

Novel vaccines and adjuvants for allergen-specific immunotherapy

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Advances in genetic engineering and biotechnology, in parallel to increased understanding of disease processes and mechanisms of protective immunity, have facilitated the development of novel rational vaccination concepts for allergy. The spectrum ranges from utilizing recombinant proteins, peptides, new adjuvants, immunomodulatory therapy and DNA vaccines, to use of new application routes for both prophylaxis and treatment of allergic disease. New therapeutic concepts based on patient-tailored immunotherapy using recombinant allergens, anti-IgE and sublingual immunotherapies are slowly moving from the bench to the clinics. The additive value of new treatments above existing therapies must be, however, focused on the improvement of efficacy for long-term cure and increased patient compliance to warrant a broad applicability that has to compete with the symptomatic control of allergic and asthmatic diseases of currently available drugs.

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Introduction

Allergic and asthmatic diseases represent a big health problem in industrialized countries, in which up to 30% of the population suffer from allergic diseases such as rhinitis, conjunctivitis, atopic dermatitis or bronchial asthma. They represent an important cause of morbidity and, occasionally, mortality. Atopic disorders have become a relevant socio-economic problem, causing a health-care burden to the society in the order of billions of US dollars. A common hallmark of these diseases is the production of allergen-specific IgE against normally innocuous environmental antigens. In sensitized individuals, allergen re-exposure induces cross-linking of high-affinity FcεR1 receptor-bound IgE on effector cells and, thus, immediate release of anaphylactogenic mediators. The clinical symptoms that derive from mild type I hypersensitivity

reactions are not normally life threatening and manifest mainly as generalised urticaria, itching, broncho-constriction, nausea, vomiting, angioedema or abdominal pain. Severe forms manifest as dyspnoea, respiratory and cardiovascular symptoms or even as anaphylactic shock with loss of consciousness, and are associated with a risk of fatal reaction [1].

Drugs for the treatment of allergic diseases and asthma such as antihistamines, corticosteroids, bronchodilators (β-adrenergic receptor antagonists) and anti-IgE antibody therapy treat allergy symptoms and concomitant inflammatory reactions and require a long-term recurrent administration of the drugs [2]. Currently, allergen-specific immunotherapy (SIT) is the only treatment able to cure allergic diseases [3] and has been shown to be successful in treatment of insect venom allergy [4] and in patients suffering from allergic rhinitis or rhinoconjunctivitis [5]. Recent studies in other complex allergic syndromes, such as atopic eczema, have demonstrated that SIT has limited efficacy in selected patients [6]. SIT is the practise of administrating gradually increasing quantities of an allergen extract to an allergic individual to ameliorate the symptoms associated with the subsequent exposure to the causative allergen. Currently, SIT has several problems related to the vaccines used, adjuvant type, route of application, duration of treatment, side effects and limited efficacy. Therefore, there is an urgent need for the development of new curative strategies aimed to treat IgE-mediated disorders, which is justified by the profound impact of the diseases on both quality of life and health care burdens.

In this review, we discuss how detailed knowledge of the immunologic mechanisms underlying atopic diseases can be used to develop novel therapeutic strategies.

Conventional allergen-specific immunotherapy

Currently, allergen-SIT is performed using extracts from natural sources that contain insufficiently characterised allergen mixtures and unwanted non-allergenic components, and spans over a period of 3–5 years. In a WHO position paper on SIT, standardized allergen extracts were designated ‘vaccines’ [7]. The vaccines are standardized in terms of total allergenic activity or potency and sometimes concentration of individual major allergens, whereas batch-to-batch consistency is assessed by biochemical methods that determine protein and allergen profiles [8]. However, commercially available extracts can show high variations in composition even of the major

allergens, which can span several orders of magnitude [9]; this might be partly responsible for the differences reported about the efficacy of SIT. Interestingly, allergen extracts from wasp, birch, house dust mite, cat and grass differ in their tendency to produce side-effects in SIT [10•] and might induce new IgE-mediated sensitizations to allergen structures contained in the extracts used for therapy. However, one of the most important problems related to current allergen-SIT protocols results from the long-term clinical treatment that lasts at least three years, which radically reduces the patient's compliance [5]. Although SIT is considered, from a medical perspective, to be the best treatment of an allergy, currently few patients can afford or are willing to invest the time for 30–80 doctor visits, each of which is more than one hour, over a period of several years.

Mechanisms underlying specific immunotherapy

The initial event responsible for the development of allergic diseases is the generation of allergen-specific CD4⁺ T_H cells [11]. Naïve CD4⁺ T cells differentiate into T helper type 1 (Th1) or Th2 cells that orchestrate an effective immune response. Th2 cells are important for the initiation and maintenance of an allergic phenotype. They secrete IL-4, IL-5, IL-9 and IL-13, which regulate effector cells by triggering B-cell IgE production, growth, differentiation and recruitment of eosinophils, and mucus production in the airways, respectively [12]. Th1 cells produce the cytokine INF- γ , which promotes the development of cell-mediated immune responses and also antagonizes the development of Th2 cells.

A further subtype of T cells that have immunosuppressive function and cytokine profiles distinct from either Th1 or Th2 cells is termed regulatory T cells (T_{Reg} cells) [13], which appear to represent the comeback of the old suppressor T cells concept [14]. Analysis of T-cell responses to food- and aero-allergens demonstrated the co-existence of Th2 and regulatory responses to allergens [15]. Thus, the ratio between allergen-specific T_{Reg} cells and Th1/Th2 cells determines the development of a healthy or an allergic immune response to allergen exposure [15].

Based on this evidence, the immune responses following SIT have been revisited. This has shown that the activity of allergen-specific T_{Reg} cells is compromised in allergic disease but can be boosted with allergen-specific immunotherapy [16–18]. The increased levels of IL-10 and TGF- β produced by T_{Reg} cells can potently suppress IgE production while increasing the production of the non-inflammatory antibody isotypes IgG₄ and IgA [19–21]. Because one of the primary goals of allergen-SIT is the induction of peripheral T-cell tolerance that counteracts the allergic Th2 response, intervention at T_{Reg} level seems to be an attractive target for therapeutic

intervention [3,22]. At the other end of the cascade, therapeutic strategies aimed to eliminate allergen-specific IgE from the circulation are emerging [23] and might efficiently complement SIT interventions by reducing the risk of anaphylactic side effects [24].

Specific immunotherapy based on recombinant allergens

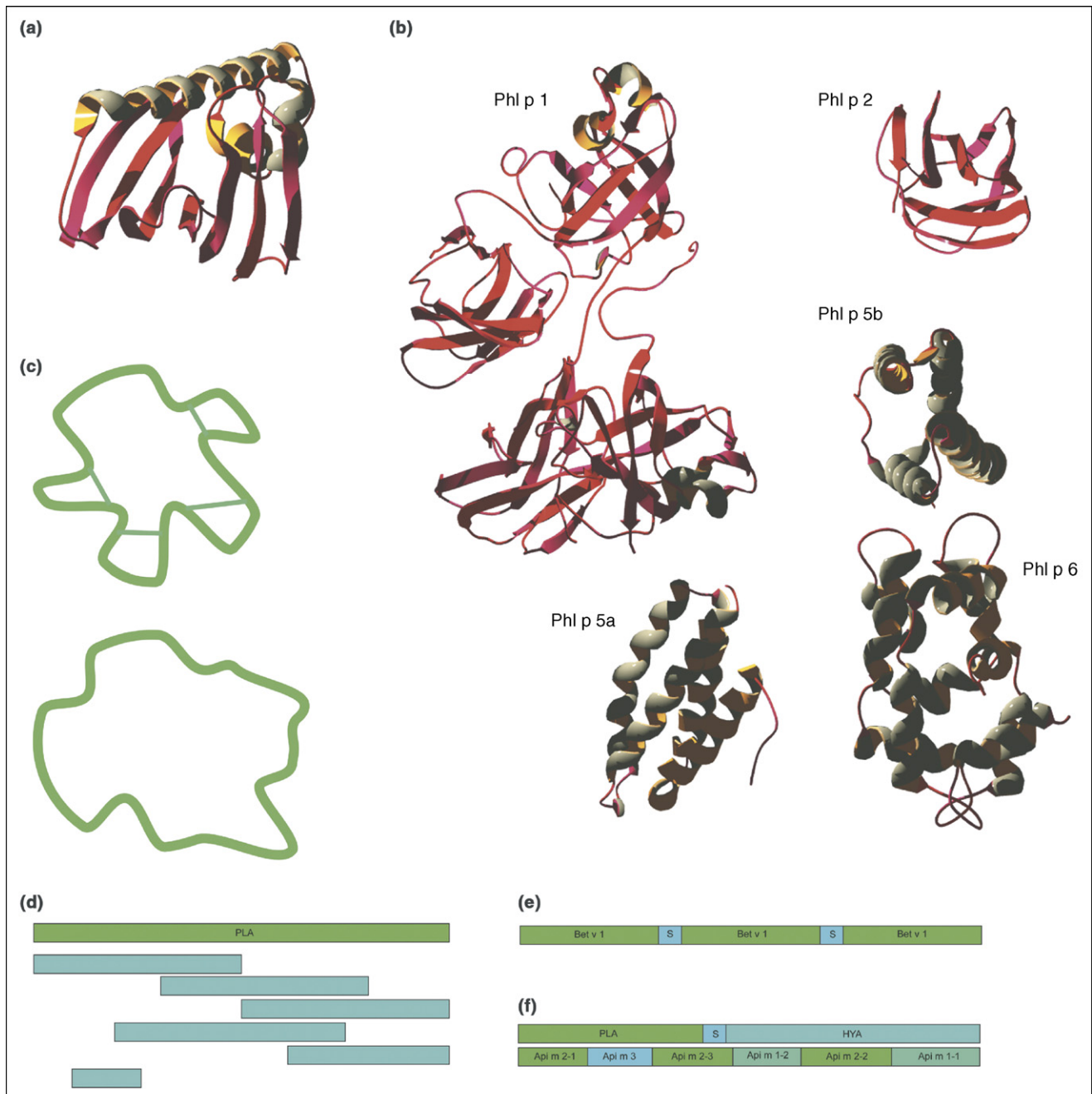
Allergic reaction to conventional extract-based SIT limits safety and efficacy of this kind of therapeutic intervention. Therefore, SIT is generally administered over a prolonged period of time starting with low doses of allergen that are sequentially increased in strength up to a high maintenance dose. Rapid progress in molecular cloning and characterization of allergen molecules allows now the production of single allergens that satisfy the requirements for pharmacological use. Most of the important allergens have been cloned, characterized and produced as recombinant proteins and can, after production under good manufacturing practice conditions, readily be used for SIT.

The relatively low number of allergenic structural entities compared with the number of known protein sequences [25] substantiates the hope to reduce the basic structures needed for efficient immunotherapy to a few protein families, as shown for the classification of pollen allergens [26]. Therefore, SIT using cocktails of recombinant perfectly standardized allergen preparations has moved from the field of speculation to clinical trials with encouraging results [27••]. Although native-like recombinant allergens retain the risk of adverse allergic events during SIT, they can be, in contrast with extracts, produced with a high batch-to-batch consistency in exactly defined pharmacological formulations [28].

The plasticity of molecular biology allows, by sequential application of genetic engineering steps, fast and reliable generation of almost any combination of hybrid molecules (Figure 1). This potential has been exploited to generate several different allergen–epitope fusion molecules as combined vaccines that cover the most important T-cell epitopes of a given allergen source [29•,30••]. These engineered vaccines can show a markedly reduced IgE-binding activity owing to a non-native conformation of the assembled proteins but fully retain the complexity of the T-cell epitopes. Although not yet tested in the clinic, hybrid vaccines have shown promising immunologic potential in animal models [31•].

An interesting approach, tested in a double-blind placebo-controlled multi-centre SIT study, used fragments or trimerised forms of Bet v 1, the major birch pollen allergen, that had reduced IgE-binding capacity to treat birch pollen allergic patients [32,33•]. The study shows that multimerisation of a single allergen increases the immunogenicity of the protein in concomitance with

Figure 1



Schematic representation of engineered allergy vaccines. **(a)** Recombinant allergens with native-like conformation, here exemplified by Bet v 1, can be produced at high yield and purity. They bind IgE and have been tested in humans [20]. **(b)** Mixtures of the recombinant grass pollen allergens Phl p 1, Phl p 2, Phl p 5a, Phl p 5b and Phl p 6 have been successfully tested in the clinic [27]. **(c)** Mutated allergens either conserving a native-like conformation [34,37] or produced as unfolded molecules [62] show a strongly reduced IgE-binding activity and are potentially useful for SIT. **(d)** Linear peptides of allergens that have strongly reduced IgE-binding activity have been successfully used in clinical trials to treat bee venom and cat allergy [38,39]. **(e)** Multimerization of Bet v 1 results in generation of a vaccine that has reduced IgE-binding activity. The molecule has been evaluated in a multi-centre study enrolling birch allergic patients [33]. **(f)** Linear fusions of major allergens [30**] or of fragments from major allergens conserving the whole T-cell epitope repertoire of the original allergens [31*] show a strongly reduced IgE-binding activity and have been successfully tested in murine models of allergy.

Table 1

Novel approaches to SIT.

Approach	Comment	References
Recombinant allergens	Recombinant allergens that can be produced as highly pure preparations have a great potential for immunotherapy and have been shown to be useful as allergy vaccines.	[20,33*]
Allergen fragments	Fragments of Bet v 1, the major birch pollen allergen, show reduced IgE-binding capacity and clinical efficacy comparable to those of Bet v 1.	[32,33*]
Allergen multimers	A trimer of Bet v 1 shows reduced IgE-binding capacity and good therapeutic efficacy in a double-blind, placebo-controlled clinical study.	[32,33*]
Hypoallergens	Recombinant allergens that have reduced IgE-binding activity can easily be engineered with different approaches and are promising reagents to improve vaccination against allergy.	[34,37,62]
Multi-allergen fusions	Sequential fusion of cDNAs that encode different allergens allows production of proteins conserving the T-cell epitopes of the fused allergens with strongly reduced IgE-binding. The efficacy of the concept has been proven in mouse models of allergy.	[29*,30**,31*]
Recombinant allergen mixtures	Mixtures of single highly pure recombinant allergens can be used to reconstitute the IgE-binding repertoire of a given allergen extract. This has been clinically tested with <i>Phleum pratense</i> allergens in a proof of concept study.	[27**]

a reduction in allergen-specific Th2 responses [32]. These few clinical studies clearly demonstrate the feasibility of using recombinant allergens in SIT, indicating that one can reduce side effects by rational modification of the three-dimensional structure of the molecules to avoid IgE–vaccine interactions [34]. The occurrence of structural polymorphism among allergen structures resulting in natural hypoallergenic variants [35,36] stimulated an intense research activity aimed to engineer hypoallergenic forms of a myriad of allergen structures. In general, engineered hypoallergens show reduced IgE binding and reduced basophil histamine release [35] as well as decreased skin prick test activity, but retain the full ability to stimulate T-cell responses [36] and to induce IgG blocking antibodies in murine models of allergy [37]. With exception of Bet v 1 derivatives [33], hypoallergens have not yet been tested in clinical studies. They bear a high therapeutic potential related to a reduced risk of anaphylactic side effects. This suggests the possibility to use higher dosage schemata that could result in a drastic reduction of the treatment time required to reach protection. The most important SIT approaches based on recombinant allergens and modifications thereof are summarized in Table 1.

Peptide immunotherapy

T-cell peptide epitopes, although capable of inducing immunologic tolerance, are short linear structures that normally have a reduced ability to cross-link mast cell- and basophil-bound IgE. These represent, therefore, an attractive approach for allergen-SIT, which was reviewed recently [38]. However, clinical trials using peptide immunotherapy have been performed only for bee venom [39] and cat allergy [40,41]. The mechanisms that lead to a successful peptide-SIT have been associated with induction of specific T-cell anergy and with a decrease in allergen-specific IgE:IgG₄ ratio initiated by the auto-crine action of IL-10 and/or TGF- β [42]. The major advantage of such an approach is the reduction in

systemic IgE-mediated adverse events compared with whole-allergen immunotherapy. A new form of peptide-SIT virus-like particles carrying a multimerized Der P 1 peptide have been recently tested in a phase I clinical study (see Update).

Adjuvants and allergen conjugates

Traditionally, SIT utilizes aluminium hydroxide as an adjuvant. These preparations have generally proved to be efficacious and have a good safety profile, but might not be the most efficient immunostimulatory reagents. A new class of adjuvants — so-called immune response modifiers — act on antigen-presenting cells through the Toll-like receptors (TLRs), which recognize pathogen-associated molecular patterns on microorganisms [43]. Ligation of TLRs activates NF- κ B and leads to the expression of several cytokines and co-stimulatory molecules. To date, 11 mammalian TLRs have been described together with a multitude of natural and synthetic ligands [44]. Depending on the TLR triggered, different signalling pathways are activated. Compounds that can control the overexpression of Th2 cytokines or skew the Th1:Th2 balance towards a Th1 profile would be of clinical relevance for the control of allergic disease [45]. Lipopoly-saccharide, polyinosinic-polycytidylic acid (polyIC), lipoproteins and GpG-containing immunostimulatory DNA sequences have been shown to be able to inhibit Th2 cell activation [46] and IgE-dependent release of Th2 cytokines [47] in murine models of allergy.

Synthetic DNA sequences that contain CpG motifs have been covalently linked to Amb a 1 — the major ragweed allergen — to generate allergen immunostimulatory conjugates. Treatment of allergic individuals with Amb a 1 allergen immunostimulatory conjugates in a placebo-controlled clinical study resulted in redirection of ragweed-specific Th2 responses toward Th1 responses, characterized by increased INF- γ , CXCL-9 and CXCL-10 expression and decreased IL-5, CCL-17 and

CCL-22 [48]. This is the first study to demonstrate the clinical efficacy of allergen immunostimulatory conjugates.

Likewise, monophosphoryl lipid A, a bacterial cell wall component, has been evaluated as an adjuvant for SIT. The allergy vaccine allergoid (Pollinex Quattro) showed clinical efficacy in a short-course SIT. Treatment resulted in improved symptom and medication scores accompanied by reduced seasonal rises in IgE and an increase in IgG₁ and IgG₄, which blocked allergen-induced basophil degranulation [49].

These results are encouraging; however, additional trials to further evaluate the safety, immunologic effects and therapeutic efficacy of the new adjuvants will be required.

Anti-IgE therapy

After almost two decades of consequent development, humanized monoclonal anti-IgE antibodies have now entered the market [50]. Omalizumab[®] has been approved in the USA, European Union and several other countries for treating patients who have moderate-to-severe asthma. The anti-IgE antibody is an IgE-neutralizing antibody and can block binding of IgE immunoglobulin to the high-affinity IgE receptor (Fc ϵ RI) on mast cells and basophils, rendering the mediator-packed inflammatory cells insensitive to allergen stimulation. The clinical data available to date support omalizumab[®] as a safe and well-tolerated add-on therapy for IgE-mediated diseases [51]; however, at present, anti-IgE immunotherapy is simply too expensive for the majority of the IgE-mediated complications [52]. Anti-IgE therapy is not expected to cure allergic diseases or asthma, and the additive value above existing therapies with respect to the induction of long-term disease remissions with infrequent drug administration remain to be critically evaluated.

DNA vaccines

DNA vaccines comprised of a plasmid DNA that encodes allergens have been proposed as prophylactic and therapeutic treatment for allergic disease [53]. Genetic immunization has proven to be a powerful method to induce anti-allergic immune responses in murine model systems [54]. However, these results are in contrast with the efficiency of naked DNA vaccines in humans, which has been disappointing to date [55].

Application routes

Strong adaptive immunologic responses are most likely to be triggered by the basic mechanisms governing antigen presentation in a dose- and time-dependent manner. If an antigen does not reach secondary lymphoid organs in the minimum required doses or for sufficiently long periods, it is ignored by immune cells; however, antigens reaching and persisting in the lymph node in excessive amounts

for long periods of time delete primed T cells to avoid exaggerated immune responses [56].

From this point of view, the application route of allergens during immunotherapy is expected to play an important role, and subcutaneous administration might not be the optimal method. An emerging concept — although still somehow controversial — consists of administration of allergens by the oral or sublingual route [57]. There is accumulating evidence showing that sublingual immunotherapy is safe and effective, and the immunological mechanisms underlying the induction of immune tolerance by mucosal application of allergens are currently under intensive investigation [58]. However, direct administration of vaccines into a lymph node, which was demonstrated to enhance DNA vaccination [59] as well as peptide immunotherapy [60[•]] in murine models, might represent an even more effective route of application for allergen-SIT. The results of the first ongoing clinical studies are awaited to give a definitive evaluation of the efficacy of intranodal immunization in humans.

Conclusions

Compared with the fast progress in characterization, cloning and production of allergens and in our understanding of the molecular mechanisms underlying allergic diseases, clinical improvements of immunotherapy have been slow. Despite emerging clinical trials that have shown that recombinant allergens and peptides are suitable reagents to cure allergic diseases, with perfectly standardized preparations satisfying the highest pharmaceutical standards, immunotherapy still suffers from extremely long treatment periods. The lack of a rapid clinical effect of immunotherapy is in direct competition with the fast and efficient control of allergic and asthmatic diseases achievable with inhaled anti-inflammatory corticosteroids and bronchodilator agents. Although these treatment modalities, in contrast to immunotherapy, are not leading to a cure of the disease, they benefit from a high compliance of patients.

One of the most formidable tasks in allergy and asthma research is turning immunotherapy into a treatment that has a high compliance. One approach consists of the development of drugs applicable without visits to the doctor, for example oral vaccines, which at present are subject to broad clinical use and intense research activity [61]. A second, perhaps even more efficient, approach aims to turn immunotherapy into vaccination. A drastic reduction of up to 80 injections scattered over a period of 3–5 years in current SIT protocols to just a few applications under strict medical control, such as direct intralymphatic injections, has a great potential to dramatically increase patient compliance. More efficient adjuvants and hypoallergens, which are able to reduce the side effects of immunotherapy and direct injection of allergens into lymph nodes, are emerging hot topics in allergy

and asthma research that have a great future potential. However, a breakthrough in SIT of allergic and asthmatic diseases will depend on the development of our understanding of the immunologic mechanisms underlying the diseases, which is still incomplete.

Update

Two recent publications have indicated two new concepts for the treatment of allergic diseases. The first one [63^{••}] shows the safety and immunogenicity of a Q β virus-like particle chemically coupled to a synthetic 16 amino acid peptide derived from the house dust mite allergen Der P 1 and known to contain a B-cell epitope in healthy human volunteers. The work shows that peptide-coupled virus-like particles that assemble spontaneously to form highly repetitive structures are efficient in rapidly inducing high antibody titers in humans without the need for adjuvants. However, antibody responses are induced not only against the allergen epitope but also against the carrier particle. Therefore, strong immune reactions against the Q β carrier protein might limit the use of vaccines based on virus-like particles to a single antigen.

The second one [64[•]] proposes a general method to produce hypoallergenic DNA vaccines based on antigen-ubiquitin fusions. Ubiquitination of allergen gene vaccines eliminates the risk of IgE cross-linking through production of hypoallergenic molecules. Mice vaccinated with ubiquitin-Bet v 1-encoding DNA vaccine are not able to induce antibody responses but lead to a Th1-biased protective T-cell memory response. This approach might have some potential as a protective allergy vaccine; however, we should keep in mind that the efficiency of naked DNA vaccination in humans has been disappointing to date [65].

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