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Molecular design of allergy vaccines

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Recombinant-allergen-based diagnostic tests enable the dissection and monitoring of the molecular reactivity profiles of allergic patients, resulting in more specific diagnosis, disease monitoring, prevention and therapy. *In vitro* experiments, animal studies and clinical trials in patients demonstrate that allergenic molecules can be engineered to induce different immune responses ranging from tolerance to vigorous immunity. The available data thus suggest that molecular engineering of the disease-related antigens is a technology that may be applicable not only for the design of allergy vaccines but also for the design of vaccines against infectious diseases, autoimmunity and cancer.

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Introduction

In a variety of diseases involving the immune system (e.g. allergy, autoimmunity, infectious diseases, cancer), antigens are either the direct or indirect cause of the disease, or can be used as targets for specific immune intervention; for example, vaccination or tolerance induction. The definition of the disease-related antigens is therefore an absolute prerequisite for developing studies of specific immune intervention strategies, which aim to explore the potency of routes and modes of application, types of adjuvants, and molecular and cellular vehicles (reviewed in [1]).

In the case of type I allergy, which is an IgE-mediated hypersensitivity disease affecting more than 25% of the population, the majority of symptoms (e.g. allergic rhinoconjunctivitis, allergic asthma, food allergy, anaphylaxis and skin manifestations) are caused by protein antigens from a variety of allergen sources, including pollens, mites, animals, insects, moulds and food (reviewed in [2,3]).

Through the application of recombinant DNA technology a tremendous explosion of knowledge regarding the characterization of disease-eliciting allergens and their molecular structure has occurred during the past 15 years. Instead of performing laborious protein chemical characterization of allergens, RNA derived from the primary allergen sources is isolated, converted into DNA and inserted into expression libraries, which can be efficiently screened for allergen-encoding cDNAs with IgE antibodies from allergic patients. Using this technology, almost complete repertoires of the disease-eliciting allergens have been produced as recombinant molecules and are already in clinical use for the diagnosis and monitoring of allergic diseases (Figure 1; [4]).

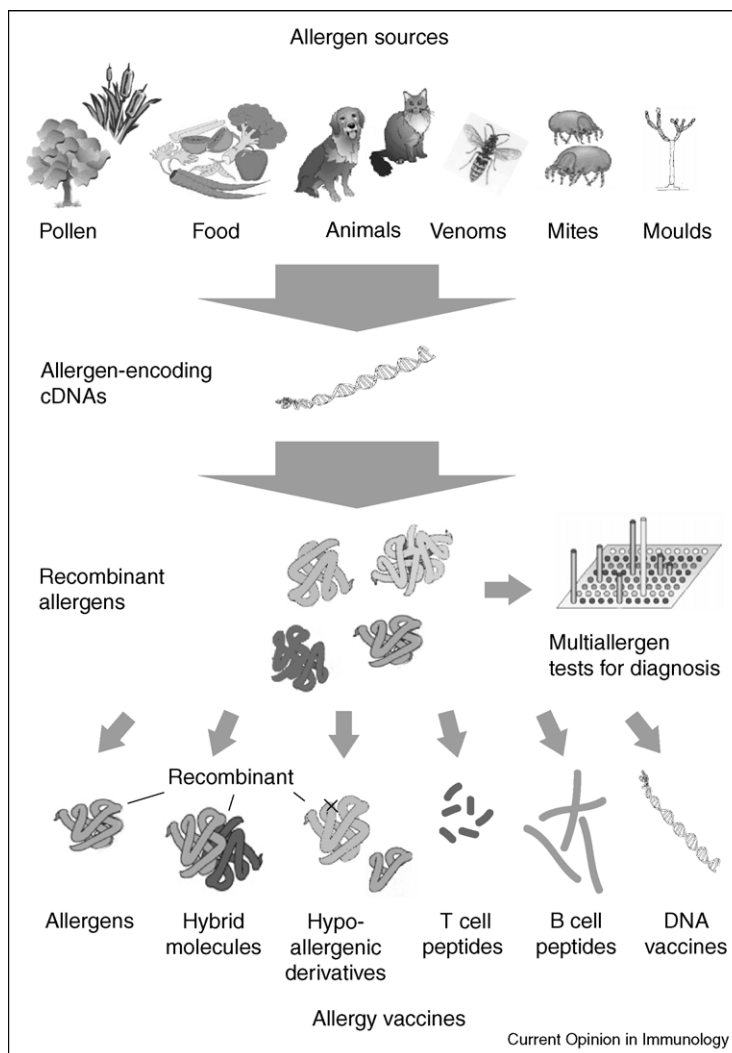
The three-dimensional structures of a variety of important allergens have been solved recently [5–11]. Using recombinant allergens as tools, several basic research studies have been conducted which have analyzed allergen trafficking [12,13], investigated allergen-specific T cell responses [14,15] and their genetic restriction [16,17], and described mechanisms of allergic inflammation [12,18–23]. Some recent review articles provide comprehensive overviews regarding the molecular nature of allergens from important allergen sources [24–30]. Databases, among them Allergome (www.allergome.org), list and describe the continuously increasing number of allergenic molecules which have been characterized by recombinant DNA technology (reviewed in [31]).

This review summarizes how detailed knowledge regarding the nature of allergens can improve diagnosis and therapy of allergy. Specifically, it focuses on the possibility of dictating the outcome of the immune response via modification of the disease-causing allergens.

Molecular reactivity profiles: a rational basis for allergy vaccination

Since the first description of a diagnostic multiallergen test, which was based on a chip containing micro-arrayed allergen molecules [32], several studies have further developed this powerful technology [33–35] (Figure 1). The implications of this technology on future allergy diagnosis and disease management have been summarized elsewhere [36]. Several studies analyzed molecular IgE reactivity profiles with recombinant allergens [37,38], and some of these were performed in specific geographical populations, revealing that reactivity profiles are determined by the allergen molecules to which patients are locally exposed [39–41]. Furthermore, several authors identified molecules in allergen sources that can be used as diagnostic reagents to confirm genuine sensitization towards the allergen

Figure 1



From allergen genes to allergy vaccines. Using allergen-encoding cDNAs, recombinant allergens for diagnosis and therapy can be obtained. Furthermore, various allergy vaccines can be engineered. In a first step, allergen-encoding RNA is isolated from the most common allergen sources and converted into cDNA. Allergen-encoding cDNAs are inserted into expression systems and recombinant wild-type allergens mimicking the properties of natural allergens are produced. These recombinant allergens can be used to develop multiallergen tests for diagnosis. On the basis of allergen sequences and the corresponding DNA it is possible to engineer a variety of allergy vaccines including wild-type-like recombinant allergens, hybrid molecules, hypoallergenic derivatives, T cell and B cell peptides as well as DNA vaccines.

source [42–47]. According to this principle, the clinician can discriminate co-sensitization from cross-sensitization to different allergen sources and hence prescribe allergen-specific forms of treatment with greater accuracy [29].

Marker allergens for the diagnosis of genuine sensitizations have been identified for some important allergen sources, such as tree [29,48], grass- [44] and weed pollens [42], food [43,49–52], mites [46], insects [28,53,54], moulds [47,55] and animal dander [45,56–59]. The diagnostic monitoring of patients undergoing allergen-specific immunotherapy with such recombinant marker allergens has demonstrated that therapy-induced protective aller-

gen-specific IgG antibodies have several beneficial roles and are important for the success of allergen-specific immunotherapy [60–65,66^{••},67[•]]. It has been impossible to measure true allergen-specific IgG responses with crude allergen extracts, because these extracts contain numerous non-allergenic components in addition to allergens, and some important allergens were poorly represented or absent from these extracts (reviewed in [68]).

Current approaches towards allergy vaccination

There are several hypotheses regarding the mechanisms underlying allergen-specific immunotherapy, ranging from

immunodeviation/modulation of Th2 to Th1 responses and tolerance induction to active vaccination inducing allergen-specific IgG antibodies that antagonize IgE-mediated effects (reviewed in [3•]). On the one hand, the immune response may be influenced without altering the antigen (i.e. allergen) in the course of allergen-specific immunotherapy. For this purpose it is possible to change the mode and site of administration, the dose, the type of adjuvant, and molecular and/or cellular vehicles. On the other hand, the outcome of the immune response can be dictated via the disease-causing allergen or modifications thereof.

As shown in Figure 1, it is possible to produce, by recombinant DNA technology, recombinant allergens that completely resemble natural, wild-type allergens. These recombinant allergens contain the relevant B cell and T cell epitopes, and also the allergenic properties of wild-type allergens.

The term 'hybrid molecules' describes molecules that consist of two or more allergens or allergen derivatives (reviewed in [69]). These molecules were developed by recombinant DNA technology to stably incorporate the epitopes of complex allergen sources within one or only a few molecules to facilitate the manufacturing of allergy vaccines. It turned out that such hybrid molecules can exhibit unexpectedly useful immunological properties, such as increased immunogenicity and reduced allergenic activity [69].

The term 'hypoallergenic allergen derivative' describes a recombinant allergen that has been modified to reduce the allergenic activity of the molecule and to preserve T cell epitopes and immunogenicity. The reduction of allergenic activity varies considerably and can be assessed by comparing the hypoallergenic derivative with the wild-type allergen using *in vitro* (e.g. basophil histamine release, CD203c expression) or *in vivo* (e.g. skin prick testing, intradermal testing, nasal provocation testing) methods. The term 'reduction of allergenic activity' thus more precisely defines the reduction of IgE-mediated effects, which should enable the administration of higher doses of the allergen derivative in comparison to the wild-type allergen in the course of treatment.

The term 'maintained immunogenicity' is used when a hypoallergenic allergen derivative can still induce IgG antibodies that both recognize the wild-type allergen and are capable of blocking the recognition of the wild-type allergen by IgE antibodies of allergic patients.

Classical allergen-derived T cell epitope-containing peptides are characterized by their ability to bind to the T cell receptors of allergen-specific T cells without reacting with IgE antibodies, and hence lack allergenic activity. T cell peptides should induce T cell tolerance and/or

anergy but they were also found to induce T-cell-dependent and MHC-dependent late phase allergic reactions [70].

Allergen-derived B cell peptides are characterized by their ability to induce upon vaccination IgG antibodies, which recognize the wild-type allergen and block IgE recognition. These peptides, which are usually rather long peptides (20-40 amino acids), contain surface exposed amino acids and can be obtained via IgE epitope mapping or three-dimensional structure data [71-73]. They are selected for lack of allergenic activity and do not need to contain allergen-specific T cell epitopes. Peptides mimicking B cell epitopes of allergens can be obtained by the mimotope technology but these peptides are rather short and hence do not seem to induce very strong allergen-specific IgG responses upon active vaccination [74].

Vaccination with allergen-encoding DNA should lead to a transfection of cells in the vaccinated host, followed by production of the corresponding protein. DNA vaccination has the goal of inducing an allergen-specific Th1 response that should prevent allergic sensitization and protect against established allergy [75-81].

The molecular design of allergen molecules dictates the outcome of immune responses

Table 1 gives an overview of the molecules used for allergen-specific immunotherapy that have been characterized recently.

Recombinant hypoallergenic allergen derivatives have been produced for several allergen sources. Most of these exhibit reduced allergenic activity in comparison to the corresponding wild-type allergen, and retain T cell reactivity and immunogenicity, as demonstrated by *in vitro* experiments and in animal models. The recombinant hypoallergenic trimer of the major birch pollen allergen Bet v 1 induces strong IgG responses and shows strong T-cell stimulating activity [82,83]. A similar reduction of allergenic activity was observed when hybrid allergens of venom and weed pollen allergens were created [86,95,99].

Hybrid allergens consisting of either two or four of the most important grass pollen allergens retain most of the features of the corresponding wild-type allergens but show a strong induction of T-cell responses and induce high levels of allergen-specific IgG [96,98•]. As exemplified for the grass pollen antigens, it was found that the fusion of molecules with low immunogenicity to other molecules from the same antigen source strongly increases the immunogenicity of the low immunogenic molecules, and this has been discussed as a potentially useful property for the construction of vaccines for other immunologically mediated diseases [69].

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Table 1

Allergy vaccines based on recombinant allergen derivatives, conjugated allergens, peptides or DNA.

	Molecule/peptide	Allergen source	Immunological properties	Induction of protective IgG antibodies	T cell reactivity/ induction of tolerance	Ref
Recombinant hypoallergenic allergen derivatives	Bet v 1 trimer	Birch pollen	Reduced allergenic activity	+	++/ND	[82,83]
	Bet v 1 fragments	Birch pollen	Reduced allergenic activity	+	+/ND	[83,84]
	Bet v 1 mutant	Birch pollen	Reduced allergenic activity	+	+/ND	[6,85]
	Par j 1 mutant	Weed pollen	Reduced allergenic activity	+	+/ND	[86]
	Hev b 6.02	Latex	Reduced allergenic activity	ND	ND	[87]
	Hev b 6.01	Latex	Reduced allergenic activity	ND	+/ND	[88]
	Phl p 7 mutant	Grass pollen	Reduced allergenic activity	+	+/ND	[73]
	Lol p 5 mutant	Grass pollen	Reduced allergenic activity	+	+/ND	[89,90]
Hybrid molecules	Ara h 1-3 + HKLM	Peanut	Reduced allergenic activity, immunomodulation	ND	+/-	[91]
	Api m 1 mutant	Venom	Reduced allergenic activity	+/-	+/ND	[92]
	Fel d 1 mutant	Cat	Reduced allergenic activity	ND	+/ND	[93]
	Gal d 1 mutant	Hen egg white	Reduced allergenic activity	ND	ND	[94]
	Ves v 5 + Pol a 5	Venom	Reduced allergenic activity	ND	+/ND	[95]
	Phl p 1 + Phl p 5	Grass pollen	Increased immunogenicity	+	++/ND	[96]
	Phl p 2 + Phl p 6	Grass pollen	Increased immunogenicity	+	++/ND	[96]
	Api m 1 + Api m 2	Venom	Reduced allergenic activity	ND	+/+	[97]
	Phl p 1+2+5+6	Grass pollen	Increased immunogenicity	+	++/ND	[98]
	Par j 1 + Par j 2	Weed pollen	Reduced allergenic activity	+	+/ND	[99]
Conjugated molecules	Amb a 1/oligonucleotides	Ragweed	Enhanced Th1 response, reduced allergenic activity	ND	+/-	[100,101]
	Bet v 1/S-layer protein	Birch pollen	Immunomodulation, reduced allergenic activity	ND	+/-	[102]
	Fel d 1/IgG Fc gamma	Cat	Inhibition of histamine release from mast cells and basophils, reduced allergenic activity	-	ND/-	[103]
T cell peptides/hybrid T cell peptides	Fel d 1-CBP	Cat	Immunomodulation	ND	ND	[104]
	Der p 2	House dust mite	Lack of allergenic activity	-	+/+	[105]
	Der p 1	House dust mite	Lack of allergenic activity	-	+/+	[106]
	Bet v 1+Phl p 1 +Phl p 5	Birch/grass pollen	Lack of allergenic activity	-	+/+	[107]
B cell peptides	Phl p 1	Grass pollen	Lack of allergenic activity	+	-/-	[71]
	Bet v 1	Birch pollen	Lack of allergenic activity	+	-/-	[72]
	Phl p 7	Grass pollen	Reduced allergenic activity	+	ND	[73]
	Phl p 5	Grass pollen	ND	+/-	ND	[74]
DNA	Der p 1	House dust mite	Enhanced Th1 response	ND	+/-	[75]
	ProDer p 1	House dust mite	Enhanced Th1 response	ND	+/-	[76]
	Bet v 1	Birch pollen	Enhanced Th1 response	ND	+/-	[77]
	Bet v 1 fragments	Birch pollen	Enhanced Th1 response	ND	+/-	[78]
	Phl p 5	Grass pollen	Enhanced Th1 response	ND	ND	[79,80]
	Bet v 1+Cor a 1 + Aln g 1	Tree pollen	Enhanced Th1 response	ND	ND	[81]

HKLM, heat-killed *Listeria monocytogenes*; ND, not done.

Conjugates with reduced allergenic activity of the major ragweed allergen, Am b a 1 with CpG oligonucleotides [100,101] and a Bet v 1 conjugate with bacterial S-layers [102] induced Th1-deviated immune responses in mice and *in vitro*, respectively.

A fusion protein of the major cat allergen Fel d 1 with a truncated human IgG Fcγ1 was made with the intention of inhibiting allergen-induced basophil and mast cell degranulation by co-crosslinking of FcεRI and Fcγ receptors [103]. The inhibition of effector cell degranulation could be shown both *in vitro*, with basophils from one atopic donor, and in transgenic mice expressing the human

FcεRI; however, several questions need to be addressed, among them whether such molecules induce autoimmunity against Fcγ1 upon repeated systemic application, and whether, upon local application, sufficient amounts of the protein penetrate the epithelium and reach the effector cells.

Fel d 1 coupled to carbohydrate-based particles has immunomodulatory effects [104]. Using T cell peptides from Fel d 1 and the major mite allergen, Der p 1, T cell tolerance can be induced in mice [105,106], and a hybrid peptide consisting of the dominating T cell epitopes of the birch pollen allergen Bet v 1 and two

grass pollen allergens induced tolerance in Balb/c mice [107].

Three studies demonstrated that B cell epitope-derived peptides from Bet v 1 and grass pollen allergens induce IgG antibodies upon immunization of animals that block the binding of allergic patient IgE to the wild-type allergens and inhibit allergen-induced basophil degranulation [71–73].

Attempts have been made to improve DNA vaccination technology by using DNA coding for hypoallergenic allergen derivatives to mitigate allergic reactions that might occur through production of allergens *in vivo* [78]. Furthermore, replicon-based DNA vaccines, which stimulate Th1-biased immune responses at low doses and induce self removal of transfected cells, were constructed [79,80].

Current state of clinical trials with designed molecules

In the past few years we have begun to see the first immunotherapy trials conducted with defined molecules and peptides in allergic patients (Table 2).

Peptides containing T cell epitopes either from the major cat allergen Fel d 1 or from bee-venom-derived phos-

pholipase have been administered without adjuvant and mainly by injection into patients [70,111–123]. These T cell epitope-containing peptides either without or with only low IgE-binding capacity were administered to induce T cell tolerance in patients. Although most of these peptides lack IgE reactivity, they induce considerable late phase systemic side effects depending on the dose and route of administration [70,114,116,118]. Current protocols aim to minimize these side effects and seek to foster the beneficial effects of such peptides on silencing allergen-specific T cells, a process in which IL-10 and regulatory T cells may be involved [120*].

Therapy with T cell peptides primarily affects T-cell-mediated late phase symptoms but does not seem to influence IgE-mediated allergic reactions [112,114]. In fact, the majority of studies could not find evidence that therapy with T cell peptides influences allergen-specific IgE levels or IgE-mediated allergic inflammation. In most studies no induction of allergen-specific IgG responses was noted. Only one study reported beneficial effects on immediate-type skin reactions, but the mechanisms behind these effects are not known [117].

Purified Amb a 1, the major allergen of ragweed pollen, was conjugated with CpG oligonucleotides to reduce the allergenic activity of Amb a 1 and to shift the Amb a 1-

Table 2

Immunotherapy trials with molecules and peptides.

	Molecule/peptide	Allergen source	No. of patients	Application	Adjuvant (alum)	Study design	Reference
Recombinant allergens	rPhl p 1 + rPhl p 2 + rPhl p 5 + Phl p 6	Grass pollen	n = 62	SC	+	DBPC	[108*]
Recombinant hypoallergenic allergen derivatives	Bet v 1 trimer/ Bet v 1 fragments	Birch pollen	n = 124	SC	+	DBPC	[66*,67*,109] ^a
T cell peptides	rBet v1-FV	Birch pollen	n = 51	SC	+	Open	[110]
	Fel d 1 chain 1-derived peptides	Cat	n = 95	SC	–	DBPC	[111]
			n = 42	SC	–	DBPC	[112]
			n = 31	SC	–	DBPC	[113]
			n = 133	SC	–	DBPC	[114]
			n = 40	ID	–	Open	[70]
			n = 34	ID	–	SBPC	[115]
	Fel d 1 chains 1+2-derived peptides	Cat	n = 24	ID	–	Open	[116]
			n = 24	ID	–	DBPC	[117]
			n = 28/24	IN/ID	–	Open	[118]
			n = 16	ID	–	DBPC	[119]
			n = 8+12	ID	–	Open	[120*]
			n = 8	ID	–	Open	[121]
	PLA-derived peptides	Bee venom	n = 15	SC	–	Open	[122]
	PLA2-derived peptides	Bee venom	n = 16	SC	–	DBPC	[123]
Conjugated molecules	Amb a 1/ oligonucleotides	Ragweed	n = 57	SC	–	DBPC	[124]

^a A Purohit *et al.*, unpublished. DBPC, double-blind placebo-controlled; FV, folding variant ID, intradermal; IM, intramuscular; IN, intranasal; PLA, phospholipase A; SBPC, single-blind placebo-controlled; SC, subcutaneous.

specific Th2 response to a Th1 response. Such results have been obtained in animal studies, but the data available so far from treated patients indicate that this vaccine induces primarily Amb a 1-specific IgG antibodies [124].

Very recently, the first results from immunotherapy studies conducted in patients with recombinant allergens and recombinant hypoallergenic allergen derivatives became available. The first and largest study was performed as a double-blind, placebo-controlled multicenter study in which 124 patients enrolled for a single treatment course (A Purohit *et al.*, unpublished; [66^{••},67[•],109]). Patients received either an aluminium hydroxide-adsorbed equimolar mix of recombinant hypoallergenic fragments of the major birch pollen allergen Bet v 1 or a hypoallergenic recombinant Bet v 1 trimer, or adjuvant alone. Both of the actively treated groups developed *de novo* IgG antibody responses against the Bet v 1 allergen that inhibited allergen-induced basophil degranulation and reduced the boost of IgE memory responses during birch pollen season [66^{••}].

A substudy demonstrated that the vaccine-induced IgG antibodies were also present in nasal secretions and associated with reduced nasal symptoms as objectively measured by nasal allergen provocation [67[•]]. The treatment improved birch pollen allergy and reduced IgE-mediated immediate type skin responses (A Purohit *et al.*, unpublished). T cell responses were assessed in a subgroup of patients and also indicated that this treatment resulted in successful vaccination [109]. Similar data were obtained in another immunotherapy trial in which patients were treated with a recombinant hypoallergenic folding variant of Bet v 1 [110]. Another study in which 62 grass pollen allergic patients were treated with an equimolar mixture of the most common and clinically relevant grass pollen allergens produced as recombinant wild type allergens reported that this treatment induced IgG₁ and IgG₄ antibodies against natural grass pollen allergens, and was clinically effective [108[•]]. At present more extensive studies are ongoing, analysing recombinant wild-type allergens and recombinant hypoallergenic allergen derivatives for the treatment of birch and grass pollen allergy.

Conclusions

Tremendous progress has been made regarding the molecular characterization of disease-eliciting allergens using recombinant DNA technology. Recombinant allergens are on their way to replacing traditional allergen extracts for the diagnosis of allergy and in allergen-specific immunotherapy. Using recombinant DNA technology, as well as peptide technology, several research groups have demonstrated that allergens can be modified to address different molecules and cells of the immune system and thus to induce immune responses of profoundly varying quality and intensity, such as tolerance or vigorous immu-

nity. These data have the potential to be applicable for a variety of diseases in which the immune system plays an important role, provided that the disease-eliciting or related antigens can be characterized.

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