

REVIEW ARTICLE

Recombinant allergens for allergen-specific immunotherapy: 10 years anniversary of immunotherapy with recombinant allergens

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Abstract

The broad applicability of allergen-specific immunotherapy for the treatment and eventually prevention of IgE-mediated allergy is limited by the poor quality and allergenic activity of natural allergen extracts that are used for the production of current allergy vaccines. Today, the genetic code of the most important allergens has been deciphered; recombinant allergens equalling their natural counterparts have been produced for diagnosis and immunotherapy, and a large panel of genetically modified allergens with reduced allergenic activity has been characterized to improve safety of immunotherapy and explore allergen-specific prevention strategies. Successful immunotherapy studies have been performed with recombinant allergens and hypoallergenic allergen derivatives and will lead to the registration of the first recombinant allergen-based vaccines in the near future. There is no doubt that recombinant allergen-based vaccination strategies will be generally applicable to most allergen sources, including respiratory, food and venom allergens and allow to produce safe allergy vaccines for the treatment of the most common forms of IgE-mediated allergies.

When Leonard Noon conducted and published the first study using grass pollen extract for desensitization of hay fever patients 100 years ago, neither the pathomechanisms underlying allergy nor the mechanisms of action of desensitization were known (1). In a pivotal experiment, Prausnitz & Küstner demonstrated that allergic reactions depend on the presence of a serum factor (i.e., IgE antibodies) in allergic patients which is highly specific for the disease-eliciting antigen (i.e., allergen) and a 'tissue component' (i.e., mast cells) occurring in allergic as well as nonallergic individuals (2). Interestingly, the Prausnitz & Küstner experiment provided the methodology that was used in another pivotal study to investigate the mechanisms underlying allergen-specific immunotherapy (SIT) (3). In fact, Cooke et al. (3) were able to demonstrate a few years after the publication of the Prausnitz & Küstner experiment that SIT induces a second type of immunity against allergens which competes with the allergic immune responses. They showed that serum from patients obtained after SIT contained allergen-specific IgG which blocked allergic inflammation in the skin in a strictly aller-

gen-specific manner. Since then, numerous SIT studies have been conducted, and SIT has been established as the only allergen-specific, disease-modifying allergy treatment with long-lasting effects (4–7). The induction of allergen-specific IgG antibodies that block the binding of allergic patients' IgE to allergen and IgE-mediated allergic inflammation as well as IgE-dependent immunoregulation has meanwhile been well established as important mechanism of SIT (5, 8).

However, since its introduction in 1911, one of the major bottlenecks of SIT has been the use of ill-defined allergen extracts that are often responsible for poor efficacy and side-effects of SIT. There have been continuous attempts to improve the quality of natural allergen extracts by various forms of standardization (e.g., determination of protein contents, assessment of biological activity, antibody-based measurement of allergens), but it is ultimately not possible to overcome the fundamental problems of natural allergen extracts. These problems include batch to batch variations, lack or poor representation of allergens, poor immunogenicity of certain allergens, varying allergenic activities of allergens,

presence of nonallergenic materials and contaminations, just to name a few. Several recent studies investigating the allergen composition of commercial allergen extracts demonstrated the shortcomings of commercial allergen extracts (9–12).

Most, if not all problems, of natural allergen extracts can now be overcome because DNA sequences of allergens became available from 1988 onwards and allowed preparing defined recombinant allergens and hypoallergenic allergen derivatives for diagnosis and SIT. In the next chapter, a summary of some developments and milestones starting from the cloning of allergen genes to the introduction of recombinant allergen-based vaccines into clinical use will be given.

From the cloning of allergen genes to recombinant allergen-based vaccines

The introduction of recombinant DNA technology into the biomedical field stimulated several research groups to apply this technology to the characterization of allergens. Figure 1 gives an overview of some milestones from the cloning of the first allergen-encoding cDNAs to the introduction of recombinant allergens into diagnosis and therapy of allergy. The first allergen-encoding cDNA sequences were reported in 1988–1989 (13–15). At the XIVth Congress of the European Academy of Allergy and Clinical Immunology 1989, one of the workshops 'Epitopes of atopic allergens' was dedicated to the molecular characterization of allergens by recombinant DNA technology (16). The close DNA and amino acid

sequence similarities of major allergens for example from birch, alder, hazel and hornbeam as well as certain plant food (e.g., apple) (17, 18) provided the molecular basis for the earlier observed extensive immunological crossreactivities between these allergen sources (19) and the clinical notion that SIT with one crossreactive allergen was equally effective as SIT with a mixture of several crossreactive allergens (20–22). Moreover, cDNAs coding for highly crossreactive allergens (e.g., profilin) were isolated, explaining crossreactivity between unrelated allergen sources through the occurrence of structurally and immunologically related and therefore crossreactive allergens in these sources (23, 24). The extensive crossreactivities observed among allergens led to and substantiated the concept of using representative allergens from highly conserved allergen families which carry most of the relevant epitopes for diagnosis and therapy instead of mixtures of crossreactive allergens (25).

Soon after the isolation of the first allergen-encoding cDNAs, recombinant allergens were produced and shown to be useful for *in vitro* allergy diagnosis (26–28). These studies demonstrated that a few recombinant allergens allowed replacing natural allergen extracts by recombinant allergens and revealed the big advantage of recombinant allergens for the determination of the molecular sensitization profiles of allergic patients, later termed component-resolved diagnosis (29, Fig. 2). With the availability of allergen sequences and purified recombinant allergens, allergen-specific cellular immune responses (i.e., T-cell responses, specific basophil activation) (30–32) were investigated, and *in vivo* animal models based on defined and clinically relevant allergens were established (33–36).

When naturally occurring hypoallergenic isoforms of allergens were discovered by PCR-based cloning and hypoallergenic allergen fragments were described, several research groups started to engineer recombinant hypoallergenic allergen derivatives for SIT to obtain vaccines with reduced allergenic activity and to increase the safety of SIT (37–44). Based on the knowledge of allergen sequences, structures and location of B- and T-cell epitopes, several approaches for the development of hypoallergenic allergy vaccines have been pursued. Figure 3 gives an overview of the various candidate molecules. The approach of using T-cell epitope-containing peptides lacking IgE reactivity (Fig. 3A) was thought to induce T-cell tolerance without causing IgE-mediated side-effects (reviewed in 45). However, immunotherapy trials mainly performed with peptides from the major cat allergen, Fel d 1, were only partially successful and showed that the peptides could induce systemic late-phase side-effects (reviewed in 45). Another problem with T-cell peptides is that rather complex mixtures of the peptides need to be administered to cover the diverse T-cell epitope repertoire of patients.

In contrast to T-cell peptides, the first generation of recombinant hypoallergenic allergen derivatives was made to exhibit reduced IgE reactivity and hence reduced allergenic activity. Furthermore, they should preserve most, if not all, allergen-specific T-cell epitopes and induce upon vaccination allergen-specific IgG antibodies that inhibit IgE recognition

Some milestones: recombinant allergens in allergy diagnosis and treatment	1988–89	First cloning of allergen-encoding cDNAs
	1989–92	DNA and amino acid sequence similarities – the basis for the 'one for all' concept
	1991–92	IgE-based diagnosis with recombinant allergens
	1992–96	Development of animal models based on recombinant allergens
	1993–97	Construction and characterization of recombinant hypoallergens for allergy vaccination
	1994–96	First studies using recombinant allergens for provocation testing in allergic patients
	1996	First population studies using recombinant allergens
	1999	Monitoring the course of SIT using recombinant allergens
	1999–2000	Evaluation of recombinant hypoallergens in patients by provocation testing
	2002	The concept of marker allergens for the identification of cross-sensitizations and for selection of patients for SIT
	2002	Microarrayed recombinant allergens for diagnosis
	2001–04	First immunotherapy trial with recombinant hypoallergens
	2002–05	First immunotherapy trial with recombinant wild-type allergens
	2002–08	Comparison of extract-, purified allergen-, and recombinant allergen-based SIT
	2008	Recombinant allergens as reference materials for allergenic products
	2009	Phase III studies with recombinant hypoallergens completed
	2006–10	First SLIT studies with recombinant allergens

Figure 1 Milestones from the cloning of first allergen-encoding cDNAs to the application of recombinant allergens to allergy diagnosis and treatment.

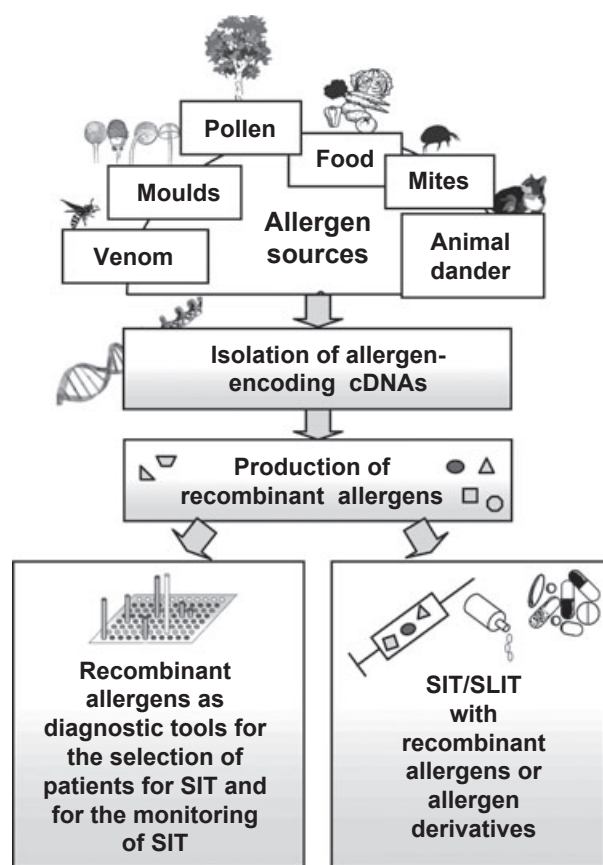


Figure 2 From allergen sources to new strategies for allergy diagnosis and treatment. Based on allergen-encoding cDNAs, recombinant allergens equalling their natural counterparts can be produced and used for component-resolved allergy diagnosis for monitoring of disease and accurate prescription of specific immunotherapy (SIT). Furthermore, recombinant allergens and hypoallergenic allergen derivatives can be produced for SIT.

of the wild-type allergen (reviewed in 8, 44) (Fig. 3). The reduction of IgE reactivity in recombinant hypoallergenic allergen derivatives is achieved by a destruction of the allergen's fold and thus of the conformational IgE epitopes. This can be achieved by the production of recombinant fragments (41, 43), denaturation of the recombinant wild-type allergen (e.g., folding variant) (46) and reassembly of allergen fragments in the form of mosaics (47). In recombinant isoforms (39) or mutants (40, 42), the reduction of IgE reactivity may be achieved either by removal of amino acids or peptides that are involved in IgE binding or by change of the allergen fold through sequence alterations. Recombinant allergen oligomers represent an interesting variation because they can preserve IgE reactivity but loose allergenic activity because of altered presentation of IgE epitopes (48, 49).

Recently, another strategy is emerging which aims at eliminating IgE- as well as T-cell-mediated side-effects. For this purpose, recombinant fusion proteins are prepared which consist of allergen-derived peptides – derived from the

conformational IgE-binding sites of allergens but lack IgE reactivity – and nonallergen-derived carrier proteins (e.g., viral proteins) (50–52). Upon immunization, these vaccines focus IgG antibodies towards the IgE-binding sites on allergens but do not activate allergen-specific T cells because the T-cell help is derived from T-cell epitopes of the carrier protein.

It is interesting to note that the first clinical immunotherapy trial performed in allergic patients was actually conducted with recombinant hypoallergenic allergen derivatives (53), and a vaccine based on recombinant hypoallergenic folding variant of the major birch pollen allergen, Bet v 1 (46), has successfully passed phase III trials (54) (Table 1).

From 1994 on, recombinant allergens were applied for *in vivo* provocation testing in allergic patients with the aim to compare their biological activity with natural allergens and to explore their usefulness for *in vivo* diagnosis (28, 55, 56). These studies confirmed the biological equivalence of most of the recombinant allergen preparations with the corresponding natural allergens, indicating that recombinant allergens can substitute natural allergen extracts also for *in vivo* application.

Allergic patients from different parts of the world grow up being exposed to different allergen sources depending on climate, culture, socioeconomic factors, nutrition habits and other factors that may influence allergen exposure during early childhood, the period when allergic sensitization most likely occurs (57). Allergic patients from different populations thus may exhibit relevant differences regarding their sensitization profiles towards certain allergen molecules. The first population studies conducted with recombinant grass pollen allergens showed a rather similar sensitization profile to recombinant grass pollen allergens in patients from different continents, most likely due to the ubiquitous distribution of grasses and the high degree of crossreactivity among the major grass pollen allergens all over the world (58). However, several other population studies performed with recombinant allergens revealed striking differences in molecular sensitization profiles in different populations which could not have been detected with the use of allergen extracts (59–61). For example, it was found that birch pollen-sensitive patients from Northern Europe react predominantly with the major birch pollen allergen, Bet v 1, whereas patients from southern parts of Europe 'appear' birch pollen sensitized because of recognition of crossreactive allergens (59). Based on these studies, the use of recombinant marker allergens was suggested to obtain an accurate diagnosis of genuine sensitizations against allergen sources and to use molecular diagnosis for improved diagnostic selection of patients for immunotherapy (62). Today, diagnostic tests based on recombinant allergens allowing the accurate diagnosis of the disease-eliciting allergen sources and thus improved prescription of SIT are available for the most common allergen sources (Fig. 2). Despite the fact that the success of immunotherapy obviously depends on more factors than only the accurate prescription of the treatment (e.g., quality of the vaccine, host immune response, etc.), it has been shown that the selection of patients who are sensitized to the major allergens of an allergen source may increase the clinical success of treatment.

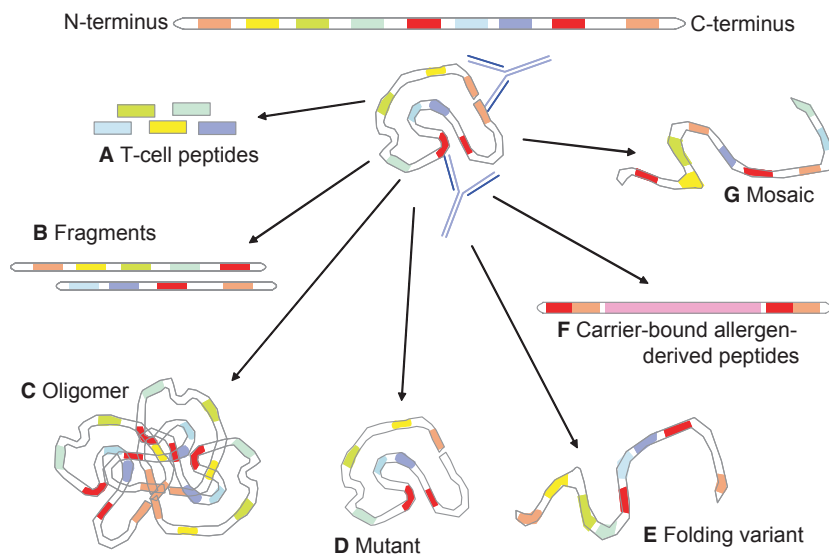


Figure 3 Approaches for the generation of hypoallergenic allergy vaccines. On the top, a schematic representation of the primary allergen structure from the N- to the C-terminus is shown. Peptides involved in the formation of conformational IgE epitopes in the folded allergen (below) are coloured in orange and red while peptides recognized by T cells are in green, blue and yellow. Isolated T-cell-reactive peptides (A) lack IgE epitopes, recombinant allergen

fragments (B), oligomers (C), folding variants (E) and mosaics (G) contain T-cell-reactive epitopes, but because of a lack of the original fold lost IgE reactivity. In recombinant allergen mutants (D), amino acids directly involved in IgE binding have been changed and/or the mutations may disturb the original fold. Carrier-bound allergen-derived peptides (F) lack IgE reactivity and allergen-specific T-cell epitopes.

Already in 1990, Birkner et al. (63) noted that patients with preferential sensitization to the major birch pollen allergen, Bet v 1, responded better to birch SIT than patients with sensitizations to other allergens, a finding that has recently been confirmed also by other investigators (64).

From 1999 onwards, first studies appeared in which recombinant allergens were used to monitor immunological effects and also efficacy during SIT (65–68). These studies provided important information that cannot be obtained with allergen extracts. For example, it was demonstrated that SIT with allergen extracts induced highly variable immune responses against allergens and nonallergenic moieties and sometime *de novo* sensitizations (67, 68). Furthermore, it was shown that lack/poor representation or poor immunogenicity of major allergens may cause insufficient production of protective allergen-specific IgG responses, which are important for the success of SIT (68).

The use of recombinant allergens for diagnosis and for the monitoring of allergen-specific IgG responses was greatly facilitated by the development of multi-allergen test systems based on micro-arrays (69). The first study describing the use of micro-arrayed allergens for diagnosis of allergy was published in 2002. It demonstrated that the IgE reactivity profile towards a large number of different allergen molecules can be determined simultaneously by probing chips containing the micro-arrayed allergens with only a few microlitres of serum. Allergen chips can be employed for IgE testing as well as for the detection of antibodies belonging to all other immunoglobulin classes and subclasses (70).

Multiallergen tests based on recombinant allergens are now available for routine allergy diagnosis and for monitoring the effects of SIT. They are also used in various clinical trials to study their usefulness for answering various clinical questions such as diagnosis of food allergy, population studies and monitoring of allergic sensitization, particularly in childhood to obtain information useful for predicting the course of allergic disease (71–74).

Clinical studies published from 1999 onwards showed that recombinant hypoallergenic allergen derivatives exhibit strongly reduced allergenic activity when compared with the corresponding wild-type allergens by provocation testing in allergic patients and thus encouraged these molecules to be used for SIT (75, 76). The first immunotherapy trial with recombinant material was therefore carried out with hypoallergenic derivatives of the major birch pollen allergen, Bet v 1 (53). Thereafter, several other clinical immunotherapy trials were initiated with recombinant wild-type allergens from grass and birch pollen (Table 1).

Current state of clinical immunotherapy studies with recombinant allergens: 10 years anniversary of recombinant allergen-based SIT

This year we are celebrating the 100 years anniversary of allergen-specific immunotherapy (1). However, it will be also the 10 years anniversary that allergic patients were treated for the first time with recombinant allergen derivatives (53). The first immunotherapy trial with recombinant

Table 1 Immunotherapy trials with recombinant allergens/allergen derivatives

Vaccine	Allergen source/active components	Study design	No. of patients	Application mode/adjuvant	References ClinicalTrials.gov ID
Recombinant wild-type allergens	Grass pollen/Phl p 1, Phl p 2, Phl p 5a, Phl p 5b, Phl p 6	DBPC Phase II; Dose response Safety and efficacy	62	SC/Alum	84 NCT00666341
	Grass pollen/Phl p 1, Phl p 2, Phl p 5a, Phl p 5b, Phl p 6	DBPC Phase III Safety and efficacy	498	SC/Alum	NCT00671268
	Birch pollen/Bet v 1	DBPC Phase II	134	SC/Alum	85 NCT00410930
	Birch pollen/Bet v 1	DBPC; Phase I; Safety, tolerability and pharmacodynamic effects	60	SL (Tablet)	NCT00396149
	Birch pollen/Bet v 1	DBPC; Phase I; Safety, tolerability and pharmacodynamic effects	112	SL (Tablet)	86 NCT00889460
	Birch pollen/Bet v 1	DBPC; Phase IIb/III Safety and efficacy	483	SL (Tablet)	87 NCT00901914
	Birch pollen/Bet v 1 fragments/trimer	DBPC Phase IIb	124	SC/Alum	53, 78, 81
Hypoallergens	Birch pollen/Bet v 1 folding variant	Open; Phase II Safety and efficacy	51	SC/Alum	83 NCT00266526
	Birch pollen/Bet v 1 folding variant	DBPC; Phase III Safety and efficacy	211	SC/Alum	54 NCT00309062
	Birch pollen/Bet v 1 folding variant	DBPC; Phase III Safety and efficacy	800	SC/Alum	NCT00554983
	Birch pollen/Bet v 1 folding variant	DBPC; Phase II; Immunological and histological evaluation	n.i.	SC/Alum	NCT00841516

DBPC, double-blind placebo-controlled; SC, subcutaneous; SL, sublingual; n.i., not indicated.

ClinicalTrials.gov ID refers to the identifier on www.ClinicalTrials.gov.

hypoallergenic derivatives of the major birch pollen allergen, Bet v 1, started in fact in 2001 (53). In this double-blind, placebo-controlled trial, 124 birch pollen allergic patients were included. Active treatment was performed with aluminium hydroxide-adsorbed recombinant Bet v 1 fragments (Fig. 3B) or a recombinant hypoallergenic Bet v 1 trimer (Fig. 3C) or aluminium hydroxide alone as placebo. This study demonstrated that subcutaneous immunotherapy with the hypoallergenic recombinant Bet v 1 derivatives induced IgG antibodies which recognized natural Bet v 1, inhibited allergic patients' IgE binding to Bet v 1 and Bet v 1-induced basophil activation (53, 77–81). It was further shown that the induction of Bet v 1-specific IgG antibodies was associated with reduced nasal sensitivity (78). In addition, reduced boosts of Bet v 1-specific IgE production caused by seasonal allergen exposure were noted for actively treated patients (53). The Bet v 1-specific IgG antibodies induced by vaccination with the recombinant Bet v 1 derivatives were directed against new epitopes, demonstrating the vaccination character of the treatment (79). Vaccine-induced IgG also blocked IgE-facili-

tated binding of allergens to antigen-presenting cells, a process that contributes to reduction of T-cell activation (80, 82). It was also found that vaccination with recombinant hypoallergenic derivatives did not induce IgE-mediated immediate type side-effects (81) or clinically relevant *de novo* IgE sensitizations because of the strong reduction of allergenic activity of the vaccine (80).

Based on the encouraging results obtained with rBet v 1 fragments and trimer, a recombinant hypoallergenic folding variant (46, Fig. 3E), resembling most of the features of the hypoallergenic Bet v 1 derivatives that had been used in the initial trial, was successfully tested in several clinical trials up to phase III and should become available for SIT in Europe soon (54, 83) (Table 1).

Besides the clinical trials with recombinant hypoallergenic Bet v 1 derivatives, two other lines of vaccine development based on recombinant allergens have been started. In one approach, a mix of recombinant major timothy grass pollen allergens (i.e., Phl p 1, Phl p 2, Phl p 5 and Phl p 6) was adsorbed onto aluminium hydroxide and used for subcutane-

ous immunotherapy of grass pollen allergic patients. The first study demonstrated that also vaccination with recombinant wild-type allergens induced allergen-specific IgG antibodies against natural grass pollen allergens and was clinically effective (84). In the Clinical Trial database of the NIH a clinical phase III study with recombinant grass pollen allergens is also registered (Table 1).

In another approach, recombinant wild-type birch pollen allergen, rBet v 1, was compared with natural purified Bet v 1 and birch pollen extract in a double-blind placebo-controlled study (85). This study showed that SIT with rBet v 1 was clinically effective and offered advantages over extract treatment because sensitizations against minor birch pollen allergens were not observed in the group of patients who had received rBet v 1. It also showed that active treatment induced allergen-specific IgG antibodies that were associated with significant clinical improvement as determined using symptom medication scores and by using objective parameters such as cutaneous sensitivity (85) (Table 1).

From all the results that are available from clinical studies with recombinant allergens or hypoallergens, it can be concluded that subcutaneous administration induced robust allergen-specific IgG antibody responses.

However, the subcutaneous immunotherapy trial with rBet v 1 wild-type allergen was carried out as a proof of principle study to compare the recombinant allergen with natural allergen extract. It was thought to serve as a pilot study for the development of a rBet v 1-based tablet for sublingual treatment. The entries in the Clinical trial database indicate that several studies up to phase II studies have been initiated using rBet v 1 tablets (86, 87) (Table 1).

Although Table 1 indicates that there are some clinical trials published and ongoing with recombinant allergens, it is quite clear that these are far too few and include only major allergen sources.

Ten years anniversary of recombinant allergen-based vaccines: now and the future?

When we celebrate this year the 100 years anniversary of allergen-specific immunotherapy, we can say without doubt that it represents the only antigen-specific, disease-modifying and long-lasting form of treatment for allergy, a disease affecting a quarter of the world's population. Through the availability of recombinant allergens and new technologies for producing safer vaccines, it has become possible to produce new forms of effective allergy vaccines which will be devoid of side-effects and may be used not only for therapeutic but even for prophylactic vaccination (52, Fig. 3).

The following five statements are confirmed by the now available data:

First, clinical immunotherapy trials clearly indicate that recombinant allergens and hypoallergenic derivatives are effective for subcutaneous immunotherapy, and first vaccines will soon be registered and become available for clinical routine use (Table 1).

Second, studies performed with recombinant allergens have clearly delineated mechanisms underlying the treatment. The induction of allergen-specific IgG antibodies that inhibit immediate and chronic allergic inflammation as well as the boosts of IgE production has been identified as one common mechanism.

Third, the preclinical characterization of numerous candidate vaccines (Fig. 3) with promising features has been completed for many allergen sources (8, 44, 88). In fact, several research groups have defined clinically relevant allergens and produced hypoallergenic versions thereof for improving the safety of immunotherapy. A variety of well-established experimental *in vitro* and *in vivo* (i.e., animal systems as well as *in vivo* provocation testing in patients) test systems have been established which allow detailed preclinical testing of the vaccines and the identification of the most suitable candidate molecules for immunotherapy trials.

Fourth, the production of recombinant allergen-based vaccines can be carried out under controlled conditions following the highest standards of good manufacture practice (GMP) at reasonable cost and independent from natural raw materials. In fact, recombinant allergens are now being considered reference substances (89) (Fig. 1).

Finally, recombinant allergen-based diagnostic tests for the accurate prescription and monitoring of immunotherapy are available (69).

Therefore, the burden is now on the pharmaceutical industry to overcome the last hurdles regarding development and clinical testing that need to be mastered to make better allergy vaccines available to allergic patients. Now, 10 years after the first application of recombinant allergen for SIT to patients, the time has come to make high-quality vaccines based on recombinant material available to patients care.

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