

Allergen-Specific Immunotherapy: Towards Combination Vaccines for Allergic and Infectious Diseases

Johanna Edlmayr, Katarzyna Niespodziana, Margarete Focke-Tejkl,
Birgit Linhart and Rudolf Valenta

Abstract IgE-mediated allergies affect more than 25% of the population. Allergen-specific immunotherapy (SIT) is an antigen-specific and disease-modifying form of treatment. It is based on the therapeutic administration of the disease-causing allergens to allergic patients. However, the fact that only allergen extracts of insufficient quality are currently available and the possible occurrence of side effects during treatment limit the broad use of SIT and prophylactic vaccination is has not yet been performed. In the last 20 years the DNA sequences of the most common allergens have been isolated and the corresponding allergens have been produced as recombinant allergens. Based on the progress made in the field of allergen characterization it is possible to improve the quality and safety of allergy vaccines and to develop new, more effective strategies for a broad application of SIT and even for prophylactic treatment. Here we discuss the development of combination vaccines for allergy and infectious diseases. This approach is based on the selection of allergen-derived peptides with reduced IgE- and T cell reactivity in order to minimize IgE- and T cell-mediated side effects as well as the potential of the vaccine to induce allergic sensitization. These peptides are fused by recombinant technology onto a viral carrier protein to obtain a combination vaccine which induces protective immunity against allergy and viral infections. The application of such combination vaccines for therapy and prophylaxis of allergy and infectious diseases is discussed.

J. Edlmayr · K. Niespodziana · B. Linhart · R. Valenta
Division of Immunopathology, Department of Pathophysiology and Allergy Research,
Center for Pathophysiology, Infectiology and Immunology,
Medical University of Vienna, Vienna, Austria

M. Focke-Tejkl · R. Valenta (✉)
Christian Doppler Laboratory for Allergy Research, Medical University of Vienna,
AKH Ebene 3Q, Waehringer Guertel 18–20, 1090 Vienna, Austria
e-mail: rudolf.valenta@meduniwien.ac.at

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1 Introduction

Allergen contact and allergic inflammation can occur in different target organs leading to mild (e.g., rhinoconjunctivitis), more severe (e.g., asthma), and systemic, life-threatening manifestations (e.g., anaphylactic shock). Mild respiratory allergy, for example, rhinitis, frequently progresses to asthma, a severe disabling respiratory form of allergy affecting the lung. Asthma is a chronic disease characterized by airway hyper-responsiveness, airway inflammation, airway remodeling and reversible airway obstruction. Patients with inadequately controlled severe persistent asthma are at a particularly high risk of exacerbations, hospitalization and death (Demoly and Bousquet 2006; Peters et al. 2006). In this context it should also be mentioned that asthma is triggered by a variety of other factors such as viral infections or pollutants (Holgate 2006; Bousquet et al. 2005).

Sensitization to allergens, i.e., the production of allergen-specific IgE antibodies, occurs in genetically predisposed individuals (i.e., atopic individuals) shortly after birth and can be modulated by a variety of environmental factors (Kulig et al. 1999; Niederberger et al. 2002; Platts-Mills 2005; Vercelli 2006; Garn and Renz 2007). Subsequent allergen contact strongly boosts the established secondary IgE response and clinical sensitivity in allergic patients (Yunginger and Gleich 1973; Henderson et al. 1975; Niederberger et al. 2007). Acute allergic inflammation is mainly due to the activation of mast cells and basophils by IgE-allergen immune complexes whereas T cells and eosinophils play an important role in chronic allergic inflammation (reviewed in Valenta 2002; Desreumaux and Capron 1996).

Mast cells and basophils bind allergen-specific IgE antibodies via the high affinity receptor for IgE, FcεRI, which can be cross-linked by allergens to release inflammatory mediators, pro-inflammatory cytokines and proteases (Bischoff 2007). These substances cause immediate allergic inflammation, tissue damage

and attract inflammatory cells. Allergen contact also activates allergen-specific T cells to release pro-inflammatory cytokines leading to chronic allergic inflammation (Larche et al. 2003). The presentation of allergens to T cells can be facilitated by IgE bound to Fc ϵ Receptors on the surface of antigen presenting cells (dendritic cells, monocytes) and B-cells or occur in an IgE-independent manner (van Neerven et al. 2006; Haselden et al. 1999; Campana et al. 2008).

Allergen-specific immunotherapy (SIT) is the only antigen-specific and disease-modifying treatment of allergy. It has been also demonstrated that SIT can prevent the progression of allergic rhinitis to asthma (Moller et al. 2002).

SIT is based on the administration of the disease-eliciting allergens (reviewed in Larche et al. 2006). This treatment has been developed exactly 100 years ago when Noon and later Freeman injected grass pollen extract into grass pollen allergic patients (Noon 1911; Freeman 1914). In the next chapter we review some milestones in the development of SIT.

2 Developments in the Field of Allergen-specific Immunotherapy

Reactions to different substances were described already in the antique, but Bostock was the first, who published in 1819 an official medical case report of hay fever, referring to his own personal experience. Wyman and especially Blackley identified pollen as source for allergens, demonstrated that pollen exposure can induce allergic reactions and measured pollen exposure (Bostock 1819; Wyman 1872; Blackley 1873). Hundred years ago it was not known that allergy is an immunologically-mediated disease but Noon injected pollen extracts into hay fever patients based on the hypothesis that they suffered from intoxication (Noon 1911).

Between 1913 and 1959 the idea was pursued that not only administration of pollen, but also bacterial vaccines may have beneficial effects on hay fever (Morrey 1913). In this context it was also reported that skin reactions and systemic reactions not only occurred on exposure to pollens and food, but also with bacterial products, such as tuberculin which led to the presumption that bacteria may cause allergic reactions (Walker and Adkinson 1917). Interestingly, a connection between respiratory tract infections and asthma or asthma exacerbations was already described 1932 (Cooke 1932). However, clinical trials with bacterial vaccines against asthma were not performed until 1955 and failed to be effective (Frankland et al. 1955; Johnstone 1957; Helander 1959).

SIT as introduced by Noon and Freeman continued to be a successful treatment also for other allergen sources. A first controlled immunotherapy trial was performed in 1954 and a first double-blind study in 1965. It could be shown that SIT resulted in fewer symptoms and lower medication scores compared to control groups (Frankland and Augustin 1954; Lowell and Franklin 1965).

The understanding of the mechanisms underlying allergy developed in parallel to the use of SIT. In an elegant experiment Prausnitz and Kuestner demonstrated in

1921 that allergen-specific sensitivity can be transferred to a non-allergic person by injection of serum from the allergic individual (Prausnitz and Kuestner 1921). This experiment indicated that serum from allergic patients contains allergen-specific factors, later termed reagents, which combine with a non-specific tissue component also present in healthy persons and then give rise to an allergic reaction induced by the allergen. It took more than 40 years until the serum factor could be isolated by K. and T. Ishizaka (1967) and was identified as immunoglobulin E through comparison with a myeloma protein discovered by Johansson and Bennich (1967). Based on the availability of the IgE myeloma the RAST test for measuring IgE antibodies in sera could be developed for the diagnosis of allergic patients (Wide et al. 1967).

Long before the characterization of “reaginic” IgE, i.e., in 1935, it could be demonstrated that patients receiving SIT developed antigen-specific serum factors (i.e., blocking IgG antibodies) that blocked allergic skin reactions (Cooke et al. 1935).

Loveless (1940) demonstrated that blocking antibodies compete with reagents for the binding sites on allergens and Lichtenstein et al. (1968) showed that the blocking antibodies belong to the IgG class.

Rocklin et al. (1980) proposed suppressor cells as another possible mechanism of SIT. They detected allergen-specific suppressor cells in ragweed allergic patients after injections with ragweed extract, but not before immunotherapy.

After introduction of the Th1/Th2 concept into human allergy (Paronchi et al. 1991) it was suggested that SIT may affect the Th1/Th2 balance (Secrist et al. 1993).

More recently the possibility has been discussed that SIT, or at least certain forms of SIT, may induce allergen-specific immune tolerance mediated through tolerogenic cytokines and possibly regulatory T cells (Akdis et al. 1998). The contribution of the various mechanisms (e.g., induction of blocking antibodies, affecting the Th2/Th1 balance and tolerogenic mechanisms) to efficacy of the various forms of SIT is a matter of intense investigation (Larche et al. 2006). However, many recent studies performed with purified allergens/recombinant allergens and recombinant hypoallergenic allergen derivatives speak for an important role of allergen-specific IgG antibodies in preventing allergen-induced inflammation and allergen-induced boosts of the allergic immune response (Valenta et al. 2010).

So far, the vast majority of immunotherapy studies and the analysis of these studies have been carried out with allergen extracts from natural sources. However, these extracts are often of poor quality. For example, the allergen concentrations vary between different extract preparations and contain biologic active allergens in different concentrations as well as non-allergenic components (Focke et al. 2008, 2009; Curin et al. 2010; Brunetto et al. 2010). The heterogeneity of allergen extracts certainly represents a major bottleneck limiting the broad use of SIT. It has hampered the analysis of the mechanisms of SIT and is responsible for the sometimes poor clinical efficacy.

The efforts to improve the quality of allergen extracts have already started early. For example, Stull et al. (1933) worked on standardization of allergen batches and developed a measurement of protein nitrogen units for the assessment of allergenic activity. King and Norman started to isolate the major allergen

fractions of ragweed pollen in 1962 (King and Norman 1962). However, despite all standardization efforts which later involved measurements of allergenic activity, IgE binding capacity and the measurement of major allergens in the extracts the fact remains that the composition of allergen extracts cannot be influenced by the manufacturer.

The administration of allergen extracts to patients in the course of SIT can cause side effects which may be mild and local but also severe, systemic and life-threatening (Mellerup et al. 2000).

Therefore several efforts for the reduction of allergenic side effects have been made. Seventy years ago aluminium-hydroxide-adsorbed allergen extracts were introduced for depot vaccination, showing improved immune stimulatory as well as reduced anaphylactic properties (Zoss et al. 1937). Already in 1969 allergen-derived peptides were used for immunotherapy. In fact, Malley and Perlman used the low-molecular-weight fragment of allergen extracts for SIT and induced blocking antibodies (Malley and Perlman 1969). During 1970 and 1980 further attempts for the reduction of side effects were made by chemical modification of allergens (Marsh et al. 1970; Lee and Sehon 1978).

In its beginning, SIT had been typically administered by subcutaneous injection. In order to facilitate SIT and make it more convenient many attempts have been made to administer SIT via other routes. Already in 1921 and 1922 MacKenzie, Caulfield and Touart tried to desensitize allergic patients by nasal, oral and throat administration of pollen in form of sprays and ointments (MacKenzie 1922; Caulfield 1922; Touart 1922). However, Feinberg et al. demonstrated in a multicenter placebo-controlled study that the oral application of high doses of ragweed pollen lacked efficacy (Feinberg 1940). Further attempts to give oral immunotherapy started in the early 1970s with controversial results until today (Laetsch and Wuthrich 1973; Urbanek et al. 1990; Mosbech et al. 1987; Scadding and Brostoff 1986). Until today several thousand individuals have been treated in various clinical studies with sublingual immunotherapy (SLIT) but the mechanisms of this treatment are still unknown. There are even studies demonstrating that SLIT induces strong boosts of allergen-specific IgE production (Durham et al. 2006). Generally, there is a lack of studies which have investigated effects of SLIT on objective outcome parameters such as allergen-specific skin or target organ sensitivity. Yet there is evidence from meta-analysis for clinical efficacy of SLIT but these effects seem to be much less than those obtained by subcutaneous SIT, although this is difficult to assess because only few studies comparing subcutaneous SIT and SLIT have been carried out (Radulovic et al. 2010).

3 The Impact of Recombinant Allergens on SIT

With the introduction of recombinant DNA technology into the field of allergen characterization it has become possible to reveal the sequences and structures of the most common allergens. A large number of the most important disease-

eliciting allergens have been characterized down to the molecular level and recombinant allergens mimicking the epitope complexity of natural allergen extracts have been produced. According to the allergen-encoding DNA sequence, recombinant allergens can be produced as highly pure proteins and used for diagnostic and therapeutic purposes (Valenta et al. 2010).

3.1 Recombinant Wildtype Allergens

The term “recombinant wildtype allergen” describes a recombinant allergen, which mimics the allergenic properties of the natural allergen derived from the natural allergen source. It contains the relevant IgE- and T cell epitopes of the natural allergen and hence can be used for diagnosis and therapy similar to allergen extracts. Advantages of recombinant allergens over extracts are that they represent well defined and highly pure proteins. When used for SIT, recombinant allergens are expected to induce similar effects as natural allergens which has indeed been shown in first immunotherapy studies using purified recombinant grass pollen allergens (Jutel et al. 2005). In a SIT trial in 2006 birch pollen allergic patients received recombinant Bet v 1a, purified natural Bet v 1 and birch pollen extract. This study showed in a direct comparison that a single recombinant allergen is as effective as treatment with the natural extract (Pauli et al. 2008).

Recombinant wildtype allergens carry the IgE and T cell epitopes of the corresponding natural allergen and upon SIT induce allergen-specific IgG blocking antibodies and depending on the dose, route and adjuvant may induce Th1 immune responses. Due to the presence of allergen-specific T cell epitopes, recombinant wildtype allergens could in principle induce T cell tolerance, which may also depend on the dose, mode and route of application. Disadvantages of recombinant wildtype allergens are that they can induce both, IgE as well as T cell-mediated side effects and that their administration may induce new sensitizations or boost an existing allergen-specific IgE response.

The first allergens made by recombinant DNA technology were Dol m V (major allergen of white-face hornet venom), Der p 1 (major house dust mite allergen) and Bet v 1 (major allergen of birch pollen) published in the late 1980's (Fang et al. 1988; Breiteneder et al. 1989; Chua et al. 1988).

Recombinant allergens were first used for allergy diagnosis in 1991. Valenta et al. tested birch pollen allergic patients' sera for Bet v 1- and Bet v 2-specific reactivity and revealed a similar IgE-binding pattern as with natural allergens (Valenta et al. 1991).

The first animal models and *in vivo* applications in patients with recombinant allergens were performed 1992 and 1994 (Sehon and Mohapatra 1992; Moser et al. 1994). Sehon and Mohapatra induced IgE antibodies specific for recombinant grass pollen antigens from *Poa pratensis* in mice. First skin test studies demonstrating the biological equivalence of recombinant and natural allergens from

Aspergillus and house dust mites were carried out in 1994 (Moser et al. 1994; Lynch et al. 1994).

Today cDNAs coding for the most common allergens have been isolated and the corresponding recombinant allergens are available for research purposes, diagnosis and treatment (reviewed in Valenta et al. 2010).

3.2 Hypoallergenic Allergen Derivatives

Recombinant allergens that resemble natural wild type allergens contain all relevant IgE epitopes and therefore retain the risk of inducing IgE-mediated side effects. Therefore modifications have been introduced to generate hypoallergenic allergen derivatives. These new molecules should show abolished or substantially reduced IgE-binding activity while retaining T cell reactivity and immunogenicity. Several strategies for the modification of an allergen were developed (Valenta 2002).

In fact, the first immunotherapy trial with recombinant molecules has been performed with genetically modified hypoallergenic derivatives of the major birch pollen allergen, Bet v 1 ten years ago (Niederberger 2004). Patients received either recombinant Bet v 1 fragments or a recombinant trimer of Bet v 1. Although patients had received only one treatment course they developed a robust IgG (IgG1, IgG2, IgG4) antibody response against natural Bet v 1, Bet v 1-related pollen allergens from alder and hazels and food allergens (apple, carrot, celery). The concept of recombinant hypoallergens has meanwhile been successfully evaluated in clinical immunotherapy trials for the treatment of birch pollen allergy up to phase III trials (Klimek et al. 2005; Rak 2009; Valenta et al. 2011).

3.3 Recombinant Hybrid Molecules

Very often certain allergens have low immunogenicity and there are allergen sources containing several allergens which need to be included in the vaccine. In order to address the problem hybrid allergens were generated by sequential fusion of cDNAs encoding for different allergens. As it has been shown for grass pollen allergy it is possible to produce recombinant hybrid molecules consisting of the most relevant allergens either as wild type allergens or as hypoallergenic allergen derivatives and thus to combine the relevant epitopes in one protein with increased immunogenicity (Linhart et al. 2002, 2005, 2008).

3.4 DNA Vaccines

Hsu et al. and Raz et al. published the first papers using DNA vaccines for the treatment of allergy in experimental animal models. The data of their studies

performed in mice demonstrated that this approach prevents the induction of IgE synthesis, IL-4 and IL-5 production as well as histamine release (Hsu et al. 1996; Raz et al. 1996).

3.5 Allergen Conjugates

Another strategy concerns immunostimulation using allergen conjugates where allergen components are fused to a foreign molecule. For example, synthetic DNA sequences that contain CpG motifs have been linked to allergens to generate immunostimulatory conjugates. Treatment of allergic patients with the major ragweed allergen in a placebo-controlled study resulted in redirection of ragweed-specific Th2 responses towards Th1 responses (Simons et al. 2004). These responses were characterized by increased production of INF- γ and decreased production of IL-5. The injection of the CpG-Amb a 1 fusion product induced no clinically significant systemic or local allergic reactions and showed promising results in an immunotherapy trial (Creticos et al. 2006).

Another conjugated product with immunostimulatory activity was developed by Bohle et al. In this study a fusion protein consisting of Bet v 1 and the bacterial cell surface protein SbsC was recombinantly produced and displayed reduced histamine-releasing capacity. Basophils of Allergic patients T cells exposed to rSbsC-Bet v 1 produced INF- γ and IL-10, but no IL-5 (Bohle et al. 2004). A reduction of allergenic activity and increase in immunogenicity was observed in murine models when the major cat allergen, Fel d 1 was conjugated to viral particles (Schmitz et al. 2009).

3.6 T Cell Epitope Peptides

A completely different strategy, for decreasing IgE-mediated side effects which is based on the administration of allergen-derived T cell-reactive peptides. Peptides are short in length and have a linear structure, which lead to a reduced ability to cross-link mast cell-and basophil-bound IgE and are therefore candidates for safe SIT. The mechanism behind the reduction of allergy symptoms using T-cell epitope containing peptides for SIT is the ability of the peptides to induce immunologic tolerance. Clinical trials using peptide immunotherapy have been mainly performed for cat and venom allergy (Ali and Larche 2005; Norman et al. 1996). T-cell epitope derived peptide immunotherapy with reduced IgE-mediated side effects, but it was reported that T cell epitope peptides of the major cat allergen, Fel d 1, induced asthma and bronchial hyper reactivity several hours after intracutaneous injection and this effect is T cell-mediated and MHC-restricted (Haselden et al. 1999).

These results indicate that the removal of IgE-reactivity diminishes IgE-mediated side effects but late phase side effects are common so attempts are currently being made to reduce them by adequate dosing.

3.7 Allergen-Derived Peptides Lacking Allergenic Activity to Induce Allergen-Specific Blocking IgG Antibodies

Long before the discovery of IgE antibodies it has been demonstrated that SIT induces allergen-specific IgG antibodies which inhibit binding of allergic patients IgE to the allergen (i.e., blocking antibodies) (Cooke et al. 1935; Loveless 1940). Several recent studies have been performed using well defined systems based on purified allergens and epitopes confirming that the induction of allergen-specific blocking antibodies is a major mechanism for the efficacy of SIT (reviewed in Larche et al. 2006; Valenta et al. 2010, 2004). Today we know that blocking IgG not only inhibits allergen-induced mast cell and basophil activation and thus immediate symptoms of allergy but also IgE-facilitated allergen presentation to T cells and thus reduces chronic allergic inflammation. Moreover, allergen-specific blocking IgG inhibits boosts of allergen-specific IgE production by capturing allergens and thus decreases allergen-specific IgE, a mechanisms which may be important for the long-lasting effects of SIT. We also know now that IgE antibodies mainly recognize conformational epitopes on allergens which require folded allergens with intact three-dimensional structure (Valenta et al. 2010). The latter has been recently visualized by the solution of the three-dimensional structures of complexes consisting of allergens and the corresponding patients IgE antibodies (Niemi et al. 2007; Padavattan et al. 2009).

It is therefore possible to engineer allergy vaccines based on allergen derivatives lacking fold and conformational IgE epitopes or in which amino acids necessary for IgE recognition have been altered (i.e., recombinant hypoallergenic allergen derivatives) (Valenta 2002). These molecules have been successfully used in immunotherapy trials and showed a lack of IgE-mediated side effects (Niederberger 2004; Klimek et al. 2005; Rak 2009; Purohit et al. 2008). However, since hypoallergenic allergen derivatives still contain allergen-specific T cell epitopes, these vaccines can still induce T cell-mediated side effects such as vaccines based on non-IgE-reactive peptides containing T cell epitopes (Haselden et al. 1999; Campana et al. 2008; Purohit et al. 2008).

Several years ago, Focke et al., have developed an approach for allergy vaccines inducing blocking antibodies using peptides derived from surface exposed areas and thus IgE recognition sites of allergens which lacked structure and hence IgE-reactivity (Focke et al. 2001, 2004). They showed that one or few peptides are sufficient to focus blocking IgG antibodies toward IgE binding sites on allergens. To induce peptide-specific and thus allergen-specific IgG antibodies they used foreign (i.e., non allergen-derived) carrier molecules such as KLH. Following the hapten carrier

principle described by the Nobel laureate Benacerraf robust IgG responses against the peptide and antigens containing the peptides can be obtained using T cell help from the carrier (Siskind et al. 1966). The big advantage of the use of allergen-derived peptides lacking allergenic activity for the induction of allergen-specific blocking antibodies is that by choosing suitable peptides a large portion of allergen-sequences containing allergen-specific T cell epitopes can be eliminated so that these vaccines hold promise of not inducing T cell-mediated side effects (Focke et al. 2010). There is more and more data accumulating that suitable peptides can be identified for the most common allergens (e.g., grass pollen, birch pollen, house dust mites, olive pollen, cat) (Focke et al. 2001, 2004; Kundig et al. 2006; Twaroch et al. 2011; Niespodziana et al. 2011) and that these vaccines also have strongly reduced allergenicity i.e., they induce no or low allergic sensitization to the corresponding allergens and thus may be useful not only for therapeutic but also for prophylactic allergy vaccination.

Kündig et al. used the peptide approach in a phase I trial using a synthetic 16-amino-acid sequence of the allergen house dust mite Der p 1 which was chemically coupled to “virus-like particles”, in fact to the bacteriophage Qbeta protein (Kundig et al. 2006). The vaccine induced IgG antibodies against Der p 1 and the virus-like particle and was well tolerated in healthy adults.

Since chemical coupling represents a poorly reproducible and cumbersome technology which is not well suited for vaccine production according to good manufacture practice (GMP) standards it is desirable to directly produce recombinant fusion proteins consisting of allergen-derived peptides lacking allergic activity and suitable carrier molecules which are safe and eventually foster a beneficial type of immune response.

4 Combination Vaccine against Allergies and Infectious Diseases

In search of suitable carrier molecules for the production of recombinant fusion proteins containing allergen-derived peptides without allergenic activity we considered pathogen-derived proteins because they may offer several advantages (Fig. 1). First, they may offer strong T cell help for robust IgG antibody production and thus have almost adjuvant-like activity, eventually twisting the resulting immune response away from the Th2 bias. Second, the pathogen-derived carrier proteins may induce anti-pathogen immunity and hence be useful for the prevention of infectious diseases (Fig. 2). Finally, it may be possible to use pathogen-derived molecules which have a proven track record of being safe vaccines in humans. In fact, vaccination against pathogens, in particular against viruses, bacteria and bacterial toxins is routinely applied in children for prophylactic purposes. In a first proof of principle study we have therefore expressed a fusion protein consisting of the capsid protein VP1 of human rhinoviruses and a peptide

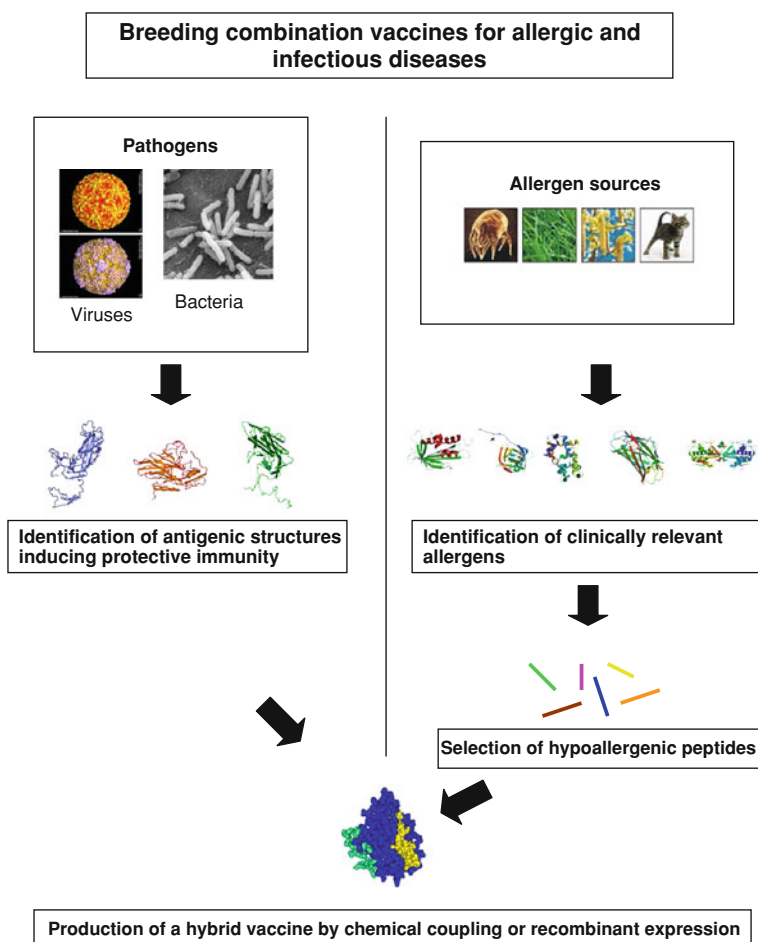


Fig. 1 Breeding combination vaccines for allergic and infectious diseases

derived from the major timothy grass pollen allergen, Phl p 1 (Edlmayr et al. 2009). The intention was to design an allergy vaccine which should not induce IgE- or T cell-mediated side effects and which may induce a protective effect against rhinovirus infections which are known as a major cause of asthma exacerbations (Holgate 2006). The rationale for selecting the viral protein VP1 was that it is involved in the interaction of the virus with ICAM 1 on epithelial cells (Greve et al. 1989). It was thus hoped that anti-VP1 antibodies would prevent the infection of epithelial cells by human rhinovirus. A recombinant fusion protein consisting of VP1 and a non-allergenic peptide of the major timothy grass pollen allergen Phl p 1 was made (Edlmayr et al. 2009). It was demonstrated that this fusion protein lacked IgE- or T-cell reactivity, but induced Phl p 1 specific blocking antibodies in mice and rabbits which inhibited allergic patients' IgE binding to the complete Phl

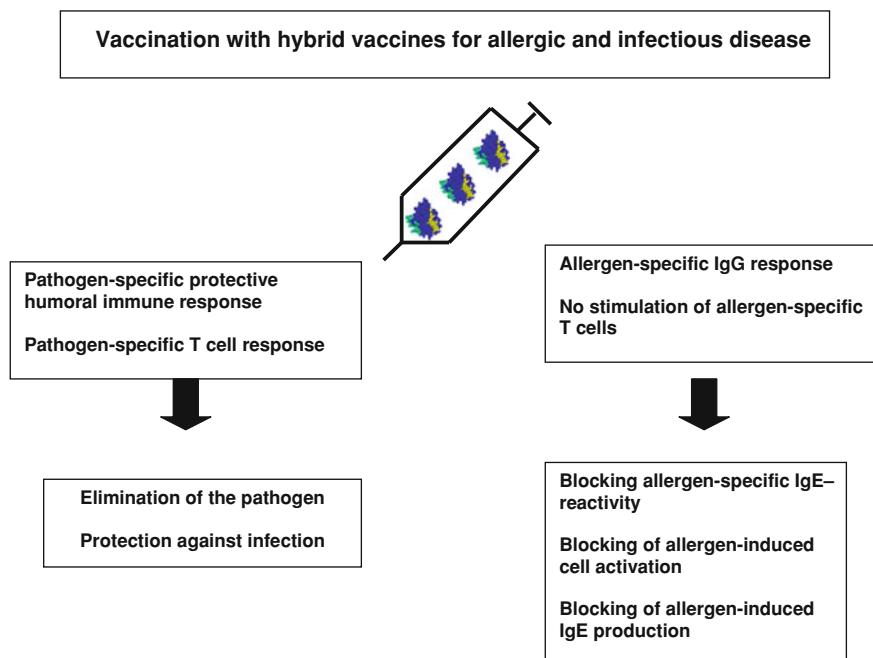


Fig. 2 Vaccination with hybrid vaccines for allergic and infectious disease

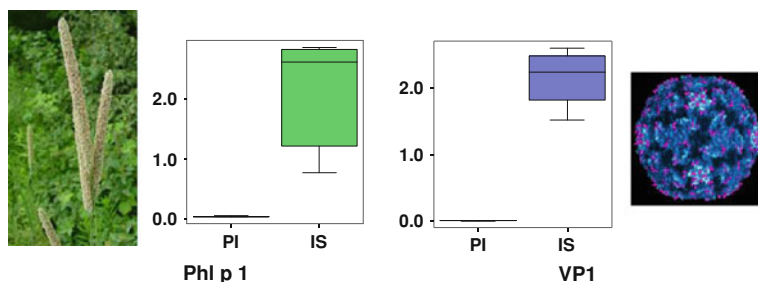


Fig. 3 Immune responses of immunized mice. Groups of mice were immunized with Phl p 1 (left) and VP1 (right). Serum samples were taken on the day of the first immunization (PI) and in three weeks interval. The immune sera (IS) were taken after three injections (*x-axis*). Phl p 1-specific IgG1 and VP1-specific titers were measured by ELISA and are expressed as optical value on the *y-axis*. The optical value corresponds to the level of IgG1 antibody in the mouse sera. The results are shown as *box plots* where 50% of the values are within the boxes and non-outliers between the bars. The lines within the boxes indicate the median values. Left: pictures of a meadow with timothy grass (*Phleum pratense*) right: rhinovirus image

p 1 allergen (Figs. 3, 4). It also blocked Phl p 1-induced immediate allergic inflammation as demonstrated by the inhibition of allergic patients' basophil degranulation. The VP1-based grass pollen vaccine induced in mice mainly

Fig. 4 Inhibition of grass pollen allergic patients IgE binding to Phl p 1 with rabbit antibodies against Phl p 1 and VP1-P5. Mean of percent inhibition of patient's ($n = 19$) IgE binding to Phl p 1 by preincubation with antisera against Phl p 1 or VP1-P5 (x-axis). The percent inhibition is shown at the y-axis

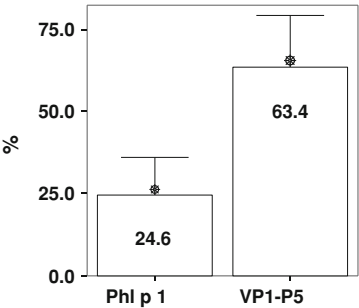
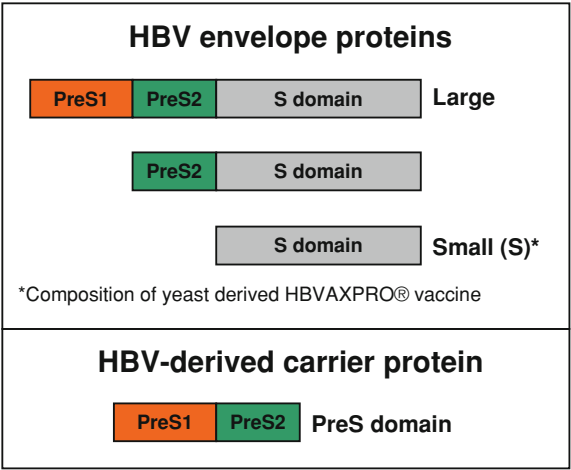


Fig. 5 Representation of the HBV envelope proteins and HBV-derived carrier protein. HBV envelope is composed of three related surface proteins known as large (L), middle (M) and small (S). These three proteins are products of the PreS1, PreS2 and S regions of the *env* gene. The small (S) envelope protein is widely and safely used worldwide for hepatitis B vaccination



VP1-specific T cell responses that shifted the Th2 response toward a mixed Th1/Th2 response. The resulting IgG subclasses were predominantly IgG1, but also IgG2a/b antibodies in mice vaccinated with the fusion protein.

In addition, the VP1 carrier induced IgG antibodies which prevented infection of epithelial cells against infection by rhinovirus and exhibited an unexpected broad cross-neutralization of several rhinovirus strains (Edlmayr et al. 2011).

Using a similar approach Niespodziana et al. produced a non-allergenic peptide vaccine consisting of the hepatitis PreS domain (Fig. 5) fused to non-allergenic peptides of the major cat allergen Fel d 1 and showed that this vaccine induced strong allergen-specific IgG responses which inhibited allergic patients IgE binding to Fel d 1 (Figs. 6, 7) (Niespodziana et al. 2011). The PreS fusion proteins could be expressed in large amounts in *Escherichia coli* under well standardized conditions and are attractive allergy vaccine candidates because PreS has been used safely for vaccination of newborns and children in clinical trials (Schumann et al. 2007).

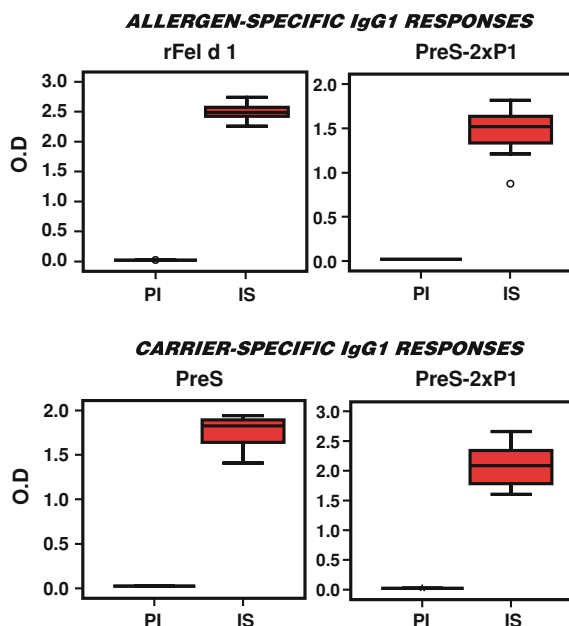
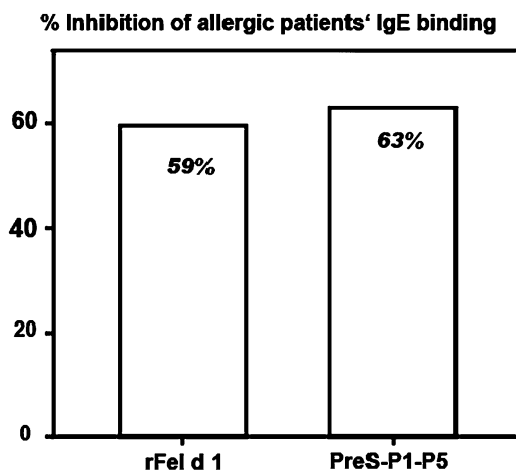


Fig. 6 Allergen-specific and carrier-specific IgG₁ responses in mice. Groups of mice were immunized subcutaneously with rFel d 1, PreS and PreS-2xP1 (*top of the boxes*). Serum samples were taken on the day before the first immunization (PI) and after the third injection (IS) (*x-axis*). rFel d 1-specific (*top*) and PreS-specific (*bottom*) IgG₁ titers were determined by ELISA and are expressed as optical values on the *y-axis*. The optical value corresponds to the level of IgG₁ antibody in mouse sera. The results are shown as *box plots* where 50% of the values are within the boxes and non-outliers between the bars. The line within the *boxes* indicates the median values

Fig. 7 Inhibition of cat allergic patients' IgE binding to rFel d 1 by anti-rFel d 1 and anti-PreS-P1-P5 rabbit IgG antibodies. Inhibitions of cat allergic patients' IgE binding to rFel d 1 by rabbit antisera (*x-axis*) are expressed as percentages of inhibition using preimmune sera as a reference. The mean percent inhibition for 26 patients is shown at the *y-axis*



At present the preclinical evaluation of a grass pollen vaccine based on non-allergenic peptides of the four major timothy grass pollen allergens, Phl p 1, Phl p 2, Phl p 5 and Phl p 6, has been performed and safety skin tests and first safety and dose-finding immunotherapy trials in allergic patients are scheduled for 2011. According to preclinical testing, the new generation of non-allergenic vaccines based on fusion proteins consisting of viral carrier proteins and allergen-derived peptides has similar properties as the recombinant hypoallergenic allergen derivatives but should not induce IgE or T cell-mediated side effects. It is thus hoped that this new generation of vaccines will break the ground for the broad, convenient (i.e., only few administrations) and side effect-free applicability of SIT and eventually be useful for prophylactic allergy vaccination.

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