

Nonallergenic Peptides from Surface-Exposed Areas or B-Cell Epitopes of Allergens for Specific Immunotherapy

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Key Words

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Type I allergy is an immunoglobulin E (IgE)-mediated hypersensitivity disease affecting almost 25% of the population [1]. The immediate symptoms of the disease (e.g. allergic rhinoconjunctivitis, asthma, anaphylactic shock) are caused by release of biologically active mediators from effector cells when allergens induce cross-linking of FcεRI-bound IgE antibodies [2]. Most allergens represent low-molecular-weight water-soluble proteins or glycoproteins. In order to cross-link effector cell-bound IgE antibodies, at least two IgE epitopes must be present on an allergen molecule. If allergens are fragmented as can be achieved by proteolytic digestion of natural allergens [3, 4] or by recombinant DNA technology [5], they will lose their anaphylactic potential but may retain their immunogenic activity. It was previously demonstrated that allergen fragments obtained by proteolytic digestion of allergen extracts lost their allergenic activity but could be used for immunotherapy [6]. Although it would be of advantage to use such allergen fragments for immunotherapy to reduce anaphylactic side effects it turned out to be difficult to prepare such extracts in a standardized form and to remove proteolytic enzymes from the preparations.

Progress made in the field of allergen characterization through the application of molecular biological techniques [7] now provides the ground for a controlled preparation of nonanaphylactic allergen fragments by recombinant DNA technology or synthetic peptide chemistry [8]. This is one possibility of using synthetic peptides containing T cell epitopes to induce tolerance and/or anergy in allergen-specific T cells [9]. T cell epitopes are short peptides in the range of 10–12 amino acids which result from the proteolytic processing of allergens by antigen-presenting cells. In contrast B cell epitopes represent surface-exposed allergen domains which make contact with IgE antibodies. Another peptide-based approach for therapy of type I allergy is thus the use of B cell epitope-derived peptides to induce and focus protective IgG antibodies to surface-exposed allergen domains recognized by IgE antibodies. To achieve this goal it is necessary to identify those allergen sources and allergens which are frequent targets for patients' IgE antibodies and which bind a high percentage of specific IgE (fig. 1). Next mRNA is isolated from the respective allergen sources and cDNAs coding for the most important allergens are synthesized and identified by expression cDNA cloning using serum IgE from allergic patients. We have selected two of the most important environmental allergens to evaluate the concept of B cell epitope-derived peptides for immunotherapy.

Bet v 1, the major birch pollen allergen, is recognized by more than 95% of birch pollen-allergic patients and more than 60% of them react exclusively with this aller-

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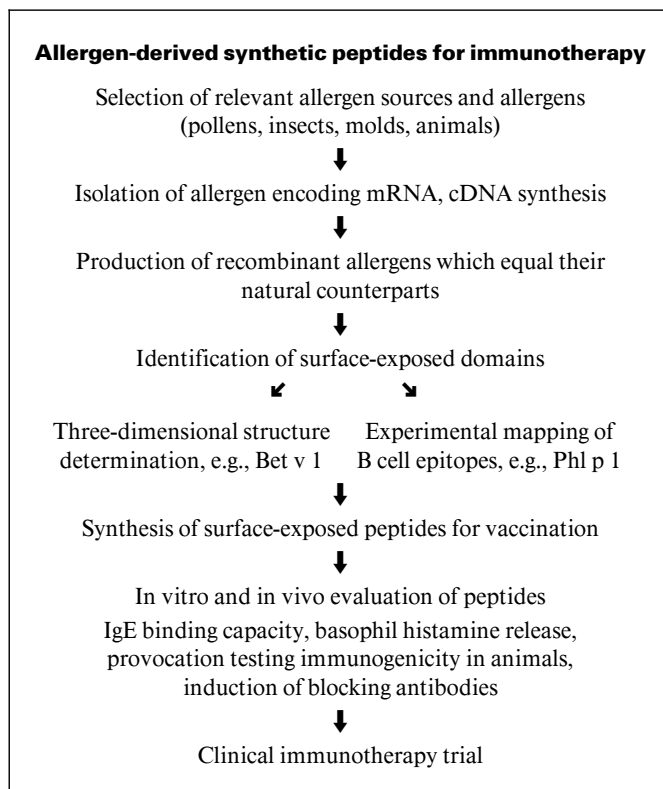


Fig. 1. Strategies for the selection of B cell epitope-derived peptides for immunotherapy.

gen [10–12]. Phl p 1, the major timothy grass pollen allergen, is recognized by more than 95% of grass pollen-allergic patients and it cross-reacts with group 1 allergens from several grass species [13, 14]. According to the three-dimensional structure of Bet v 1 [15] and the experimentally determined IgE epitopes of Phl p 1 [16] we synthesized solvent-exposed peptides with an average length of 30 amino acids. In vitro and in vivo evaluation of the peptides was performed to demonstrate lack of IgE-binding capacity and allergenic activity (basophil histamine release, skin prick testing) (fig. 1). Nevertheless the peptides could be used to induce IgG antibodies in mice and rabbits which reacted with the complete allergen molecule, immunologically related allergens from other sources and which inhibited human IgE binding. B cell epitope-derived peptides may now be evaluated in clinical immunotherapy trials in patients for their clinical efficacy.

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References

- Kay AB: Allergy and Allergic Diseases. Oxford, Blackwell Science, 1997.
- Ravetch JV, Kinet JP: Fc receptors. *Annu Rev Immunol* 1991;9:457–492.
- Malley A, Baecher L, Mackler B, Perlman F: Further characterization of a low-molecular-weight allergen fragment isolated from green pea. *Clin Exp Immunol* 1976;25:159–164.
- Chakrabarty S, Ekramoddoullah AK, Kisil FT, Sehon AH: Allergens of Kentucky Blue Grass. II. Isolation of hapten-like components from Kentucky Blue Grass pollen by preparative isoelectrofocussing. *Int Arch Allergy Appl Immunol* 1980;63:369–382.
- Ball T, Vrtala S, Sperr WR, Valent P, Susani M, Kraft D, Valenta R: Isolation of an immunodominant IgE-hapten from an epitope expression cDNA library; dissection of the allergic effector reaction. *J Biol Chem* 1994;269:28323–28328.
- Malley A, Perlman F: Induction of both reagenic and blocking antibodies with a low molecular weight fraction of timothy pollen extract. *J Allergy* 1969;43:59–64.
- Valenta R, Vrtala S, Laffer S, Spitzauer S, Kraft D: Recombinant allergens. *Allergy* 1998;53:552–561.
- Valenta R, Vrtala S, Focke-Tejkl M, Bugajska-Schretter A, Ball T, Twardosz A, Spitzauer S, Grönlund H, Kraft D: Genetically engineered and synthetic allergen derivatives: Candidates for vaccination against type I allergy. *Biol Chem* 1999;380:815–824.
- Norman PS: Modern concepts of immunotherapy. *Curr Opin Immunol* 1993;5:968–973.
- Jarolim E, Rumpold H, Endler AT, Ebner H, Breitenbach M, Scheiner O, Kraft D: IgE and IgG antibodies of patients with allergy to birch pollen as tools to define the allergen profile of *Betula verrucosa*. *Allergy* 1989;44:385–395.
- Breiteneder H, Pettenburger K, Bito A, Valenta R, Kraft D, Rumpold H, et al: The gene coding for the major birch pollen allergen, Bet v 1, is highly homologous to a pea disease resistance response gene. *EMBO J* 1989;8:1935–1938.
- Niederberger V, Pauli G, Grönlund H, Fröschl R, Rumpold H, Kraft D, Valenta R, Spitzauer S: Recombinant birch pollen allergens (rBet v 1, rBet v 2) contain most of the IgE epitopes present in birch, alder, hornbeam, hazel and oak pollen. A quantitative IgE inhibition study using sera from different populations. *J Allergy Clin Immunol* 1998;102:579–591.
- Laffer S, Vrtala S, Duchene M, et al: IgE-binding capacity of recombinant timothy grass (*Phleum pratense*) pollen allergens. *J Allergy Clin Immunol* 1994;94:88–94.
- Niederberger V, Laffer S, Fröschl R, Kraft D, Rumpold H, Kapiotis S, Valenta R, Spitzauer S: IgE antibodies to recombinant pollen allergens (Phl p 1, Phl p 2, Phl p 5 and Bet v 2) account for high percentage of grass pollen-specific IgE. *J Allergy Clin Immunol* 1998;101:258–264.
- Gajhede M, Osmark P, Poulsen FM, Joost van Neerven RJ, Schou C, Lowenstein H, Spangfort MD: X-ray and NMR structure of Bet v 1, the origin of birch pollen allergy. *Nat Struct Biol* 1996;3:1040–1045.
- Ball T, Fuchs T, Sperr WR, Valent P, Vangelista L, Kraft D, Valenta R: B cell epitopes of the major timothy grass pollen allergen, Phl p 1, revealed by gene fragmentation as candidates for immunotherapy. *FASEB J* 1999;13:1277–1290.

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