

Monovalent fusion proteins of IgE mimotopes are safe for therapy of type I allergy¹

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SPECIFIC AIMS

By screening phage-display random peptide libraries with purified immunoglobulin E (IgE) from birch pollen allergic patients, we previously defined peptide structures mimicking natural IgE epitopes (mimotopes) of the major birch pollen allergen *Bet v 1*. Phages displaying IgE mimotopes were shown to be excellent immunogenic vectors to precisely induce blocking IgG antibodies. However, due to their high density on the phage surface, these mimotopes have the potential to cross-link cytophilic IgE in sensitized individuals. Consequently, in the present study we aimed to generate a monovalent carrier for the IgE mimotopes. Therefore, we expressed the selected mimotopes as fusion proteins with albumin binding protein (ABP) and examined their capacity to induce an IgG immune response in mice and to trigger histamine release.

PRINCIPAL FINDINGS

1. ABP-fused mimotopes resemble the natural epitope

As previously described, we screened phage-display peptide libraries with *Bet v 1*-specific human IgE antibodies (huIgE_{Betv1}). Phages displaying the peptides CQQTLSVRALC and CSEYREPLLC, both derived from a pVIII9aa.Cys library, were characterized as IgE mimotopes (designated Bet mim E_{CQQ} and Bet mim E_{CSEY}, respectively). The sequences of these mimotopes were selected for generation of fusion proteins. The mimotope for profilin-specific human IgE CAISGGYPVC, referred to as Prof mim E, was expressed as fusion protein with ABP for control purposes. To produce recombinant mimotopes fused to ABP, the corresponding DNA sequences were inserted into the pSB 511 vector. The gene coding for the desired protein was directionally inserted via two noncompatible *Sfi*I sites. The gene was fused to the ABP-6xHis module, which codes for the ABP and a His tag. Gene expression was driven by the *lac* promoter and the protein is directed into the periplasmic space of *Escherichia coli* via the *pelB* leader sequence. The fusion proteins examined by Western blotting with an anti-His antibody showed a

dominant signal of 18 kDa, corresponding to the calculated mass of the recombinant fusion protein.

The huIgE_{Betv1} binding capacity of the phage-displayed mimotopes and the expressed fusion proteins were further compared by ELISA experiments. Coated human IgE antibodies bound both Bet mim E_{CQQ} and Bet mim E_{CSEY} when displayed by phage as well as fused to ABP, but not to the control mimotope Prof mim E. Remarkably, Bet mim E_{CSEY} showed the same IgE binding capability displayed on phage or fused to ABP. In contrast, IgE binding to the Bet mim E_{CQQ}-ABP fusion protein was reduced when compared with the phage-displayed peptide. No signal was obtained by coating human hybridoma IgE to the ELISA plates.

2. Induction of blocking antibodies to ABP-fused mimotopes

The expressed fusion proteins (ABP-Bet mim E_{CQQ}, ABP-Bet mim E_{CSEY}, and ABP-Prof mim E) were used for immunization of BALB/c mice. The specific immune response in sera of mice was analyzed by ELISA (Fig. 1A). Four of five BALB/c mice immunized with ABP-Bet mim E_{CQQ} and five of five BALB/c mice immunized with ABP-Bet mim E_{CSEY} developed *Bet v 1*-specific IgG antibodies. In contrast, sera of mice immunized with Prof mim E fusion protein did not show any *Bet v 1*-specific reactivity.

To provide further evidence that induced IgG immune response was directed toward natural IgE epitopes on *Bet v 1*, we performed inhibition experiments. The results are presented in Fig. 1B. Preincubation of r*Bet v 1* with a sera pool from both groups of mice immunized with Bet mim E fusion proteins (ABP-Bet mim E_{CQQ} and ABP-Bet mim E_{CSEY}) resulted in 33% inhibition of human IgE binding. The inhibition capability of these sera was lower than of sera obtained from mice immunized with phage-displayed Bet mim E.

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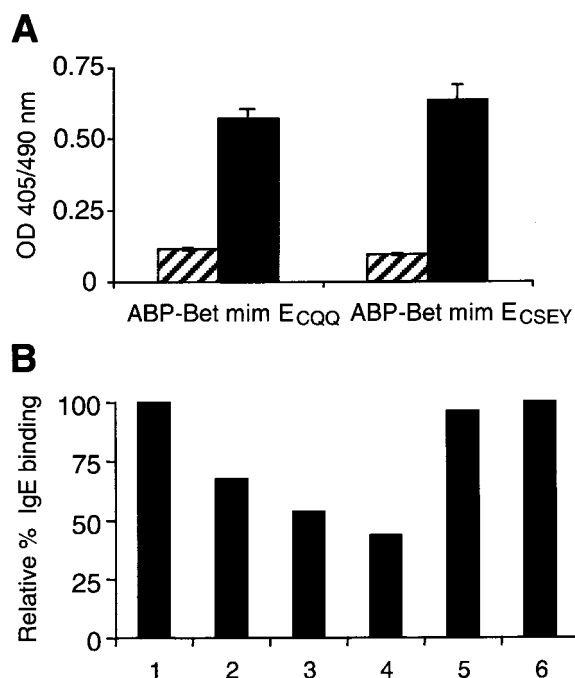


Figure 1. Anti-mimotopes antibodies and their properties. *A*) Mice immunized with ABP-Bet mim E_{CQQ} and ABP-Bet mim E_{CSEY}, respectively, form specific IgG to rBet v 1. Antibodies in sera of mice were measured by ELISA. Plates were coated with rBet v 1, incubated with preimmune and immune sera of mice, and bound IgG detection was performed with peroxidase-labeled anti-mouse IgG antibody. The mean values of both mouse groups (each *n*=5) are shown. ELISA results with preimmune sera (dashed bars) are compared with results with immune sera (black bars). *B*) Preincubation with mouse anti-Bet mim E antibodies reduces human IgE binding to rBet v 1 in ELISA. Plates were coated with rBet v 1 and preincubated with mouse sera. Lane 1: preimmune serum; lane 2: serum pool of mice immunized with ABP-Bet mim E; lane 3: serum pool of mice immunized with phage-displayed Bet mim E; lane 4: inhibiting monoclonal antibody '0590B2'; lane 5: serum pool of mice immunized with ABP-Prof mim E; lane 6: preincubation with serum pool of mice immunized with phage-displayed Prof mim E. Thereafter, plates were incubated with a pool of Bet v 1 allergic patients' sera. Bound IgE was detected by alkaline phosphatase-labeled anti-human IgE. Maximum absorption was set equal to 100%.

3. ABP-fused mimotopes cannot cross-link cell-bound IgE antibodies

To test the IgE cross-linking capability of ABP-fused Bet mim E, BALB/c mice were immunized twice with recombinant Bet v 1 and Al(OH)₃ as adjuvant, a schedule usually rendering a Th2 type response and IgE formation. Skin testing was performed with the Bet mim E fusion proteins (ABP-Bet mim E_{CQQ} or ABP-Bet mim E_{CSEY}), the control fusion protein (ABP-Prof mim E), Bet v 1, and profilin. The histamine-releasing compound 48/80 was used as positive control and PBS as negative control. All mice developed Bet v 1-specific immune response but showed no skin reaction with the Bet mim E fusion proteins. A representative skin testing experiment is shown in **Fig. 2**.

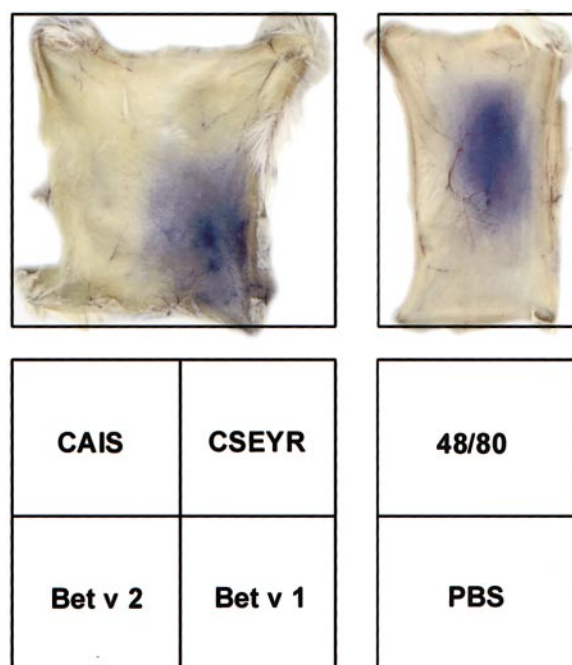


Figure 2. Skin testing of rBet v 1 allergic mice with ABP fusion proteins shows that ABP-Bet mim E does not cross-link cell-bound IgE antibody. Mice were immunized twice with rBet v 1 and Al(OH)₃ as adjuvant. Skin testing was performed 7 days after the last injection. Mice were challenged intradermally with ABP-Bet mim E (CYESR), ABP-Prof mim E (CAIS), rBet v 1, rProf (Bet v 2), mast cell degranulation substance 48/80 as positive, or PBS as negative control. One representative skin testing experiment of 10 mice tested is shown.

CONCLUSIONS AND SIGNIFICANCE

Immunotherapy with monovalent IgE mimotopes may represent a novel concept for treating type I allergy. Native and artificial IgE epitopes have the potential to cross-link IgE bound to the high-affinity IgE receptor (FcεRI), leading to release of histamine and other inflammatory mediators in sensitized individuals. To avoid binding of allergens to IgE on FcεRI-bearing

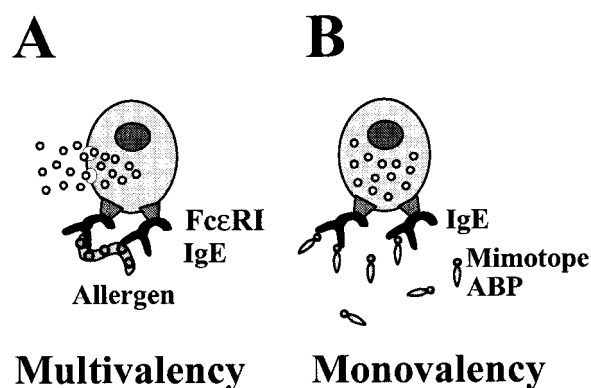


Figure 3. Schematic diagram showing the principle of IgE cross-linking bound to effector cells via the high-affinity receptor (FcεRI). Multivalent allergens can trigger histamine release (*A*) whereas monovalent ABP mimotopes do not (*B*).

cells, it is important to develop monovalent carriers for the IgE mimotopes. Therefore, we expressed the mimotopes as fusion proteins with ABP and demonstrated correct folding of the fusion protein by its recognition through *Bet v* 1-specific human IgE. The mimotopes presented by ABP obviously adapted the same shape as during selections when displayed on phage. In a previous study, we used phage particles as immunogenic vectors to generate IgE mimotopes. Here we demonstrate that the expressed Bet mim E fusion proteins were also able to induce a *Bet v* 1-specific B cell response in mice. These mice produced IgG, which recognized the natural conformational IgE epitope of

the allergen and reduced consecutive binding to IgE. The results from skin testing of *Bet v* 1-sensitized mice support the notion that fusion proteins may be safely used for vaccination of allergic individuals, as they do not cross-link cell-bound IgE and all mice apparently were healthy until the day of death. A synthesis of the conceptual approach for the action of fusion proteins with IgE mimotopes is schematically depicted in **Fig. 3**.

Based on previous findings and the present results, we conclude that the use of monovalent constructs with artificial IgE haptens, epitopes, or mimotopes may reduce the risk of anaphylactic reactions during epitope-specific immunotherapy. **[FJ]**