Bip 1, a Monoclonal Antibody with Specificity for the Major Birch Pollen Allergen Bet v 1, Modulates IgE Binding to the Allergen

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Introduction

Results and Discussion

More than 95% of patients allergic to tree pollen, fruit and vegetable display IgE cross-reactivity to the major birch pollen allergen Betv1 [1]. The cDNA coding for Bet v 1 has been isolated and showed significant homology to pathogenesis related plant proteins [2]. Recombinant Bet v 1 was expressed in *Escherichia coli* and shown to possess IgE-binding capacity and biological activity comparable to natural Betv 1 [3–5]. In contrast to other major allergens, no continuous IgE epitopes could be identified for Bet v 1 as yet, indicating that IgE epitopes of Bet v 1 belong to the conformational type. We have therefore used mouse and human monoclonal antibodies to study the interaction of patients' IgE with Bet v 1 using competition experiments [6,7]. Among the human and mouse monoclonal antibodies with specificity for Bet v 1, three groups of antibodies could be identified. Antibodies of the first group did not affect IgE binding to Bet v 1, while the second group of antibodies strongly inhibited IgE binding to Bet v 1. The blocking antibodies also inhibited Betv 1-induced histamine release from patients' basophils and may therefore be considered as tools for passive therapy of Bet v 1-induced allergy. Unexpectedly, the third group of antibodies, represented by the mouse monoclonal antibody Bip 1, enhanced IgE-binding to Bet v 1. We expressed the Bip 1 Fab in *E. coli* and characterized the purified recombinant Fab and its interaction with Bet v 1 by immunologic and spectroscopic methods [8]. To express the Bet v 1-specific mouse monoclonal antibody Bip 1 as recombinant Fab in *E. coli*, RNA was isolated from the hybridoma cells [9]. Using primers specific for the heavy chain

fragment and light chain of mouse immunoglobulins, cDNA was synthesized and PCR amplified. The cDNAs coding for the heavy chain fragment and light chain were inserted into plasmid pComb 3H [10]. Clones producing Bet v 1-specific Fabs were identified using a colony lift procedure. In a first step, nitrocellulose-immobilized colonies were exposed to recombinant Bet v 1. Filters were then washed and incubated with serum IgE from patients allergic to Bet v 1. Finally ¹²⁵I-labeled antihuman IgE antibodies (RAST, Pharmacia) were used to detect colonies which had bound Bet v 1 and specific IgE antibodies. Clones were further tested for their ability to bind ¹²⁵I-labeled recombinant Bet v 1. Soluble recombinant Bip 1 Fabs could be purified by affinity chromatography to Bet v 1. Complete natural-hybridoma-derived Bip 1 antibody, recombinant Bip 1 Fabs cross-reacted with Bet v 1 homologous allergens present in alder pollen, hazel pollen and apple. The effect of the complete hybridoma-derived Bip 1 antibody and the recombinant Bip 1 Fab on binding of patients' IgE antibodies was further investigated. Recombinant Bet v 1 which had been checked by circular dichroism analysis for proper folding was dotted to nitrocellulose and incubated with an excess of Bip 1 antibodies, Bip 1 Fabs, an antibody with different specificity or buffer. After washing, the dot blots were in-

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incubated with serum IgE from individuals allergic to Bet v 1, washed, and bound IgE antibodies were detected with ¹²⁵I-labeled anti-human IgE antibodies. Differences in IgE binding were evaluated by densitometric analyses of the autoradiographies and by γ -counting. With most sera an up to fivefold enhancement of IgE binding to Bet v 1 was observed after preincubation of Bet v 1 with Bip 1 compared to the controls. Figure 1 illustrates the proposed mechanism for the observed enhancement of IgE binding. Patients were apparently sensitized against different conformations of Bet v 1 so that they produced IgE antibodies (IgE1, IgE2...) which are able to specifically discriminate these Bet v 1 conformations. The monoclonal antibody Bip 1 might then stabilize a certain Bet v 1 conformation in a chaperonin-like manner so that other IgE epitopes are exposed. As we have also observed this effect for human monoclonal IgG antibodies [7], we believe that the enhancement of antibody binding to a given antigen (probably antigens with conformational epitopes) by other antibodies may represent a biologically relevant mechanism to regulate humoral immune responses in a complex network.

Fig. 1. Proposed mechanism for the Bip 1 induced modulation of patients' IgE binding to Bet v 1. It is proposed that Bet v 1 occurs preferentially in one conformation (a) so that only a certain group of IgE antibodies specific for this conformation (IgE1) can bind. IgE antibodies (IgE2) which were induced against a different Bet v 1 conformation or epitopes of this conformation occur in the serum but are unable to bind. In a chaperonin-like manner, Bip 1 is apparently able to stabilize a different Bet v 1 conformation or to expose additional IgE epitopes so that other and/or additional groups of IgE antibodies (IgE2) can bind to Bet v 1 (b).

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