

Induction of Blocking Antibodies with T Cell Epitope-Containing Hypoallergenic Recombinant Bet v 1 Fragments

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Key Words

Birch pollen allergy · Bet v 1 · Immunotherapy ·
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antibodies

Bet v 1, the major birch pollen allergen, represents one of the most prominent allergens. It is recognized by serum IgE from more than 95% of birch pollen-allergic patients and 60% of these patients react exclusively with this allergen [1, 2]. Bet v 1 contains conformational IgE epitopes and shares epitopes with major allergens from trees and plant-derived food [3]. We produced by genetic engineering two rBet v 1 fragments, comprising amino acids (aa) 1–74 and aa 75–160 of complete Bet v 1, which due to a loss of their three-dimensional conformation, failed to bind IgE antibodies from allergic patients and had more than 100-fold reduced allergenic activity in histamine release assays and skin tests [4–7]. Here we show that the recombinant Bet v 1 fragments induced proliferation of PBMC from birch pollen-allergic patients comparable to the rBet v 1 wild type, indicating that both fragments contain most of the Bet v 1-specific T cell epitopes. Interestingly, the C-terminal rBet v 1 fragment aa 75–160 induced significantly higher IFN- γ secretion compared to

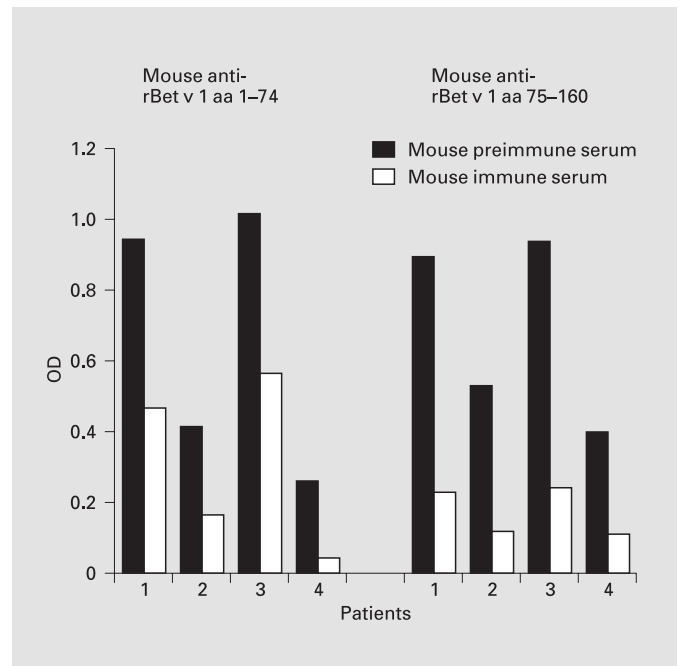


Fig. 1. Mouse anti-rBet v 1 fragment antisera inhibit human IgE binding to rBet v 1. ELISA plate-coupled rBet v 1 was preincubated with mouse preimmune sera and anti-rBet v 1 fragment immune sera, then incubated with sera from 4 birch pollen-allergic patients (1–4). IgE binding to rBet v 1 is displayed as OD values.

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the N-terminal fragment [4, 8]. Immunization of rabbits and mice with purified rBet v 1 fragments induced IgG antibodies which reacted with rBet v 1 wild type, natural Bet v 1 and cross-reacted with Bet v 1-related allergens present in pollens of trees of the order of *Fagales* and in plant-derived food. Moreover, rBet v 1 fragment-induced IgG antibodies recognized epitopes defined by patients' IgE antibodies and inhibited patients' IgE binding to complete Bet v 1 wild type and to Bet v 1 homologous allergens. Figure 1 shows the inhibition of patients' IgE binding to rBet v 1 wild type after preincubation with mouse anti-rBet v 1 fragment antibodies. ELISA plate-coupled rBet v 1 was preincubated with mouse anti-rBet v 1 fragment antisera (anti-aa 7–74 antibodies; anti-aa 75–160 antibodies), or, for control purposes, with the corresponding preimmune sera and then incubated with serum IgE from birch pollen-allergic patients. Mouse sera raised against rBet v 1 aa 1–74 in allergic patients inhibited IgE

binding to Bet v 1 between 45 and 83% (59.5% mean inhibition) and mouse sera raised against rBet v 1 aa 75–160 inhibited IgE binding to rBet v 1 between 73 and 78% (74.8% mean inhibition) [8] (fig. 1).

Hypoallergenic rBet v 1 fragments may, therefore, be considered as safe and effective vaccines for specific immunotherapy of tree pollen and associated food allergies. High allergen doses can be administered with a reduced risk of anaphylactic side effects, and thus induce a preferential Th1 response to Bet v 1 accompanied by the induction of high titers of protective Bet v 1-specific IgG antibodies.

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