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Mimotopes identify conformational B-cell epitopes on the two major house dust mite allergens Der p 1 and Der p 2

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Abstract

House dust mite allergy occurs in 10–20% of the population. Improvement of the present immunotherapy requires detailed knowledge about the structure of the allergens. Mimotopes selected from phage peptide libraries imitate the conformational epitopes of a natural allergen. The aim of our study was to generate epitope mimics for the two major allergens of house dust mite. When the monoclonal anti-Der p 1 and anti-Der p 2 antibodies were used for biopannings, mimotopes were selected which bound also specific IgE from human allergic patients' sera. The conformational matching of these mimotopes on the 3D structure of the natural allergens determined discontinuous epitopes in both cases, representing conformational B-cell epitopes relevant for binding of human IgE. Therefore, these mimotopes are potential candidates for the directed induction of blocking antibodies and epitope-specific immunotherapy of mite allergy.

Keywords: Allergy; Conformational epitopes; House dust mite allergens; Immunotherapy; Mimotopes

1. Introduction

Type I hypersensitivity reactions affect about 25% of the population in the industrialized countries. The key event in these reactions is the cross-linking of IgE antibodies on effector cells by allergens, which induces a release of biologically active mediators like histamine and leukotrienes leading to different symptoms of allergy (Ishizaka et al., 1966; Ravetch and Kinet, 1991). For better understanding of the mechanism of type I allergy and the mechanism of specific-immunotherapy several studies are ongoing (Akdis and Blaser, 2000; Moingeon et al., 2006), including attempts to use T-cell epitope peptides which were hampered by the occurrence of late phase reactions in the lungs (Oldfield et al., 2001).

For the generation of epitope-specific vaccines detailed knowledge of the antibody-binding sites, i.e. allergen epitopes, is required. B-cell epitopes are mainly conformational or even discontinuous, where several amino acid sections widely separated in the primary structure are assembled on the molecule surface when the polypeptide chain folds the native protein (Laver et al., 1990; Sela, 1969). These are exactly the regions of the allergen which are recognized by the complementary determining regions (CDRs) of the specific antibody (Crameri, 2003).

Mimotopes are peptides or proteins that mimic antibody epitopes, and do, consequently, not necessarily correspond to the linear sequence of the natural allergen. They do, moreover, rep-

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resent pure B-cell epitopes without direct stimulation capacity of allergen-specific T-cells (Scholl et al., 2002). This may be advantageous in future immunotherapy due to avoidance of late phase reactions. Last but not least, mimotopes allow the visualization of B-cell epitopes and the directed induction of epitope-specific antibodies against allergens (Hantusch et al., 2004; Jensen-Jarolim et al., 1998; Leitner et al., 1998; Untersmayr et al., 2006) or oncogene products (Riemer et al., 2004; Spillner et al., 2003).

This study focuses on the generation of mimotopes of major house dust mite allergens Der p 1 and Der p 2 (Chapman and Platts-Mills, 1980; Lind and Lowenstein, 1983) to characterize their conformational B-cell epitopes for future immunotherapy. Due to the wide cross-reactivity among mite species, active immunotherapy against Der p 1 and Der p 2 would potentially cure 80% of all mite-allergic patients globally (Meyer et al., 1994; van der Zee et al., 1988).

2. Materials and methods

2.1. Biopanning

To select specific ligands from a random peptide phage library, which was kindly provided by Prof. Dr. L. Mazzucchelli (Locarno, Switzerland), the biopanning technique was employed. This phage library, carrying the kanamycin resistance, presented 1.04×10^9 different nonamer peptides (nine amino acids) on the pIII minor coat protein of the M13 filamentous bacteriophage (Mazzucchelli et al., 1999). For selection, Der p 1- and Der p 2-specific human monoclonal antibodies (derived from patients' memory B cells) were used, kindly provided by Dr. J.M. Saint-Remy (Leuven, Belgium). Specific antibodies (40 μg/ml for the first and second rounds, 20 μg/ml for the third round) were coated in 0.1 M $NaHCO_3$, pH 8.5 on ELISA plate (Maxisorp, Nunc, Roskilde, Denmark) overnight (ON) at 4 °C. On the next day, wells were washed with 1% dry milk powder (DMP) in 0.1 M $NaHCO_3$, pH 8.5 and blocked with blocking buffer, 1% DMP in phosphate-buffered saline (PBS) for 2 h at room temperature (RT). After washing with blocking buffer, wells were incubated for 2 h at RT either with the original phage library or with the amplificate of the previous round diluted in blocking buffer/0.1% Tween 20. After washing away unspecific phages with PBS/0.05% Tween (PBST) and with PBS, specifically bound phages were eluted with acidic glycine buffer (0.1 M, pH 2.2) for 2 min, and immediately neutralised with 1 M Tris–HCl, pH 8.0. Eluted phage particles were amplified in *Escherichia coli* (K91), and were used for the next selecting biopanning round, or were stored at −80 °C. After three consecutive rounds of biopanning, the enrichment of the Der p 1- and Der p 2-specific phage particles was verified by capture ELISA and colony forming units test.

2.2. Colony forming units test (cfu) and capture enzyme-linked immunosorbent assay (ELISA)

For colony forming units test, diluted ON-culture of K91 with $OD_{600\,nm} = 0.8–1.0$ was infected with the eluted phage particles. Afterwards several dilutions of the infected bacteria were prepared in Luria-Bertani-medium (LB-medium). From each dilution, 10 μl were dropped on LB-kanamycin plates and incubated ON at 37 °C. The number of grown bacteria colonies reflects the concentration of infectious phage particles carrying the kanamycin resistance in cfu/ml.

For capture ELISA, Der p 1- and Der p 2-specific human IgG, respectively, and as a negative control, myeloma IgG were coated (1 μg/ml in 50 mM $NaHCO_3$, pH 9.6) overnight at 4 °C on ELISA plates. Thereafter, plates were washed with PBST and blocked for 1 h at RT and 1 h at 4 °C with PBST/1% bovine serum albumin (BSA). From each biopanning round, phage amplificates were diluted in PBST/0.1% BSA to the concentration of 10^{10} cfu/ml, and 100 μl/well were incubated ON at 4 °C. After washing the plates, bound phages were detected with a peroxidase-labeled mouse anti-phage antibody (Amersham Pharmacia Biotech, Little Chalfont, UK) and the reaction was developed by adding 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) in citric acid (pH 4.0) and hydrogen peroxide. The optical density (OD) was measured at 405–490 nm in an ELISA-Reader (Spectramax Plus, Molecular Devices, Munich, Germany).

2.3. Colony screening assay

Specific phage clones were screened on replica plates. *E. coli*, K91 bacteria were infected with different dilutions of phage amplificates from the last biopanning round and 100 μl were plated on LB-kanamycin plates and incubated ON at 37 °C to select phage clones carrying the kanamycin resistance.

Microtiter plates were filled with LB-kanamycin medium (50 μg/ml kanamycin), and 120 single clones were picked from the replica plates and transferred into the intended microtiter well (ELISA 1). A non-infected K91 colony and LB-medium were considered as controls. Plates were incubated ON at 37 °C, shaking at 160 rpm to let the bacteria grow. In the meantime new microtiter plates (ELISA 2) were coated either with 1 μg/ml Der p 1-or Der p 2-specific IgG and incubated ON at 4 °C. On the next day, ELISA 2 plates were washed and blocked as described previously. ELISA 1 with the overnight grown bacteria, each well representing one phage clone, was centrifuged for 30 min with 3000 rpm at 4 °C. Bacteria were pelleted on the bottom of the wells, whereas the phage particles remained in the supernatant. Phage supernatants of each well were transferred in duplicates to the intended wells of ELISA 2 and incubated ON at 4 °C. After washing the ELISA 2 plates, specifically bound phage particles were detected with mouse anti-phage antibody.

2.4. Specificity ELISA of selected phage clones

ELISA plates were coated with 1 μg/ml of Der p 1-, Der p 2-specific or myeloma IgG as negative control, ON at 4 °C. After washing and blocking, single phage amplificates were adjusted to the same concentration (10^9 cfu/ml) and were incubated on coated antibodies ON at 4 °C. Repeating the washing step, bound phage particles were detected as described in Section 2.2.