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
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

Results and Discussion

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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 Betv1. Finally 251-labeled anti-human 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 25 I-labeled recombinant Betv 1. Soluble recombinant Bip 1 Fabs could be purified by affinity chromatography to Betv 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-

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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References


Modulation of IgE Binding to Bet v 1 by a Recombinant Antibody Fragment

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