may be life-threatening reactions [33–36]. The sublingual forms of treatment show a lower rate of side-effects than injection immunotherapy. However, SLIT requires frequent self-administration and significant side-effects may still occur [37–41].

Because of the allergenic activity of natural allergen extracts, the dose has to be increased gradually up to a clinically effective maintenance dose requiring multiple administrations. Treatment for periods of 2 years or longer was found to be more effective than the treatment for shorter periods (e.g. 1 year) [42, 43]. In the case of sublingual immunotherapy, it is even necessary to administer the allergen extracts on a daily basis for long periods [44].

There are many reasons why SIT with allergen extracts is often not effective. One possibility is that a therapeutically effective maintenance dose cannot be reached because of the side-effects. Another possibility is that important allergens are either not present in the allergen extracts in sufficient amounts or they exhibit poor immunogenicity [21, 31]. Furthermore, it has been reported that administration of natural allergen extracts can induce new IgE specificities against allergens present in the extracts, which were not recognized by the patient before treatment [45–47].

The administration of natural allergen extracts also increases initially allergen-specific IgE responses. Often treatment-induced increases of allergen-specific IgE are moderate and might be overcome by the induction of high levels of allergen-specific IgG [21, 48–53]. Treatment-induced increases of allergen-specific IgE up to the four-fold baseline levels have been observed in a recent SLIT study whereas allergen-specific IgG remained very low [44, 54]. So far, there are almost no studies in which objective provocation testing (e.g. quantitative skin testing, nasal provocation) was used to investigate whether the allergen-specific sensitivity changes in patients undergoing sublingual treatment [55–59].

A further drawback of SIT is that it can only be applied by highly specialized allergologists who are able to adjust the treatment to the patients needs and who can handle the therapy-related side-effects. With the increasing availability of new component-resolved forms of allergy diagnosis, correct diagnosis of the disease-causing allergens becomes easier and should facilitate the accurate prescription of immunotherapy [60–63]. Diagnostic tests based on the major allergens of the most common allergen sources are now available, but treatment is still based on allergen extracts.

Finally, SIT has to compete with symptomatic pharmacotherapy, which does not require a detailed identification of the disease-causing allergens.

Unwanted immunological effects during allergen-specific immunotherapy

Figure 1 gives a brief overview regarding the immunological mechanisms underlying the unwanted effects during immunotherapy. The most severe and best-known unwanted effects during SIT result from the cross-linking of mast cell and basophil-bound IgE by the administered allergen (Fig. 1, left, upper image). If the allergen becomes systemically spread in the body, it can cross-link mast cells and basophils to release large amounts of biologically active mediators, which may cause life-threatening anaphylactic reactions. If the allergen remains bound to an adjuvant locally, mediator release will be limited to the injection site and only local side-effects will be observed. The effects of mast cell and basophil degranulation typically occur immediately (i.e. <30 min after the allergen administration). Mast cell and basophil products (i.e. mediators, cytokines) may also exhibit chemotactic activity and induce the migration of basophils, eosinophils and T cells to the site of allergen exposure and cause activation of these inflammatory cells, which can give rise to late-phase inflammation after several hours [6]. There are at least two more IgE-dependent mechanisms for unwanted side-effects during SIT. First, it is possible that the administered allergens become presented by IgE antibodies via Fcε receptors on APCs (Fig. 1, left, lower image), which will activate allergen-specific T cells to proliferate and release pro-inflammatory cytokines. Second, administration of allergens may induce allergen-specific IgE antibody production either by boosting of already existing B epsilon cells or by inducing *de novo* an allergen-specific IgE response [45–47]. The more the administered allergen resembles the structure of the natural allergen the more it will be able to boost already existing IgE responses and to exhibit sensitizing capacity. Allergens, which have been modified in their structure by chemical treatment or recombinant DNA technology to reduce their IgE reactivity, may give rise to the induction of milder IgE responses [22]. There is also good evidence that the route of admin-

istration has an impact on the induction of IgE responses because it was found that mucosal application (e.g. nasal administration, sublingual administration) induces high IgE boosts [44, 64]. The induction of allergen-specific IgE antibodies during SIT is unwanted because it is known that increased IgE antibody levels up-regulate receptors for IgE on mast cells, basophils and APCs and prolong their survival, which consecutively cause increases of allergen sensitivity in the target organs of allergy [65–68].

It has become increasingly apparent that non-IgE-mediated side-effects can also occur during SIT. In the past, when patients received immunotherapy with allergoids, which represent chemically denatured allergen extracts with reduced IgE reactivity, side-effects and, in particular, late-phase side-effects were still observed despite a reduction of IgE reactivity (Table 1) [69, 70]. The unambiguous demonstration of non-IgE-mediated side-effects was given, observing patients who had received T cell epitope-containing peptides without IgE reactivity. These patients showed late-phase side-effects, which occurred hours after the injection of the peptides (Table 1) [8, 71]. Late-phase side-effects were also observed when patients received immunotherapy with recombinant allergen derivatives of the major birch pollen allergen, Bet v 1, which showed a more than 100-fold reduced IgE reactivity compared with the natural Bet v 1 allergen [22, 72]. In fact, it could be demonstrated in an atopy patch test model that the rBet v 1 derivatives indeed induced IgE-independent but T cell-mediated cutaneous inflammation [10]. All these findings indicate that certain late-phase side-effects during SIT are independent from IgE and result from the activation of allergen-specific T cells by allergen-derived T cell epitopes (Fig. 1, right lower image).

Past and present attempts for the improvement of allergen-specific immunotherapy

Already in the 1930s, the first major improvement of SIT was achieved by the use of adjuvants (e.g. aluminium hydroxide), which keep the allergens at the injection site, increase the immunogenicity of the allergy vaccines and reduce the risk of systemic side-effects [73, 74]. Furthermore, adjuvant-bound allergens induce higher levels of allergen-specific IgG antibodies than aqueous extracts. More recently, novel adjuvants (e.g. monophosphoryl lipid A [21]) have been introduced into the field of SIT, which may induce stronger humoral and cellular immune responses than the traditional aluminium hydroxide and may favour Th1 immune responses [75, 76].

In attempts to reduce IgE-mediated side-effects and thus the potentially life-threatening immediate-type side-effects of SIT, several research groups started in the late 1960s to develop procedures for the modification of allergen extracts. The IgE reactivity of the allergen extracts was reduced by denaturation using aldehydes

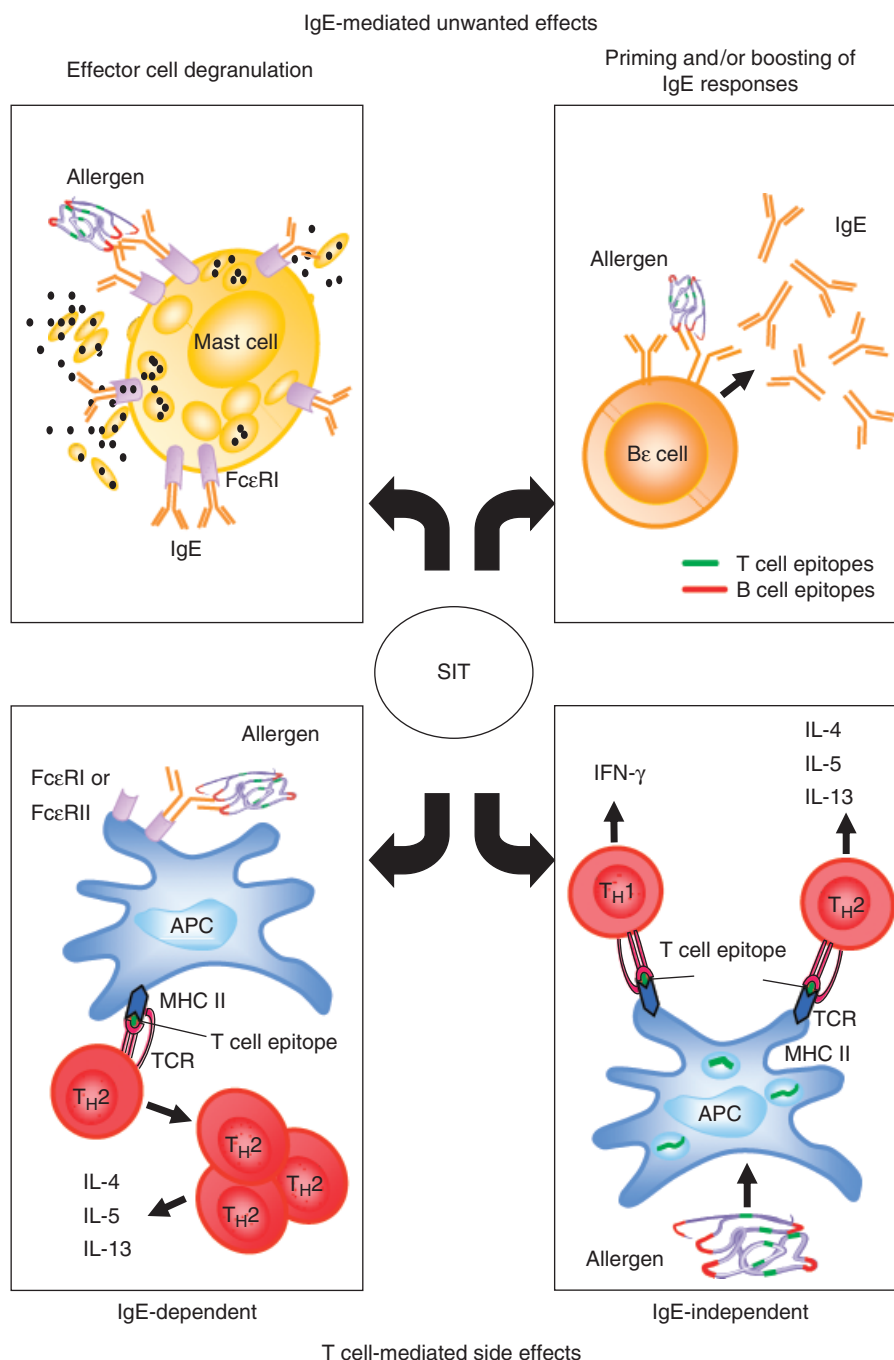


Fig. 1. Mechanisms underlying the side-effects of currently performed immunotherapy (SIT). IgE-mediated unwanted effects (upper panels): allergens can cross-link effector cell-bound IgE leading to the degranulation and release of mediators causing immediate allergic inflammation (left). Administration of allergens can induce a *de novo* IgE response and the boosting of an existing IgE response (right). T cell-mediated side-effects (lower panels): IgE-dependent (left) or IgE-independent (right) presentation of allergens by antigen-presenting cells (APCs) can activate allergen-specific T cell responses.

resulting in allergoids [69, 70, 77, 78], by proteolysis [79–81] or by conjugation of the extracts to chemical groups (e.g. using mPEG) [82, 83]. Allergoids representing denatured allergen extracts with reduced IgE reactivity but largely retained T cell reactivity are the basis of many allergy vaccines used today [84].

For many years, considerable efforts have been put into the development of other routes of administration besides the traditional subcutaneous application, such as the sublingual application. These efforts are driven by the idea of obtaining a more convenient (i.e. non-injective) form of SIT, which can be self-administered and

Table 1. Strategies for improvement of protein- and peptide-based immunotherapy

Strategies	IgE reactivity	T cell reactivity	Induction of protective antibodies	Possible side-effects		References
				IgE-mediated	T cell-mediated	
Approaches retaining IgE and T cell reactivity based on defined molecules						
Recombinant allergens	+	+	+	+	+	[53, 106, 107]
Hybrid molecules	+	+	+	+	+	[108–113]
Approaches reducing IgE and retaining T cell reactivity						
Chemically modified allergens	Reduced	+	+	Reduced	+	[70, 81, 83, 84]
Recombinant allergen derivatives (mutants, fragments, oligomers, mosaics)	Reduced	+	+	Reduced	+	Reviewed in [102, 104–106]
Conjugated allergens (CpG)	Reduced	+	+	Reduced	+	[23, 99–101]
T cell epitope-containing peptides	–	+	–	–	+	[8, 9, 86–95]
Approaches reducing IgE and T cell reactivity						
Peptides derived from surface-exposed regions of allergens	–	–	+	–	–	[116, 117, 120]

should also enable to treat patients reluctant to frequent injections. Numerous immunotherapy trials have been conducted with allergen extracts, which have been administered sublingually in the form of drops or tablets. Even when given at relevant doses, SLIT seems to be of a lower clinical efficacy as traditional injection immunotherapy [55–57]. It requires numerous, often daily applications for prolonged periods and also exhibits side-effects [37–41]. Furthermore, the immunological mechanisms behind SLIT are not understood and further elucidation is limited as long as crude allergen extracts are used, which make a systematic molecular and cellular analysis difficult, if not impossible.

In the last two decades, considerable progress has been made in the field of allergen characterization. The primary, secondary and tertiary structures of the most common allergens have been elucidated and research in the field of molecular allergen characterization has provided us with recombinant allergens, which can substitute the allergen repertoires of the most common allergen sources [85]. With the availability of the first allergen sequences, a new strategy for SIT was developed that was based on experimental results showing that tolerance against a complete allergen can be induced using allergen-derived peptides, which are recognized by allergen-specific T cells [86, 87]. The fundamental idea of using allergen-derived T cell epitopes was that such peptides are too small to be recognized by allergen-specific IgE antibodies, which would eliminate the side-effects due to IgE recognition. On the other hand, it was expected that the administration of T cell epitope-derived peptides would induce immune tolerance against the allergen. In

the beginning of the 1990s, clinical trials evaluating the concept of SIT with allergen-derived peptides targeting T cells for treating cat allergy were initiated [88–91]. Since then, the application of allergen-derived peptides containing T cell epitopes has been tested in several clinical studies and indeed no immediate type symptoms were elicited. However, treatment with peptides of the major cat allergen, Fel d 1, was still associated with adverse events, primarily the late-onset symptoms of rhinitis, asthma and pruritus [88–91]. The incidence of late adverse events was related to both peptide dose and the severity of disease [9, 92, 93]. It could be demonstrated that side-effects occurring during SIT with 'T cell peptides' (i.e. allergen-derived peptides recognized by T cells) are non-IgE-mediated and result from the MHC-dependent activation of allergen-specific T cells [8, 9]. This activation seemed to precede the induction of T cell tolerance and currently doses and application modes are investigated, which should reduce the side-effects in favour of tolerance induction. Clinical trials are also ongoing to further investigate the extent to which therapy with T cell peptides has beneficial effects on IgE-mediated immediate allergic symptoms (reviewed in Larche [24]).

A similar T cell epitope-based approach was investigated for the treatment of bee venom allergy. After immunotherapy with short (11–18 amino acids) and long synthetic peptides (45–60 amino acids) of the major bee venom allergen, Api m 1, patients reported no or only mild adverse reactions [94, 95]. In the study described by Mueller et al. [95] performed with a mixture of three short peptides, subcutaneous allergen challenge was tolerated

without systemic allergic symptoms but the number of patients was very limited.

With the availability of allergen cDNAs, vaccination with allergen-encoding DNA has been proposed as another innovative strategy. Injection of plasmids that carry an allergen gene should result in the expression of the corresponding protein in the host cells and the induction of an allergen-specific Th1 cell response, which should prevent from allergen sensitization and protect against established allergy [96, 97]. However, the approach of DNA vaccination was not further pursued in clinical trials after the finding that this treatment may lead to the uncontrolled release of allergens in treated animals [98].

With the aim of reducing the allergenicity and enhancing the immunogenicity of allergen vaccines, the approach of conjugating allergens to immunostimulatory sequences, either DNA oligonucleotides containing CpG motifs [99] or genes encoding bacterial cell surface proteins, has also been proposed for SIT [100]. Fusion proteins obtained by this approach showed reduced allergenic activity and an increase in allergen-specific Th1 cell responses [99, 100]. Immunotherapy with a conjugate of the major ragweed pollen allergen, Amb a 1, and an immunostimulatory oligonucleotide resulted in a decrease in chest symptoms and a reduction in nasal symptoms [101].

Another approach that has been successfully applied for the reduction of IgE reactivity of numerous important allergens is based on recombinant technologies and results in the so called 'recombinant hypoallergenic allergen derivatives'. With the aim of reducing the IgE-binding capacity, recombinant allergens have been modified by the introduction of point mutations, by deletion of parts of their sequences, by fragmentation, oligomerization, by chemical modification of the recombinant protein and by fusion of allergen variants (reviewed in Valenta [16], Linhart and Valenta [102]). Such hypoallergenic derivatives are characterized by reduced IgE reactivity and allergenic activity, but they retain immunogenicity and T cell reactivity of the wild-type allergens. In fact, the first immunotherapy study, which has been performed in patients with recombinant allergens, was based on hypoallergenic derivatives of the major birch pollen allergen Bet v 1 (two recombinant fragments and a recombinant trimer) [22, 72]. Actively but not placebo-treated patients developed robust IgG antibody responses against natural Bet v 1 and Bet v 1-related pollen and food allergens, which inhibited allergen-induced basophil degranulation and were associated with an increased ability of the patients to tolerate the allergen in controlled allergen provocation tests [22, 103]. In a further study conducted with a folding variant of rBet v 1, reductions in symptom medication scores were observed [104, 105].

Additional successful immunotherapy studies using recombinant allergens, allergen derivatives, have been

performed recently (reviewed in Linhart and Valenta [102], Valenta and Niederberger [106]).

It is assumed that vaccines containing defined amounts of the clinically relevant allergens in the form of recombinant allergens should be at least equally effective as an allergen extract containing the same components. Several studies have supported this concept. Recently, a clinical study has compared the recombinant major allergen of birch, rBet v 1, with a birch pollen extract containing the same amount of purified natural Bet v 1 [53]. The rBet v 1-based vaccine showed even additional advantages over the birch pollen extract because vaccination with rBet v 1 induced higher levels of Bet v 1-specific IgG, a greater reduction of skin sensitivity and no *de novo* sensitizations to other allergens were found when compared with the group of patients having received birch pollen extract.

Grass pollen-allergic patients have also been successfully treated with a mix of recombinant grass pollen allergens [107]. For allergen sources such as grass pollen, which contain several clinically relevant allergens, hybrid molecules consisting of the individual allergens have been developed [108]. These hybrid molecules offer the advantage that they contain the clinically relevant allergens of the allergen source in the form of one molecule and also offer as an additional advantage of an increase of the immunogenicity (i.e. high induction of protective IgG antibodies) of low immunogenic allergens through their fusion with other allergens [108–112]. Using this technology, the IgG responses against weakly immunogenic grass pollen allergens such as Phl p 2 and Phl p 6 could be strongly increased when the animals were immunized. A hybrid molecule containing the major grass pollen allergens was found to be equivalent to allergen extracts when used for skin prick testing of allergic patients [113].

The use of recombinant allergen mixes or hybrid molecules consisting of several recombinant allergens (Table 1) will address problems of poor efficacy and poor specificity of the current traditional allergen extract-based vaccines. Vaccines based on wild-type recombinant allergens or hybrids contain the allergen-specific IgE epitopes and T cell epitopes and hence can be used for tolerance induction strategies as well as for active vaccination with the goal to induce protective allergen-specific IgG antibodies. However, such vaccines will in principle still be able to induce IgE- as well as T cell-mediated side-effects, such as the degranulation of mast cells, the boosting of allergen-specific IgE responses and the induction of T cell activation (Fig. 1). These potential problems will need to be carefully investigated in clinical studies for the individual vaccines and dose-finding studies will be needed to determine the most convenient and safe doses and application regimens for such recombinant allergen-based vaccines.

Utilization of the hapten-carrier concept for the design of allergy vaccines with reduced or no immunoglobulin E- and T cell-reactivity: towards side-effect-free allergen-specific immunotherapy

Based on the work of the Nobel laureate B. Benacerraf [114, 115], a robust antibody response can be obtained against a haptenic structure lacking T cell epitopes, if this structure is covalently bound to an unrelated carrier molecule, which contains T cell epitopes. In a series of elegant experiments, Benacerraf and co-workers have shown that IgG antibody responses can be induced against haptenic structures when the hapten was conjugated to a carrier molecule, which provided the T cell epitopes supporting the antibody production against the hapten.

The hapten-carrier principle can be used for the design of allergy vaccines lacking IgE and allergen-specific T cell reactivity. For this purpose, peptides (between 20 and 40 amino acids) derived from the surface and thus from or close to the IgE-binding sites of the allergen need to be selected, which *per se* show no or only minimal IgE reactivity. The second selection criterion for these peptides is that they should contain no or minimal allergen-derived T cell epitopes (Table 1; Fig. 2). Requirements for the selection of suitable allergen-derived peptides are the knowledge about the primary sequence of the allergen, information regarding the IgE-binding sites on the allergen and/or data regarding the three-dimensional allergen structure and finally information regarding the T cell epitopes recognized by allergic patients. Because of the progress made in the field of allergen characterization, the data required for the design of peptide vaccines are available for the most common and relevant allergens today. The selection is based on the information obtained from IgE-epitope mapping data, the three-dimensional allergen structure and/or based on computer programs allowing the predicting of surface-exposed allergen areas.

We have exemplified the construction and characterization of peptide vaccines for two major allergens, i.e. for the major timothy grass pollen allergen Phl p 1 and for the major birch pollen allergen, Bet v 1 [116, 117]. It seems to be sufficient to define one to three allergen-derived peptides per allergen, which represent <40% of the complete allergen sequence. Meanwhile, we have applied the principle to the major grass pollen allergens, Phl p 5, Phl p 2, Phl p 6, the major cat allergen, Fel d 1, the major olive pollen allergen, Ole e 1 and for the major mould allergen, Alt a 1.

Figure 2 gives an overview of the design and an evaluation of the peptide vaccines. For evaluation whether the peptides are suitable vaccine candidates, the allergen-derived peptides are tested for IgE reactivity, allergenic activity and T cell reactivity, using standard assays (i.e. IgE reactivity by serology, allergenic activity using basophil activation assays or skin testing and T cell

reactivity by proliferation assays, cytokine measurements in supernatants from peripheral blood mononuclear cells and atopy patch testing). Peptides with low or no allergenic activity and with low or no activation of allergen-specific T cell responses are considered to be suitable vaccine candidates.

As part of the peptide vaccine evaluation, peptides are coupled to well-established carrier molecules, which have already been used for vaccination in humans, such as KLH (keyhole limpet haemocyanin), a protein from the giant sea mollusc *Megathura crenulata* [118, 119]. The peptide conjugates are then used to immunize animals (e.g. mice, rabbits) in order to investigate, if they induce IgG responses against the corresponding allergen and if the IgG antibodies can block the binding of allergic patients IgE to the allergen as well as inhibit allergen-induced basophil activation. Furthermore, the extent to which the peptide conjugate induces IgE responses against the complete allergen needs to be established in order to estimate its allergenicity and compare it with the allergenicity of the wild-type allergen with the goal to select the conjugates with the lowest possible allergenicity [116, 117].

Upon immunization, carrier-conjugated peptides may bind to membrane immunoglobulin molecules on B lymphocytes and become internalized and processed in endosomal vesicles. B cells then present carrier-derived peptides in association with MHC II molecules to carrier-specific T cells. This results in a carrier-specific T cell response and in the induction of anti-peptide IgG antibodies (Fig. 2). It has been shown that peptide vaccination can induce for certain allergens even higher titres of allergen-specific IgG responses, which block IgE-binding better than those IgG antibodies induced by vaccination with the natural allergen [116]. However, generally the titres of anti-allergen IgG may be lower than those that can be obtained with the complete natural allergen or with hypoallergenic derivatives. This small disadvantage should be overcome by the possibility of injecting higher doses and the advantage of having no risk of side-effects [116, 117].

Our results indicate that peptides with the characteristics summarized above can be defined for the most common allergens. We are currently focusing on the selection of suitable carrier molecules, which can be used for vaccination in humans and offer advantages over KLH. As vaccination against viral pathogens is an important issue, we are currently testing several viral proteins for their suitability as carrier molecules for the design of combination vaccines against allergy and viral infections. In order to avoid the laborious and difficult-to-standardize process of chemical coupling of synthetic peptides to carrier molecules during the production of the vaccine, we have designed expression plasmids that allow fusion of cDNAs coding for one or more allergen-derived peptides to the cDNA coding for the carrier molecule in order that

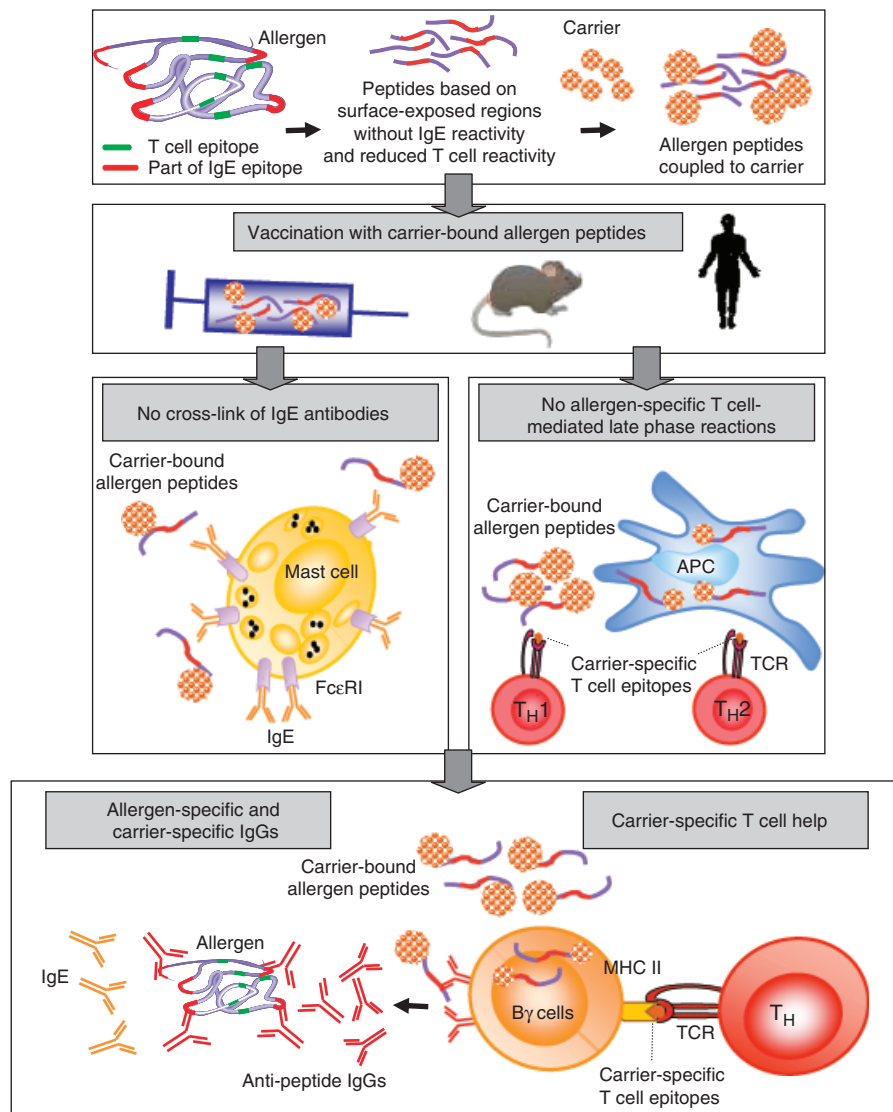


Fig. 2. Immunological effects of immunotherapy performed with carrier-coupled, allergen-derived peptides. Peptides from the allergen surface derived from or close to IgE binding sites (red) are selected in order to bypass allergen-specific T cell epitopes (green). These peptides are then covalently bound to allergen-unrelated carrier molecules by chemical coupling or expression as recombinant fusion proteins. Such peptide vaccines will not cross-link effector cell-bound IgE antibodies and therefore should not induce immediate allergic inflammation. Allergen-derived peptides are selected to have no or minimal T cell reactivity and hence should not induce T cell-dependent side-effects. The peptide vaccine induces peptide-specific and hence allergen-specific IgG antibodies receiving T cell help from carrier-derived T cell epitopes. Carrier-bound peptides are tested in experimental animal models to select those peptide vaccines that induce high-titre, high-affinity allergen-specific IgG responses, which strongly block allergic patients' IgE binding to the allergen.

recombinant fusion proteins can be easily expressed and purified [120]. The fusion of more than one allergen-derived peptide to the carrier molecule may increase the immunogenicity of the vaccine.

First results indicate that viral capsid proteins are especially suited for this purpose because they also exhibit an immuno-modulatory activity towards a Th1 response and have been shown to increase the immunogenicity of the conjugated peptide [120]. In a similar approach, vaccination of healthy adults with a peptide antigen covalently coupled to highly repetitive virus-like bacter-

iophage particles induced high IgG antibody titres against the allergen [121].

Outlook, visions and possible advantages of the peptide vaccines

The possible advantages of peptide allergy vaccines are summarized in Textbox 1. The peptide vaccines can be designed for each allergen by selecting appropriate peptides and fusing them to a non-allergenic but immunogenic carrier molecule. The peptide vaccines are therefore

Textbox 1.

Possible advantages of allergy vaccines based on non-allergenic peptides derived from allergen surfaces

- High specificity – no induction of new sensitisations
- Should not induce any IgE- or T cell-mediated side effects
- Low ability to boost allergen-specific IgE responses
- Convenient and safe application of a few high doses
- Technology applicable for all allergens

highly specific for a given allergen. Sensitization to another unrelated allergen is therefore impossible. As the peptide vaccines contain little or no allergen-derived T cell epitopes and lack IgE reactivity, it is expected that these vaccines do not induce any side-effects, i.e. neither IgE- nor T cell-mediated inflammation. The peptide vaccines contain unfolded allergen-derived peptides, which represent only a relatively small percentage of the allergen-derived sequences and lack most T cell epitopes. It is, therefore, expected that these vaccines will not boost the allergen-specific IgE responses, an assumption, which is supported by data from animal immunization experiments [117]. Immunotherapy trials are now needed to deliver the proof of concept in patients, to demonstrate whether the by-passing of allergen-derived T cell epitopes is not a disadvantage, to study clinical efficacy and to confirm the lack of any side-effects. It is envisioned that the immunization protocol will comprise three to four high dose administrations per year, which can be given for long periods without any side-effects.

According to our results, it appears that the peptide technology can be applied for all common allergens for which the sequences are available. It should thus be possible to design peptide vaccines for the most important and common allergen sources such as mites, pollen, animals, moulds and even food allergens so that vaccination can be performed for respiratory and food allergens as well as for seasonal and perennial allergens. The lack of any side-effects should also allow that the procedure of vaccination can be performed safely on the basis of a solid diagnosis and prescription. We thus hope that the peptide vaccines will heavily expand SIT and eventually also break the ground towards prophylactic allergy vaccination.

References

- 1 Flostrup H, Swartz J. The Parsifal Study G. *et al.* Allergic disease and sensitization in Steiner school children. *J Allergy Clin Immunol* 2006; 117:59–66.
- 2 Kay A. *Allergy and allergic diseases*. Oxford, UK: Blackwell Science, 2008.
- 3 Kulig M, Bergmann R, Klettke U, Wahn V, Tacke U, Wahn U. Natural course of sensitization to food and inhalant allergens during the first 6 years of life. *J Allergy Clin Immunol* 1999; 103:1173–9.
- 4 Niederberger V, Niggemann B, Kraft D, Spitzauer S, Valenta R. Evolution of IgM, IgE and IgG(1–4) antibody responses in early childhood monitored with recombinant allergen components: implications for class switch mechanisms. *Eur J Immunol* 2002; 32:576–84.
- 5 Bischoff SC. Role of mast cells in allergic and non-allergic immune responses: comparison of human and murine data. *Nat Rev Immunol* 2007; 7:93–104.
- 6 Macfarlane AJ, Kon OM, Smith SJ *et al.* Basophils, eosinophils, and mast cells in atopic and nonatopic asthma and in late-phase allergic reactions in the lung and skin. *J Allergy Clin Immunol* 2000; 105:99–107.
- 7 Bradding P, Walls AF, Holgate ST. The role of the mast cell in the pathophysiology of asthma. *J Allergy Clin Immunol* 2006; 117:1277–84.
- 8 Haselden BM, Kay AB, Larche M. Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reactions. *J Exp Med* 1999; 189:1885–94.
- 9 Oldfield WL, Kay AB, Larche M. Allergen-derived T cell peptide-induced late asthmatic reactions precede the induction of antigen-specific hyporesponsiveness in atopic allergic asthmatic subjects. *J Immunol* 2001; 167:1734–9.
- 10 Campana R, Mothes N, Rauter I *et al.* Non-IgE-mediated chronic allergic skin inflammation revealed with rBet v 1 fragments. *J Allergy Clin Immunol* 2008; 121:528–30.e1.
- 11 Hansel TT, Barnes PJ. *New drugs for asthma, allergy and COPD*. Basel, CH: Karger, 2001.
- 12 Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 1998; 102:558–62.
- 13 Durham SR, Walker SM, Varga EM *et al.* Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 1999; 341:468–75.
- 14 Frew AJ, Powell RJ, Corrigan CJ, Durham SR. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2006; 117:319–25.
- 15 Larche M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. *Nat Rev Immunol* 2006; 6:761–71.
- 16 Valenta R. The future of antigen-specific immunotherapy of allergy. *Nat Rev Immunol* 2002; 2:446–53.
- 17 Valenta R, Ball T, Focke M *et al.* Immunotherapy of allergic disease. *Adv Immunol* 2004; 82:105–53.
- 18 van Neerven RJ, Knol EF, Eijraes A, Würtzen PA. IgE-mediated allergen presentation and blocking antibodies: regulation of T-cell activation in allergy. *Int Arch Allergy Immunol* 2006; 141:119–29.
- 19 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* 1999; 163:2944–52.
- 20 Wachholz PA, Soni NK, Till SJ, Durham SR. Inhibition of allergen-IgE binding to B cells by IgG antibodies after grass pollen immunotherapy. *J Allergy Clin Immunol* 2003; 112:915–22.

- 21 Mothes N, Heinzkill M, Drachenberg KJ *et al*. Allergen-specific immunotherapy with a monophosphoryl lipid A-adjuvanted vaccine: reduced seasonally boosted immunoglobulin E production and inhibition of basophil histamine release by therapy-induced blocking antibodies. *Clin Exp Allergy* 2003; 33:1198–208.
- 22 Niederberger V, Horak F, Vrtala S *et al*. Vaccination with genetically engineered allergens prevents progression of allergic disease. *Proc Natl Acad Sci USA* 2004; 101 (Suppl. 2):14677–82.
- 23 Creticos PS, Schroeder JT, Hamilton RG *et al*. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. *N Engl J Med* 2006; 355:1445–55.
- 24 Larche M. Immunoregulation by targeting T cells in the treatment of allergy and asthma. *Curr Opin Immunol* 2006; 18:745–50.
- 25 Schwartz HJ. Problems with skin testing in patients with allergic bronchopulmonary aspergillosis. *Ann Allergy Asthma Immunol* 1995; 75:533–5.
- 26 van der Veen MJ, Mulder M, Witteman AM *et al*. False-positive skin prick test responses to commercially available dog dander extracts caused by contamination with house dust mite (*Dermatophagoides pteronyssinus*) allergens. *J Allergy Clin Immunol* 1996; 98:1028–34.
- 27 Rosenbaum MR, Esch RE, Schwartzman RM. Effects of mold proteases on the biological activity of allergenic pollen extracts. *Am J Vet Res* 1996; 57:1447–52.
- 28 Akkerdaas JH, Wensing M, Knulst AC *et al*. How accurate and safe is the diagnosis of hazelnut allergy by means of commercial skin prick test reagents? *Int Arch Allergy Immunol* 2003; 132:132–40.
- 29 Trivedi B, Valerio C, Slater JE. Endotoxin content of standardized allergen vaccines. *J Allergy Clin Immunol* 2003; 111:777–83.
- 30 Valerio CR, Murray P, Arlian LG, Slater JE. Bacterial 16S ribosomal DNA in house dust mite cultures. *J Allergy Clin Immunol* 2005; 116:1296–300.
- 31 Focke M, Marth K, Flicker S, Valenta R. Heterogeneity of commercial timothy grass pollen extracts. *Clin Exp Allergy* 2008; 38:1400–8.
- 32 Focke M, Marth K, Valenta R. Molecular composition and biological activity of commercial birch pollen allergen extracts. *Eur J Clin Invest* 2009; 39:429–36.
- 33 Bernstein DI, Wanner M, Borish L, Liss GM. Twelve-year survey of fatal reactions to allergen injections and skin testing: 1990–2001. *J Allergy Clin Immunol* 2004; 113:1129–36.
- 34 Malling HJ. Sublingual immunotherapy: efficacy–methodology and outcome of clinical trials. *Allergy* 2006; 61 (Suppl. 81):24–8.
- 35 Winther L, Arnved J, Malling HJ, Nolte H, Mosbech H. Side-effects of allergen-specific immunotherapy: a prospective multi-centre study. *Clin Exp Allergy* 2006; 36:254–60.
- 36 Rezvani M, Bernstein DI. Anaphylactic reactions during immunotherapy. *Immunol Allergy Clin N Am* 2007; 27:295–307.
- 37 Dunskey EH, Goldstein MF, Dvorin DJ, Belecanech GA. Anaphylaxis to sublingual immunotherapy. *Allergy* 2006; 61:1235.
- 38 Antico M, Pagani, Crema A. Anaphylaxis by latex sublingual immunotherapy. *Allergy* 2006; 61:1236–37.
- 39 Agostinis F, Foglia C, Landi M *et al*. The safety of sublingual immunotherapy with one or multiple pollen allergens in children. *Allergy* 2008; 63:1637–9.
- 40 Esch RE, Bush RK, Peden D, Lockey RF. Sublingual-oral administration of standardized allergenic extracts: phase 1 safety and dosing results. *Ann Allergy Asthma Immunol* 2008; 100:475–81.
- 41 Wahn U, Tabar A, Kuna P *et al*. Efficacy and safety of 5-grass-pollen sublingual immunotherapy tablets in pediatric allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2009; 123:160–6.
- 42 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* 2002; 109:777–83.
- 43 Gadermaier E, Flicker S, Aberer W *et al*. Dissection of the antibody responses induced by subcutaneous injection immunotherapy with aluminium hydroxide-absorbed birch and *Fagales* pollen extracts. *Int Arch Allergy Immunol* 2010; 151:17–27.
- 44 Durham SR, Yang WH, Pedersen MR, Johansen N, Rak S. Sublingual immunotherapy with once-daily grass allergen tablets: a randomized controlled trial in seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2006; 117:802–9.
- 45 van Ree R, Antonicelli L, Akkerdaas JH, Garritani MS, Aalberse RC, Bonifazi F. Possible induction of food allergy during mite immunotherapy. *Allergy* 1996; 51:108–13.
- 46 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* 2002; 57:423–30.
- 47 van Hage-Hamsten M, Valenta R. Specific immunotherapy – the induction of new IgE-specificities? *Allergy* 2002; 57:375–8.
- 48 Pastorello EA, Incorvaia C, Gerosa A, Vassellatti D, Italia M, Pravettoni V. Allergen specific IgG subclass antibody response in hyposensitization with *Dermatophagoides pteronyssinus* extract. *N Engl Reg Allergy Proc* 1987; 8:417–21.
- 49 McHugh SM, Lavelle B, Kemeny DM, Patel S, Ewan PW. A placebo-controlled trial of immunotherapy with two extracts of *Dermatophagoides pteronyssinus* in allergic rhinitis, comparing clinical outcome with changes in antigen-specific IgE, IgG, and IgG subclasses. *J Allergy Clin Immunol* 1990; 86:521–31.
- 50 Ball T, Sperr WR, Valent P *et al*. Induction of antibody responses to new B cell epitopes indicates vaccination character of allergen immunotherapy. *Eur J Immunol* 1999; 29:2026–36.
- 51 Ball T, Fuchs T, Sperr WR *et al*. B cell epitopes of the major timothy grass pollen allergen, phl p 1, revealed by gene fragmentation as candidates for immunotherapy. *FASEB J* 1999; 13:1277–90.
- 52 Rossi RE, Monasterolo G. Evaluation of recombinant and native timothy pollen (rPhl p 1, 2, 5, 6, 7, 11, 12 and nPhl p 4)- specific IgG4 antibodies induced by subcutaneous immunotherapy with timothy pollen extract in allergic patients. *Int Arch Allergy Immunol* 2004; 135:44–53.
- 53 Pauli G, Larsen TH, Rak S *et al*. Efficacy of recombinant birch pollen vaccine for the treatment of birch-allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2008; 122:951–60.
- 54 Didier A, Melac M, Montagut A, Lhéritier-Barrand M, Tabar A, Worm M. Agreement of efficacy assessments for five-grass

- pollen sublingual tablet immunotherapy. *Allergy* 2009; **64**:166–71.
- 55 Gidaro GB, Marcucci F, Sensi L, Incorvaia C, Frati F, Ciprandi G. The safety of sublingual-swallow immunotherapy: an analysis of published studies. *Clin Exp Allergy* 2005; **35**:565–71.
 - 56 Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy* 2005; **60**:4–12.
 - 57 Calamita Z, Saconato H, Pela AB, Atallah AN. Efficacy of sublingual immunotherapy in asthma: systematic review of randomized-clinical trials using the Cochrane Collaboration method. *Allergy* 2006; **61**:1162–72.
 - 58 Nettis E, Colanardi MC, Soccio AL *et al.* Double-blind, placebo-controlled study of sublingual immunotherapy in patients with latex-induced urticaria: a 12-month study. *Br J Dermatol* 2007; **156**:674–81.
 - 59 Alvarez-Cuesta E, Berges-Gimeno P, González-Mancebo E, Fernández-Caldas E, Cuesta-Herranz J, Casanovas M. Sublingual immunotherapy with a standardized cat dander extract: evaluation of efficacy in a double blind placebo controlled study. *Allergy* 2007; **62**:810–7.
 - 60 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* 2002; **127**:259–68.
 - 61 Pittner G, Vrtala S, Thomas WR *et al.* Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens. *Clin Exp Allergy* 2004; **34**:597–603.
 - 62 Valenta R, Twaroch T, Swoboda I. Component-resolved diagnosis to optimize allergen-specific immunotherapy in the Mediterranean area. *J Invest Allergol Clin Immunol* 2007; **17** (Suppl. 1):36–40.
 - 63 Hiller R, Laffer S, Harwanegg C *et al.* Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. *FASEB J* 2002; **16**:414–6.
 - 64 Niederberger V, Ring J, Rakoski J *et al.* Antigens drive memory IgE responses in human allergy via the nasal mucosa. *Int Arch Allergy Immunol* 2007; **142**:133–44.
 - 65 Yamaguchi M, Sayama K, Yano K *et al.* IgE enhances Fc epsilon receptor I expression and IgE-dependent release of histamine and lipid mediators from human umbilical cord blood-derived mast cells: synergistic effect of IL-4 and IgE on human mast cell Fc epsilon receptor I expression and mediator release. *J Immunol* 1999; **162**:5455–65.
 - 66 Saini SS, Klion AD, Holland SM, Hamilton RG, Bochner BS, Macglashan DW Jr. The relationship between serum IgE and surface levels of Fc epsilon R on human leukocytes in various diseases: correlation of expression with Fc epsilon RI on basophils but not on monocytes or eosinophils. *J Allergy Clin Immunol* 2000; **106**:514–20.
 - 67 Katoh N, Kraft S, Wessendorf JH, Bieber T. The high-affinity IgE receptor (Fc epsilon RI) blocks apoptosis in normal human monocytes. *J Clin Invest* 2000; **105**:183–90.
 - 68 Kalesnikoff J, Huber M, Lam V *et al.* Monomeric IgE stimulates signaling pathways in mast cells that lead to cytokine production and cell survival. *Immunity* 2001; **14**:801–11.
 - 69 Marsh DG, Lichtenstein LM, Campbell DH. Studies on “allergoids” prepared from naturally occurring allergens. I. Assay of allergenicity and antigenicity of formalinized rye group I component. *Immunology* 1970; **18**:705–22.
 - 70 Bousquet J, Maasch HJ, Hejjajoui A *et al.* Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. III. Efficacy and safety of unfractionated and high-molecular-weight preparations in rhinoconjunctivitis and asthma. *J Allergy Clin Immunol* 1989; **84**:546–56.
 - 71 Haselden BM, Kay AB, Larché M. Peptide-mediated immune responses in specific immunotherapy. *Int Arch Allergy Immunol* 2000; **122**:229–37.
 - 72 Purohit A, Niederberger V, Kronqvist M *et al.* Clinical effects of immunotherapy with genetically modified recombinant birch pollen Bet v 1 derivatives. *Clin Exp Allergy* 2008; **38**:1514–25.
 - 73 Sledge R. Treatment of alum-precipitated pollen. *US Naval Med Bull* 1938; **36**:18.
 - 74 Zoss A, Koch CA, Hirose R. Alum-Ragweed precipitate: preparation and clinical investigation; preliminary report. *J Allergy Clin Immunol* 1939; **8**:29.
 - 75 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* 2001; **126**:135–9.
 - 76 Drachenberg KJ, Wheeler AW, Stuebner P, Horak F. A well-tolerated grass pollen-specific allergy vaccine containing a novel adjuvant, monophosphoryl lipid A, reduces allergic symptoms after only four preseasonal injections. *Allergy* 2001; **56**:498–505.
 - 77 Marsh DG, Norman PS, Roebber M, Lichtenstein LM. Studies on allergoids from naturally occurring allergens. III. Preparation of ragweed pollen allergoids by aldehyde modification in two steps. *J Allergy Clin Immunol* 1981; **68**:449–59.
 - 78 Norman PS, Lichtenstein LM, Kagey-Sobotka A, Marsh DG. Controlled evaluation of allergoid in the immunotherapy of ragweed hay fever. *J Allergy Clin Immunol* 1982; **70**:248–60.
 - 79 Attallah NA, Sehon AH. Isolation of haptenic material from ragweed pollen. *Immunochemistry* 1969; **6**:609–19.
 - 80 Malley A, Begley D, Forsham A. Chemical modification of timothy grass pollen antigen B – I. Loss of antibody binding properties. *Mol Immunol* 1979; **16**:929–34.
 - 81 Litwin A, Pesce AJ, Fischer T, Michael M, Michael JG. Regulation of the human immune response to ragweed pollen by immunotherapy. A controlled trial comparing the effect of immunosuppressive peptic fragments of short ragweed with standard treatment. *Clin Exp Allergy* 1991; **21**:457–65.
 - 82 Lee WY, Sehon AH. Abrogation of reaginic antibodies with modified allergens. *Nature* 1977; **267**:618–9.
 - 83 Mosbech H, Dreborg S, Pahlman I, Stahl Skov P, Steringer I, Weeke B. Modification of house dust mite allergens by monomethoxypolyethylene glycol. Allergenicity measured by in vitro and in vivo methods. *Int Arch Allergy Appl Immunol* 1988; **85**:145–9.
 - 84 Corrigan CJ, Kettner J, Doemer C, Cromwell O, Narkus A. Efficacy and safety of preseasonal-specific immunotherapy with an aluminium-adsorbed six-grass pollen allergoid. *Allergy* 2005; **60**:801–7.
 - 85 Valenta R, Kraft D. Recombinant allergens for diagnosis and therapy of allergic diseases. *Curr Opin Immunol* 1995; **7**:751–6.
 - 86 Hoyne GF, O’Hehir RE, Wraith DC, Thomas WR, Lamb JR. Inhibition of T cell and antibody responses to house dust mite

- allergen by inhalation of the dominant T cell epitope in naive and sensitized mice. *J Exp Med* 1993; **178**:1783–8.
- 87 Briner TJ, Kuo MC, Keating KM, Rogers BL, Greenstein JL. Peripheral T-cell tolerance induced in naive and primed mice by subcutaneous injection of peptides from the major cat allergen Fel d 1. *Proc Natl Acad Sci USA* 1993; **90**:7608–12.
 - 88 Norman PS, Ohman JL Jr, Long AA *et al*. Treatment of cat allergy with T-cell reactive peptides. *Am J Respir Crit Care Med* 1996; **154**:1623–8.
 - 89 Simons FE, Imada M, Li Y, Watson WT, HayGlass KT. Fel d 1 peptides: effect on skin tests and cytokine synthesis in cat-allergic human subjects. *Int Immunol* 1996; **8**:1937–45.
 - 90 Pene J, Desroches A, Paradis L *et al*. Immunotherapy with Fel d 1 peptides decreases IL-4 release by peripheral blood T cells of patients allergic to cats. *J Allergy Clin Immunol* 1998; **102**:571–8.
 - 91 Maguire P, Nicodemus C, Robinson D, Aaronson D, Umetsu DT. The safety and efficacy of ALLERVAX CAT in cat allergic patients. *Clin Immunol* 1999; **93**:222–31.
 - 92 Oldfield WL, Larche M, Kay AB. Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. *Lancet* 2002; **360**:47–53.
 - 93 Alexander C, Tarzi M, Larche M, Kay AB. The effect of Fel d 1-derived T-cell peptides on upper and lower airway outcome measurements in cat-allergic subjects. *Allergy* 2005; **60**:1269–74.
 - 94 Fellrath JM, Kettner A, Dufour N *et al*. Allergen-specific T-cell tolerance induction with allergen-derived long synthetic peptides: results of a phase I trial. *J Allergy Clin Immunol* 2003; **111**:854–61.
 - 95 Mueller U, Akdis CA, Fricker M *et al*. Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. *J Allergy Clin Immunol* 1998; **101**:747–54.
 - 96 Raz E, Tighe H, Sato Y *et al*. Preferential induction of a Th1 immune response and inhibition of specific IgE antibody formation by plasmid DNA immunization. *Proc Natl Acad Sci USA* 1996; **93**:5141–5.
 - 97 Hsu CH, Chua KY, Tao MH *et al*. Immunoprophylaxis of allergen-induced immunoglobulin E synthesis and airway hyperresponsiveness in vivo by genetic immunization. *Nat Med* 1996; **2**:540–4.
 - 98 Slater JE, Paupore E, Zhang YT, Colberg-Poley AM. The latex allergen Hev b 5 transcript is widely distributed after subcutaneous injection in BALB/c mice of its DNA vaccine. *J Allergy Clin Immunol* 1998; **102**:469–75.
 - 99 Tighe H, Takabayashi K, Schwartz D *et al*. Conjugation of immunostimulatory DNA to the short ragweed allergen Amb a 1 enhances its immunogenicity and reduces its allergenicity. *J Allergy Clin Immunol* 2000; **106**:124–34.
 - 100 Bohle B, Breitwieser A, Zwolfer B *et al*. A novel approach to specific allergy treatment: the recombinant fusion protein of a bacterial cell surface (S-layer) protein and the major birch pollen allergen Bet v 1 (rSbsC-Bet v 1) combines reduced allergenicity with immunomodulating capacity. *J Immunol* 2004; **172**:6642–8.
 - 101 Tulic MK, Fiset PO, Christodoulouopoulos P *et al*. Amb a 1-immunostimulatory oligodeoxynucleotide conjugate immunotherapy decreases the nasal inflammatory response. *J Allergy Clin Immunol* 2004; **113**:235–41.
 - 102 Linhart B, Valenta R. Molecular design of allergy vaccines. *Curr Opin Immunol* 2005; **17**:646–55.
 - 103 Reisinger J, Horak F, Pauli G *et al*. Allergen-specific nasal IgG antibodies induced by vaccination with genetically modified allergens are associated with reduced nasal allergen sensitivity. *J Allergy Clin Immunol* 2005; **116**:347–54.
 - 104 Kahlert H, Suck R, Weber B *et al*. Characterization of a hypoallergenic recombinant Bet v 1 variant as a candidate for allergen-specific immunotherapy. *Int Arch Allergy Immunol* 2008; **145**:193–206.
 - 105 Klimek L, Bachert C, Doemer C, Meyer H, Narkus A. Specific immunotherapy with recombinant birch pollen allergen Bet v 1-FV is clinically efficacious. *Allergy Clin Immunol Int* 2005; (Suppl. 1):15.
 - 106 Valenta R, Niederberger V. Recombinant allergens for immunotherapy. *J Allergy Clin Immunol* 2007; **119**:826–30.
 - 107 Jutel M, Jaeger L, Suck R, Meyer H, Fiebig H, Cromwell O. Allergen-specific immunotherapy with recombinant grass pollen allergens. *J Allergy Clin Immunol* 2005; **116**:608–13.
 - 108 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* 2002; **16**:1301–3.
 - 109 King TP, Jim SY, Monsalve RI, Kagey-Sobotka A, Lichtenstein LM, Spangfort MD. Recombinant allergens with reduced allergenicity but retaining immunogenicity of the natural allergens: hybrids of yellow jacket and paper wasp venom allergen antigen 5s. *J Immunol* 2001; **166**:6057–65.
 - 110 Linhart B, Valenta R. Vaccine engineering improved by hybrid technology. *Int Arch Allergy Immunol* 2004; **134**:324–31.
 - 111 Linhart B, Hartl A, Jahn-Schmid B *et al*. A hybrid molecule resembling the epitope spectrum of grass pollen for allergy vaccination. *J Allergy Clin Immunol* 2005; **115**:1010–6.
 - 112 Linhart B, Mothes-Luksch N, Vrtala S, Kneidinger M, Valent P, Valenta R. A hypoallergenic hybrid molecule with increased immunogenicity consisting of derivatives of the major grass pollen allergens, Phl p 2 and Phl p 6. *Biol Chem* 2008; **389**:925–33.
 - 113 Metz-Favre C, Linhart B, Focke-Tejkl M *et al*. Skin test diagnosis of grass pollen allergy with a recombinant hybrid molecule. *J Allergy Clin Immunol* 2007; **120**:315–21.
 - 114 Katz DH, Paul WE, Goidl EA, Benacerraf B. Carrier function in anti-hapten immune responses. I. Enhancement of primary and secondary anti-hapten antibody responses by carrier preimmunization. *J Exp Med* 1970; **132**:261–82.
 - 115 Paul WE, Katz DH, Goidl EA, Benacerraf B. Carrier function in anti-hapten immune responses. II. Specific properties of carrier cells capable of enhancing anti-hapten antibody responses. *J Exp Med* 1970; **132**:283–99.
 - 116 Focke M, Mahler V, Ball T *et al*. Nonanaphylactic synthetic peptides derived from B cell epitopes of the major grass pollen allergen, Phl p 1, for allergy vaccination. *FASEB J* 2001; **15**:2042–4.
 - 117 Focke M, Linhart B, Hartl A *et al*. Non-anaphylactic surface-exposed peptides of the major birch pollen allergen, Bet v 1, for preventive vaccination. *Clin Exp Allergy* 2004; **34**:1525–33.

- 118 Musselli C, Livingston PO, Ragupathi G. Keyhole limpet hemocyanin conjugate vaccines against cancer: the Memorial Sloan Kettering experience. *J Cancer Res Clin Oncol* 2001; 127:R20–6.
- 119 Demoly P, Persi L, Dhivert H, Delire M, Bousquet J. Immunotherapy with keyhole limpet hemocyanin-conjugated decapeptide vaccine in cypress pollen allergy. *Clin Exp Allergy* 2002; 32:1071–6.
- 120 Edlmayr J, Niespodziana K, Linhart B *et al.* A combination vaccine for allergy and rhinovirus infections based on rhinovirus-derived surface protein VP1 and a non-allergenic peptide of the major timothy grass pollen allergen, Phl p 1. *J Immunol* 2009; 182:6298–306.
- 121 Kündig TM, Senti G, Schnetzler G *et al.* Der p 1 peptide on virus-like particles is safe and highly immunogenic in healthy adults. *J Allergy Clin Immunol* 2006; 117:1470–6.