

IgE Mimotopes of Birch Pollen Allergen Bet v 1 Induce Blocking IgG in Mice

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Key Words

Bet v 1 · BIP 1 · IgE epitope · Phage · Mimotope · Bet mim
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Abstract

Background: The induction of nonanaphylactogenic 'blocking' IgG antibodies capable of inhibiting the IgE/allergen interaction represents a favorable therapeutic concept for type I allergy. However, IgG antibodies to allergens may block or enhance specific IgE binding, depending on the recognized epitope. Taking the major birch pollen allergen Bet v 1 as a model, we developed a strategy for the precise induction of IgG antibodies of a desired epitope specificity. **Methods:** Random phage display peptide libraries were applied to define peptide structures mimicking natural epitopes (mimotopes) of Bet v 1. Selections were performed with BIP 1, a murine monoclonal antibody known to enhance the IgE binding to Bet v 1, and with anti-Bet v 1 IgE purified from patients' sera. The characterized Bet v 1 mimotopes were used to localize the corresponding epitope at the surface of Bet v 1 by a computer-aided mathematical approach based on the three-dimensional structure and the chemical char-

acter of the amino acids. The Bet v 1 mimotopes were further used to immunize BALB/c mice. The specificity of the induced antibodies was tested by immunoblotting and inhibition assays. **Results:** With the three-dimensional epitope search it became possible to localize a discontinuous IgE epitope on the surface of Bet v 1 in a substantial distance from the IgG epitope of the monoclonal antibody BIP 1. Moreover, we could demonstrate that phage displaying mimotopes are immunogenic vectors for the precise induction of epitope-specific IgG. Immunization with BIP 1 mimotopes induced IgG enhancing the IgE binding to Bet v 1, whereas immunization with IgE mimotopes resulted in IgG capable of blocking human IgE binding in vitro. **Conclusion:** Allergen mimotopes can be used for the induction of anti allergen IgG of desired specificity. We propose that mimotope immunotherapy based on IgE mimotopes generated by biopannings may represent a future concept for therapy of type I allergy.

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Introduction

Type I allergic symptoms are a consequence of the interaction of allergens and cytophilic IgE and the subsequent release of vasoactive mediators. The theory of 'blocking antibodies' capable of inhibiting the IgE/allergen interaction is a long-known concept in the allergy field [1]. Immunotherapy with allergen extracts is associated with an increase of allergen-specific IgG, however, without correlation to the outcome of therapy [2]. This may be explained by the fact that immunizations with whole allergen molecules induce IgG that, depending on the epitope specificity, may enhance or inhibit the allergic reaction [3, 4]. We developed a novel strategy for the precise induction of monoepitope-specific IgG by immunizations with artificial epitopes, using the major birch pollen allergen Bet v 1 [5] as a model. This molecule is recognized by IgE from more than 90% of the birch pollen-allergic patients, and from 60% solely [6]. Therefore, Bet v 1 represents a suitable and relevant model for a reinvestigation of the concept of blocking antibodies.

We applied phage display technique using filamentous phages displaying random peptides on their surface for the definition of peptide structures mimicking natural epitopes (mimotopes) [7, 8]. Selections were performed with a monoclonal anti-Bet v 1 antibody known to enhance the IgE binding (BIP 1) [9], and with polyclonal human anti-Bet v 1 IgE. The generated mimotopes were further used to immunize mice and the induced IgG was tested for its blocking capability in a serological assay.

Material and Methods

Biopanning with Phage Libraries

Biopannings were performed using phage display libraries expressing random linear [7] or circular [8] nonapeptides fused to pVIII of the filamentous phage VSCM 13. These libraries were kindly provided by IRBM (Istituto di Ricerche Biologia Molecolare, Rome, Italy). Four successive panning rounds were performed using monoclonal antibody BIP 1 [9] and affinity-purified human antibodies, respectively.

Three-Dimensional Epitope Search

The three-dimensional coarse-grained epitope search was done based on the X-ray structure of Bet v 1 (EMBL-protein database, entry: 1BV1). Our approach was based on homology between neighboring residues permitting gaps. The amino acids were further classified in four categories depending on the chemical character of their residues. Alignment with the derived sequences was done without admission of gaps. All hits were statistically evaluated.

Immunization of BALB/c Mice

Female BALB/c mice, 3–5 weeks of age, were used for intraperitoneal immunization with phages (Biovendor Biotechnology, Brno, Czech Republic). Mice were immunized on day 0, 14 and 28 and blood samples from the tail vein were taken on day 0 (preimmune serum), day 21 and day 38.

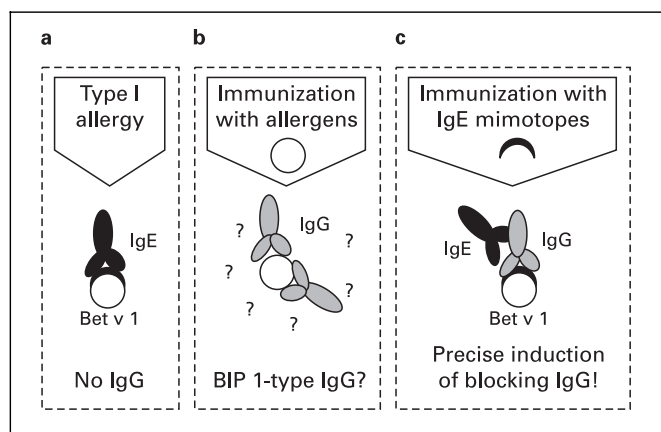
Results

We defined peptide structures mimicking natural epitopes (mimotopes) of anti-Bet v 1 antibodies by using random nonapeptide phage libraries. Selections were performed with monoclonal IgG (BIP 1) known to enhance the IgE binding to Bet v 1 and with polyclonal human IgE, purified from allergic patients' sera. Selected phage ligands were analyzed and the deduced amino acid sequences were determined: alignments with the Bet v 1 amino acid sequence showed no linear homologies. To localize the corresponding epitopes at the surface of Bet v 1, we used a computer-aided mathematical approach based on the three-dimensional structure and the chemical character of the amino acids. The program identified the IgG epitope of BIP 1 at position 58–67, confirming previous speculations based on sequence alignments [10]. For the IgE epitope significant similarities were found in two independent regions including amino acids 9–22 and 104–113. These two regions, although separated on the linear sequence, form a discontinuous IgE epitope. To induce epitope-specific antibodies, phage mimotopes were further used in immunization experiments with BALB/c mice. Whereas BIP 1 mimotopes led to the induction of Bet v 1-specific IgG [11] that enhanced type I skin reactions in mice [12], immunization with IgE mimotopes induced IgG able to inhibit consecutive IgE binding to Bet v 1 in immunoblotting [13].

Discussion

We report a strategy to define and localize discontinuous IgE epitopes with the phage display technology. More importantly, the mimotopes selected were capable of inducing B cell responses to the natural epitopes. It has been speculated earlier that IgG may enhance or inhibit IgE binding depending on the epitope specificity. In a direct approach, using mimotopes of BIP 1 antibody for immunization of BALB/c mice, we could recently confirm that IgG of the BIP 1 type enhances allergic reactions [12]. Moreover, we could demonstrate that immunization

Fig. 1. Novel concept for the treatment of type I allergy: mimotope immunotherapy. **a** Allergic patients reveal IgE but no IgG antibodies specific for the allergen. **b** Immunization with whole allergen molecules (as during hyposensitization therapy) induces IgG with multiple epitope specificities. **c** Immunization with IgE mimotopes results in the precise induction of blocking IgG, able to inhibit the IgE/allergen interaction.



with an IgE mimotope was in fact able to block consecutive IgE binding to Bet v 1. Based on these observations, we propose that immunotherapy with IgE mimotopes generated by biopannings may result in formation of blocking IgG. We conclude that mimotope immunotherapy possibly represents a new promising concept for treatment of type I allergic disease (fig. 1).

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