

Nonanaphylactic synthetic peptides derived from B cell epitopes of the major grass pollen allergen, Phl p 1, for allergy vaccination¹

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SPECIFIC AIMS

We aimed to develop a B cell epitope-based peptide allergy vaccine for Phl p 1, a major timothy grass pollen allergen that is recognized by more than 200 million allergic patients. Five synthetic peptides derived from the Phl p 1 IgE epitopes were analyzed by nuclear magnetic resonance (NMR) for fold and their allergenic activity was studied by IgE binding assays, basophil histamine release, and skin tests in allergic patients. Anti-peptide antibodies induced in mice and rabbits were studied for their ability to recognize the complete Phl p 1 wild-type allergen, group 1 allergens from other monocots, and whether they could inhibit the binding of grass pollen-allergic patients IgE to Phl p 1.

PRINCIPAL FINDINGS

1. Phl p 1-derived synthetic peptides lack secondary and tertiary structure

Five peptides representing portions of the major IgE epitope-bearing domains of Phl p 1, a 26 kDa allergen, were synthesized: peptide 1, aa 151–177; peptide 2, aa 87–117; peptide 3, aa 1–30; peptide 4, aa 43–70; peptide 5, aa 212–241. The peptides ranged from 2920.3 to 3482.9 daltons and the isoelectric points of the peptides were in the range of 4.07 (P2) to 9.41 (P4). Only peptide 2 contained a T cell epitope recognized by allergic patients. One- and 2-dimensional NMR experiments clearly showed absence of secondary or tertiary structure in all five peptides.

2. Phl p 1-derived peptides lack IgE binding capacity and allergenic activity

The five Phl p 1-derived peptides were compared by ELISA with complete rPhl p 1 regarding IgE binding capacity using sera from 60 grass pollen-allergic patients. All patients contained rPhl p 1-specific IgE antibodies, but no serum displayed IgE antibody reactivity to any of the five peptides.

The in vitro allergenic activity of the Phl p 1-derived peptides was compared with complete rPhl p 1 by basophil histamine release tests using granulocytes from grass pollen-allergic individuals. None of the five peptides elicited histamine release up to concentrations of 10 µg/ml, whereas complete rPhl p 1 induced a dose-dependent histamine release starting between 10⁻³ and 10⁻⁴ µg/ml, with a maximal release at 0.1 µg/ml. On the basis of the histamine release tests, Phl p 1-derived peptides had at least a 10,000- to 100,000-fold reduced allergenic activity compared to rPhl p 1. The in vitro test results were confirmed by skin prick test experiments performed in eight grass pollen-allergic patients and two nonatopic controls (Table 1). None of the eight grass pollen-allergic patients reacted with any of the peptides or the peptide mixture even when they were tested at a concentration of 100 µg/ml for the individual peptides or as a mix containing 20 µg/ml of each of the five peptides (Table 1). In contrast, complete rPhl p 1 induced immediate type skin reactions in seven patients at a concentration of 5 µg/ml and in one patient at a concentration of 10 µg/ml. All grass pollen-allergic patients displayed immediate skin reactions to timothy grass pollen extract. All individuals reacted after testing with histamine. The nonatopic persons showed no reactions to timothy grass pollen extract, rPhl p 1-, or rPhl p 1-derived peptides (Table 1, #9, #10).

3. Anti-peptide antibodies react with Phl p 1, group 1 allergens from various monocots and inhibit grass pollen-allergic patients IgE binding to Phl p 1

In mice, all five peptides induced IgG₁ anti-rPhl p 1 antibody responses that could be detected 4–8 wk after

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TABLE 1. Induction of immediate skin reactions with rPhl p 1 and Phl p 1-derived peptides (mean wheal diameter)

	rPhl p 1 $\mu\text{g/ml}$					timothy	histamine	100 $\mu\text{g/ml}$						
	5	10	25	50	100			P1	P2	P3	P4	P5	P1-P5 mix	
1	5	6	5	9	10	9	6	0	0	0	0	0	0	
2	3	3	5	6	8	10	3	0	0	0	0	0	0	
3	4	4	4	4	5	6	4	0	0	0	0	0	0	
4	2.5	3.5	4	4.5	5.5	10.5	4.5	0	0	0	0	0	0	
5	3	3	4	5	7	12	4	0	0	0	0	0	0	
6	4.5	6.5	8	10	16	13	7	0	0	0	0	0	0	
7	2	3.5	4	4.5	9	5.5	6	0	0	0	0	0	0	
8	0	2	2.5	4	5	2.5	5	0	0	0	0	0	0	
9	0	0	0	0	0	0	4	0	0	0	0	0	0	
10	0	0	0	0	0	0	5	0	0	0	0	0	0	

the first immunization and increased after the immunizations. Alu-adsorbed KLH-coupled peptides gave lower mouse IgG₁ titers than KLH-coupled CFA-adsorbed peptides but were of similar magnitude as the IgG₁ responses induced with CFA-adsorbed unconjugated peptides. In mice, peptides 2–5 induced higher levels of Phl p 1-specific antibody responses than peptide 1 and thus seemed to be more immunogenic than peptide 1.

Almost all mouse anti-peptide antisera exhibited broad cross-reactivity with group 1 allergens from three grasses (*Lolium perenne*, *Poa pratensis*, *Dactylis glomerata*) and a corn (*Secale cereale*). The capacity of rabbit anti-Phl p 1 peptide antibodies to inhibit the binding of allergic patients IgE to complete rPhl p 1 was examined by ELISA competition experiments. Strong inhibition of IgE binding was observed in the majority of patients ($n=46$).

Rabbit anti-peptide 5 antibodies strongly inhibited IgE binding to complete rPhl p 1 in all 46 patients (10–87% inhibition; average: 53.8%). Anti-peptide 1–4 antisera gave a much lower inhibition of IgE binding (mean inhibitions: anti-P1: 5.5%; anti-P2: 9.2%; anti-P3: 19.2%; anti-P4: 15.3%). The inhibition of IgE binding achieved with a mixture of all five antisera was not stronger than that achieved with the anti-peptide 5 antiserum alone (average inhibition with anti-P5: 53.8%; average inhibition with anti-P1-P5: 45.8%).

CONCLUSION

In this study, we provide evidence that vaccination with nonanaphylactic peptides derived from B cell epitopes may be a generally applicable strategy for the therapy of Type I allergies. We demonstrate that synthetic peptides derived from the IgE epitopes of one of the most important environmental allergens, the major timothy grass pollen allergen Phl p 1, lack allergenic activity but induce protective antibody responses.

The approach of using B cell epitope-derived hypoallergenic peptides for allergy vaccination is different from the strategy of using T cell epitope-derived peptides for allergy treatment, because T cell peptides

exert their effects preferentially via the induction of tolerance or anergy in allergen-specific T cells.

The possibility of obtaining hypoallergenic peptides by proteolytic digestion of allergen extracts for immunotherapy of allergic diseases has been indicated by classical experiments performed more than 30 years ago. However, difficulties in preparing standardized peptide mixtures from crude allergen extracts may have hampered a broader use of this technology. Recombinant DNA technology has now made it possible to produce defined allergen components as recombinant proteins that equal their natural counterparts and has facilitated the mapping of allergen epitopes. The importance of individual allergen components can now be evaluated in vitro and in vivo, and IgE epitopes can be determined by controlled allergen fragmentation and/or structural biology methods.

According to the experimentally determined IgE epitopes of the major timothy grass pollen allergen Phl p 1, we have synthesized five epitope-derived peptides. In contrast to the IgE-reactive β -galactosidase-fused peptides previously described, the synthetic peptides did not bind IgE antibodies and failed to elicit allergic reactions in vitro and in vivo up to concentrations of 100 $\mu\text{g/ml}$. In agreement with the lack of allergenic activity, NMR analysis of the peptides showed that none of the peptides exhibited signs of stable secondary or tertiary structure. The lack of IgE binding capacity and allergenic activity may therefore be due to the low likelihood that IgE epitopes capable of cross-linking effector cells are present on the isolated fragments and due to the destruction of conformational IgE epitopes.

Despite their lack of IgE reactivity, all five peptides induced in vivo IgG antibodies that reacted with the complete wild-type Phl p 1 allergen and cross-reacted with group 1 allergens from other grass and corn species. This indicates that the peptides contained sufficient sequence motifs for the induction of antibodies, which are capable of recognizing complete group 1 allergens. Synthetic peptides derived from the Phl p 1 allergen may thus be used not only to treat timothy grass pollen allergy, but allergies to other grass and corn species as well. The latter assumption is supported by the demonstration that Phl p 1 contained many of

the relevant epitopes present in group 1 allergens from other monocots and by our finding that peptide-induced antibodies cross-reacted with group 1 allergens from a variety of monocots.

More important is the finding that the peptide-induced antibodies were able to inhibit the binding of grass pollen-allergic patients' IgE to the complete Phl p 1 wild-type allergen. The most potent inhibition of IgE binding (up to 87%, average 53.8%) was achieved with antibodies induced against one particular peptide (peptide 5), whereas antibodies directed against other IgE binding sites were less potent inhibitors of IgE recognition. The latter result may be explained by the fact that Phl p 1-specific antibody levels in the anti-P1-P4 antisera were lower than in the anti-P5 antiserum. Since a mixture of antibodies against all five peptides did not yield stronger inhibition of IgE binding than the anti-P5 antibodies, the peptide P5 may be sufficient to treat a high percentage of patients allergic to group 1 grass pollen allergens. This assumption is supported by our finding that the anti-P5 antiserum inhibited IgE binding to Phl p 1 as well as did an antiserum raised against the complete recombinant Phl p 1 allergen.

Although we could not achieve inhibition of IgE binding in all of the patients tested with the peptide-induced antibodies, we think that the approach of using B cell epitope-derived peptides has many advantages. First, it is possible to produce well characterized hypoallergenic and thus safe vaccines for allergy treatment. Second, the peptides can be used to focus blocking antibodies directly to or close to the major IgE binding sites of important allergens. The importance of focusing blocking antibodies to the IgE-reactive portions of allergens is supported by other studies showing that IgG antibodies induced by immunotherapy may also recognize epitopes other than those recognized by IgE, and thus may fail to inhibit IgE binding or even enhance the IgE recognition of allergens.

From classical experiments performed more than 60 years ago, we know that blocking antibodies induced in allergic patients and in nonatopic persons can suppress the IgE mediated activation of effector cells and thus the immediate symptoms of Type I allergy. More recently, it has even been demonstrated that immunotherapy-induced blocking antibodies can also inhibit the IgE-mediated presentation of allergens to T cells and thus the chronic manifestations of atopy.

Based on our data and those of others, we expect that blocking antibodies induced by vaccination with Phl p 1 peptides will have several beneficial effects. Peptide-induced antibodies will reduce Phl p 1-induced effector cell activation, prevent production of Phl p 1-specific IgE synthesis, and inhibit IgE-mediated T cell activation. However, the hypoallergenic Phl p 1-derived peptides described now need to be evaluated in con-

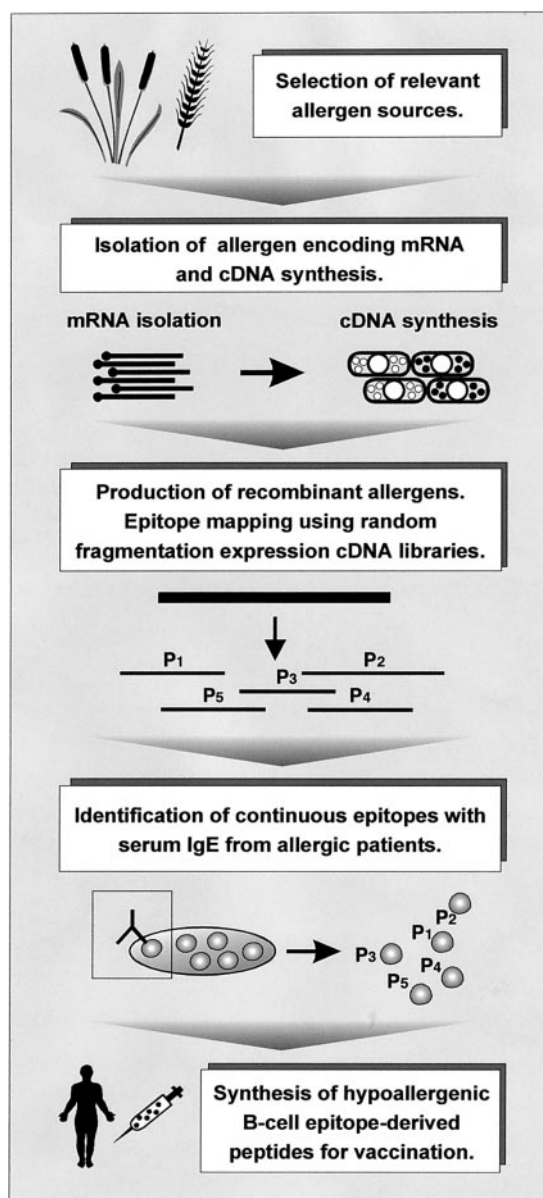


Figure 1. Schematic diagram for the preparation of allergy vaccines based on synthetic hypoallergenic B cell epitope-derived peptides.

trolled immunotherapy studies for the therapy of grass pollen allergy. Should the clinical immunotherapy studies confirm the *in vitro* results, the principle of using B cell epitope-derived peptides may be applied as a general strategy to produce safe allergy vaccines for all allergens of which the IgE epitopes or 3-dimensional structures are known (Fig. 1).

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