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Humoral Immune Responses to Recombinant Tree Pollen Allergens (*Bet v I* and *Bet v II*) in Mice: Construction of a Live Oral Allergy Vaccine

Abstract

Recombinant tree pollen allergens (recombinant *Bet v I* and recombinant birch profilin, *Bet v II*) were purified and used to immunize BALB/c and B6D2F1 mice with Al(OH)₃ to elicit a specific IgE response. Serum from immunized mice was then used to detect immunoblotted natural tree pollen allergens. The onset of the humoral immune response was monitored using antimouse IgE, IgG1, IgG2a/b, IgG3 and IgA. In both strains, a specific and long-lasting IgE response could be elicited with both recombinant allergens. Mice immunized continuously with recombinant *Bet v I* + Al(OH)₃ showed a significant decrease of specific IgE antibodies indicating that continuous application of allergens can reduce specific IgE responses. The possibility of inducing a different type of immune response is indicated by the fact that mice fed with *Bet v I* expressed in apathogenic *Salmonella* strains showed a Th1 immune response to *Bet v I* accompanied by specific IgG2a/b without detectable IgG1 or IgE. Recombinant allergens can hence be used to decrease or even modulate specific IgE responses in vivo.

Key Words

Recombinant tree pollen allergens
Bet v I
Bet v II
 Humoral immune response
 Mice
 Recombinant vaccines

Specific IgE Responses in Mice Induced with Recombinant Allergens Are Long Lasting and Show a Similar Degree of Cross-Reactivity as Observed in Allergic Patients

The induction of IgE antibodies with aluminium hydroxide in mice has been described extensively [1, 2]. When BALB/c mice were immunized with recombinant *Bet v I* or recombinant birch profilin (*Bet v II*), IgE antibodies could be induced which cross-reacted with *Bet v I*- and *Bet v II*-homologous allergens. Both allergens share significant sequence similarities and similar immunological properties to

a number of related natural allergens. IgE antibodies of immunized mice hence displayed similar cross-reactivities as IgE antibodies of allergic patients [3–9]. Mice which had been immunized with *Bet v I* had IgE antibodies that cross-reacted with the major allergens of alder and hazel whereas *Bet v II*-allergic mice reacted with profilins from grasses and weeds.

It is known that IgE responses in allergic patients are long lasting. We have therefore investigated the duration of IgE responses which had been induced in mice with recombinant *Bet v I*. Figure 1 shows that B6D2F1 mice immunized with 1 µg recombinant *Bet v I* at 2- to 3-week intervals for

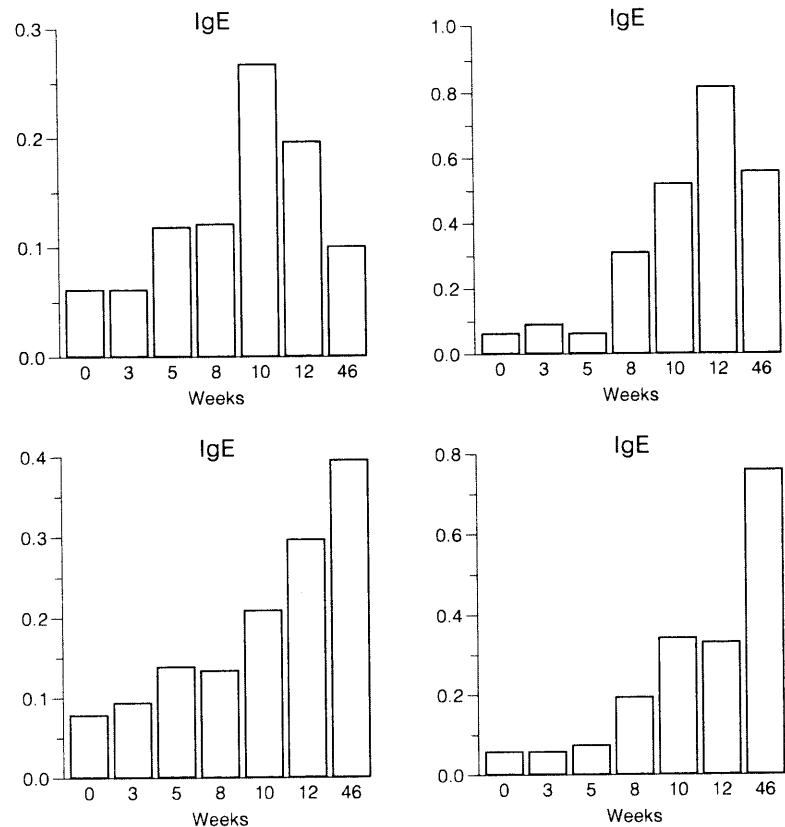


Fig. 1. Long-lasting *Bet v* I-specific IgE response in B6D2F1 mice. The amount of *Bet v* I-specific IgE which was induced by subcutaneous immunization of four representative B6D2F1 mice with recombinant *Bet v* I and aluminium hydroxide was measured by ELISA. Mice were immunized and bled until week 12. Immunization was then stopped and mice were bled again at week 46. For detection of IgE, serum was diluted 1:20 and ELISA values reflect OD measurements at 405 nm.

12 weeks had a high titer of anti *Bet v* I IgE antibodies after 8 further months without immunization.

We conclude that immunization with single recombinant allergens induces IgE antibodies in mice which display a similar degree of cross-reactivity as observed in allergic patients. The IgE responses against recombinant *Bet v* I, representing a typical seasonal allergen, are as long lasting as observed in allergic patients.

Continuous Immunization with Recombinant Allergens Using Aluminium Hydroxide as Adjuvant Leads to a Decrease of Specific IgE

Current hyposensitization treatment which is generally accepted as an efficient form of specific-allergy treatment is based on the subcutaneous injection of increasing doses of crude allergen extracts in combination with aluminium hydroxide. Although aluminium hydroxide is known to be a potent inducer of IgE responses, it is currently the only adjuvant which is permitted for hyposensitization. To investi-

gate the kinetics of specific-IgE responses induced in mice with recombinant *Bet v* I and aluminium hydroxide, mice were continuously immunized and bled for a period of 38 weeks. Figure 2 shows the *Bet v* I-specific IgE measured in two representative mice after continuous allergen injection. At approximately weeks 26–28, a peak of *Bet v* I-specific IgE responses was observed which decreased during the treatment to lower levels.

Bet v I-specific IgG1 responses increased later and were still high at week 38. It is likely that specific immunotherapy induces a similar decrease of specific IgE responses in allergic patients. It would therefore be interesting to monitor the efficacy of immunotherapy on the basis of purified recombinant allergens with currently available adjuvants. It is likely that immunotherapy with those recombinant allergens against which patients display a high percentage of specific IgE may lead to significant reduction of allergic symptoms.

Fig. 2. Continuous immunization of BALB/c mice with recombinant *Bet* v I and aluminium hydroxide leads to a decrease in specific IgE responses. The *Bet* v I-specific IgE and IgG1 response was measured in two representative BALB/c mice (left and right) which had been immunized and bled at the times indicated. For the detection of specific IgE, serum was diluted 1:20. Specific IgE responses peak at weeks 26–28 and then decrease continuously.

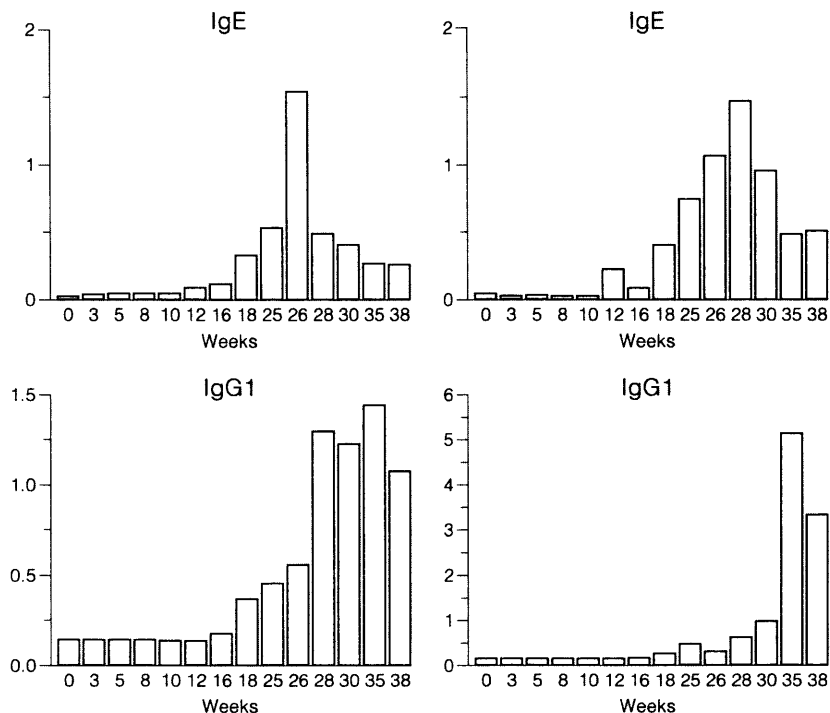
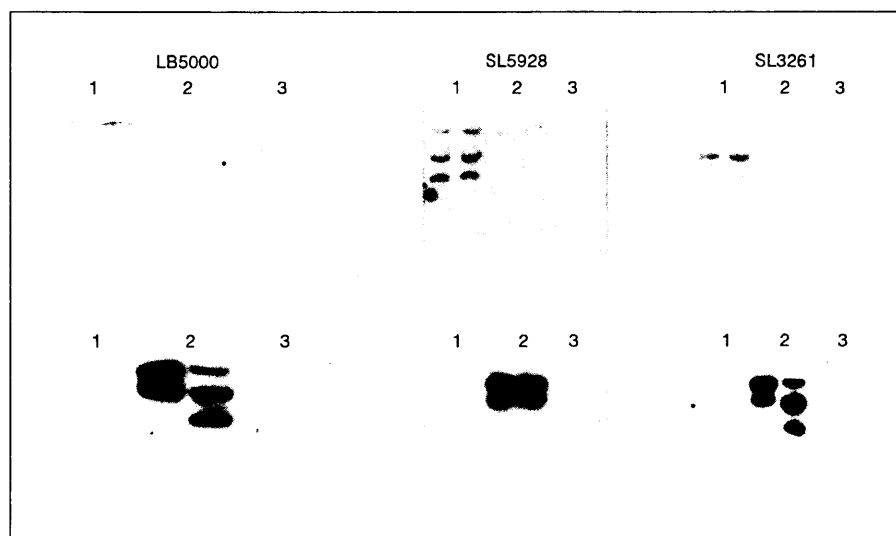


Fig. 3. Transport of plasmid pKK 223–3 expressing recombinant *Bet* v I and *Bet* v II into apathogenic *Salmonella* strains. Plasmid pKK 223–3 expressing recombinant *Bet* v I or *Bet* v II was transformed first into *Salmonella* strain LB5000. Plasmid DNA was then isolated and further transformed into the live vaccine strains SL5928 and SL3261. The successful transfer of the plasmid DNA is proven by hybridization of *Salmonella* DNA with oligonucleotides specific for *Bet* v I (upper block) and *Bet* v II (lower block). Lanes 1 = DNA isolates from *Salmonella* transformed with the *Bet* v I cDNA; 2 = DNA from *Bet* v II-transformed *Salmonella*; 3 = *Salmonella* DNA transformed with the expression plasmid without insert.



Construction of a Live Oral Allergy Vaccine in Apathogenic *Salmonella* Strains

The modification of allergens such as polymerization by glutaraldehyde [10] or injection with Freund's adjuvant [11] is known to induce a specific TH1 immune response. *Salmonella* strains which have a deficiency in their aromatic ami-

no acid metabolism and which therefore are apathogenic have been developed for the delivery of foreign antigens [12, 13]. The oral application of these *Salmonella* strains induces a Th1 immune response against *Salmonella* antigens and those antigens which are expressed in the *Salmonella*. Figure 3 shows the transport of plasmids pkk 223–3 expressing *Bet* v I and *Bet* v II [14] into apathogenic *Salmonella* live



Fig. 4. Immune electron microscope localization of *Bet v I* and *Bet v II* in live *Salmonella* vaccine strains. *Bet v I* and *Bet v II* were localized in the cytoplasm of apathogenic *Salmonella* SL5928. *Salmonella* were fixed in aqueous paraformaldehyde, embedded in Lowicryl and ultrathin sections were incubated with specific antibodies and secondary antibodies coupled with gold particles. **a** *Salmonella* expressing *Bet v I*. **b** *Salmonella* expressing *Bet v II*.

vaccine strains SL5928 and SL3261. Both recombinant allergens are expressed in the cytoplasm of the *Salmonella*, as demonstrated by immune electron microscopy in figure 4. The amount of recombinant *Bet v I* and *Bet v II* expressed by the pKK plasmids in *Salmonella* is, however, low, but both *Salmonella*-expressed allergens bound patients' IgE and specific antibodies.

Mice Fed Salmonella expressing Bet v I Can Develop a Bet v I-Specific Th1 Immune Response

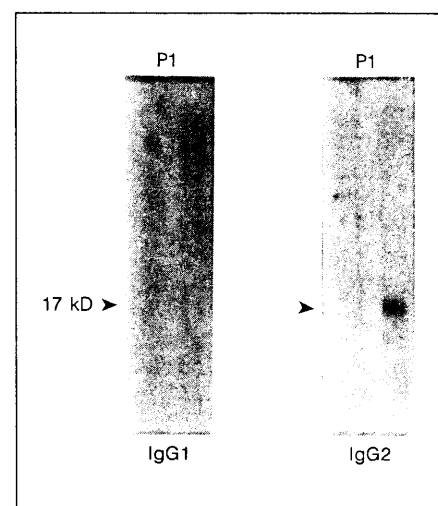


Fig. 5. A mouse fed the *Bet v I* live oral allergy *Salmonella* vaccine displays a Th1 response to nitrocellulose-blotted natural *Bet v I*. Nitrocellulose strips containing natural birch pollen extract were incubated with the 1:50 diluted preimmune serum (P) and the immune serum (I) of a mouse which was immunized orally with *Salmonella* SL5928 expressing *Bet v I*. *Bet v I*-specific IgG2 could be detected, whereas no *Bet v I*-specific IgG1 could be measured.

BALB/c mice fed *Salmonella* expressing the major birch pollen allergen *Bet v I* developed high titers of IgG2 antibodies against *Salmonella* antigens. In about 10% of immunized mice, IgG2 responses specific for *Bet v I* could be measured, whereas not *Bet v I*-Specific IgE or IgG1 could be detected. This indicates that apathogenic *Salmonella* strains which are genetically engineered to express allergens can induce allergen-specific Th1 immune responses (fig. 5). Such live allergy vaccines might therefore be useful for specific immunotherapy of allergic patients. The live vaccine strains can be transformed with any cDNA coding for the respective allergen without need for extensive protein purification, and offers the advantage of oral application. There are, however, a number of technical and ethical problems before such live allergy vaccines could be used for therapy of type I allergy in patients. To yield an allergen-specific Th1 immune response in a high percentage of immunized hosts, the yield of allergens expressed in the *Salmonella* has to be increased. In addition, the application dose has to be optimized to avoid any side effects due to the infection. Nevertheless, we believe that the use of recombinant vaccination techniques may add to allergen-specific immunotherapy in the future [15].

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