

IgE-Related Peptide Mimotopes

Basic Structures for Anti-Allergy Vaccine Development

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The interaction between IgE and its high affinity receptor (FcεRIα) is a crucial event in the development of anaphylactic reactions. The efficacy of the treatment of allergic disease by inhibition of receptor binding using monoclonal anti human IgE antibodies has been demonstrated in clinical trials. The strategy of introducing such inhibitory antibodies by vaccination with large fragments of human IgE encounters two problems: (a) the possible generation of an anaphylactogenic immune response supposed to cause severe side effects, and (b) the expected low immunogenicity of these autoantigen-derived IgE fragments in humans. The monoclonal antihuman IgE antibody BSW17 recognizes IgE in solution thereby inhibiting receptor attachment but also binds to FcεRIα-bound IgE [1]. However, BSW17 is non-anaphylactogenic [2]. Here we present a vaccination strategy based upon peptides (mimotopes) mimicking the natural epitope of BSW17. Such mimotopes can be used as immunogens which present 'semi-foreign' structures to the human immune system which are at the same time able to elicit a specific and protective anti-human IgE response.

Costar RIA plates were coated with BSW17 and used for biopanning of random peptide phage libraries. Both pIII and pVIII libraries were screened displaying either linear or

disulfide bridge-constrained peptides from 6 to 15 amino acids in length. Phage particles strongly binding to BSW17 were enriched and analyzed by DNA sequencing. Two groups of mimotope peptides were identified which mimic parts of the original BSW17 epitope. Alignment with a IgG homology-based structural computer model for hIgE [3] revealed that BSW17 recognizes a complex conformational epitope comprising regions of both the Cε3 and Cε4 domain. The Cε3 epitope sequence (C3E; amino acids 370–379) belongs to a region which takes part in receptor binding [3], the Cε4 epitope sequence (C4E; amino acids 497–506) represents a region hypothetically involved in the induction of target cell triggering [4].

Mimotope peptides consisting of amino acid sequences deduced from phage display (BSW17 mimotopes) were chemically synthesized, coupled to keyhole limpet hemocyanin (KLH) as a protein carrier and used to immunize rabbits. Crude rabbit sera as well as IgG fractions purified by affinity chromatography on a Sepharose-mimotope peptide column were tested for the presence of specific antihuman IgE antibodies by ELISA. As shown in figure 1a, anti-mimotope antibodies obtained from immunized rabbits were able to recognize human IgE and to inhibit IgE/FcεRI binding.

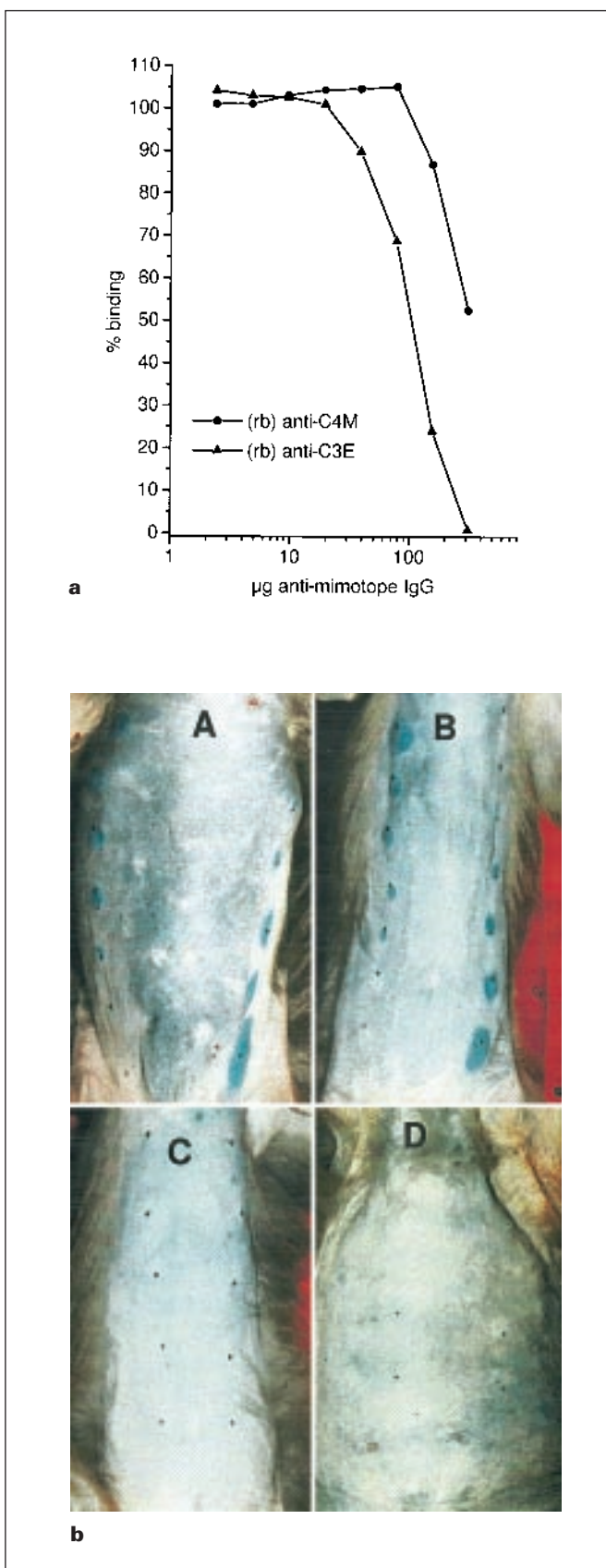
The protective effect of mimotope vaccination could also be demonstrated in monkeys by inhibition of the passive cutaneous anaphylaxis (PCA) reaction, a frequently used model for testing the anti-allergic potential of compounds in vivo [5]. PCA was performed in rhesus monkeys by inject-

ing increasing concentrations of human IgE (JW8) intradermally and inducing the anaphylactic skin reaction by i.v. administration of the JW8-specific allergen 4-hydroxy-3-nitro-4-hydroxy-iodophenyl acetic acid (NIP)-BSA together with Evan's blue resulting in the development of blue areas around the IgE (JW8) injection sites. As shown in figure 1b, anaphylactic skin reaction could be suppressed by vaccination of monkeys (primary immunization and two boosting injections) with either a C3 mimotope or a C4 mimotope-KLH conjugate.

References

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Fig. 1. a Inhibition of IgE/FcεRIα binding by affinity-purified polyclonal anti-mimotope immunoglobulins obtained from rabbits immunized either with the C3E peptide VNLTSRASG or the C4M peptide GEFCINHRGYWVCGDPA coupled to KLH as immunogenic carrier protein. Microtiter plate wells were each coated with 5 µg/ml BSA-NIP and incubated for 2 h at 37°C with 1 µg/ml of human IgE (JW8) and increasing amounts of the purified anti-mimotope Ig preparations. 1 µg/ml recombinant FcεRIα-horseradish peroxidase conjugate was used to bind uncomplexed IgE. The optical density readout is expressed as percentage of the value obtained without inhibitor addition. **b** Suppression of passive cutaneous anaphylaxis in rhesus monkeys vaccinated with BSW17 mimotope-KLH conjugates. Panels A and B show an untreated and a KLH-treated monkey, respectively. The monkey shown in panel C was immunized with the C3E peptide-KLH conjugate, the monkey shown in panel D was vaccinated with the C4M peptide-KLH conjugate.



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