

Mapping of conformational IgE epitopes of food allergens

Food allergies reduce the quality of life of affected individuals and may result in anaphylaxis or even death. Allergen-specific immunotherapy (AIT) is based on vaccination with the disease-causing allergens. However, the administration of food allergens can produce severe side effects. Engineered food allergens with altered IgE epitopes hold the promise to improve the safety and efficacy of AIT. Food allergens contain linear and conformational IgE epitopes. Linear epitopes are easy to map by peptide microarrays. Conformational epitopes are challenging as they are created by protein folding which brings distantly located amino acid residues into spatial proximity. Knowledge of the allergen structure is required to map conformational IgE epitopes by various technologies (Table 1). Although linear and conformational epitopes are equally relevant for the clinical manifestation of food allergy, less information is available on conformational epitopes. The Immune Epitope Database (www.iedb.org) of experimentally mapped B- and T-cell epitopes lists 3986 linear and 87 conformational epitopes of allergens (accessed 07.08.2018).

The phage peptide display technology allows the expression of large libraries of random peptides fused to phage coat proteins. Phage libraries can be screened with monoclonal or polyclonal antibodies to identify peptide mimics of epitopes. Such mimotopes do not necessarily contain amino acid residues identical to the ones present in the epitope. Epitopes have to be located by sophisticated computer algorithms. IgE specific for the nonspecific lipid-transfer protein Pru p 3 of peach was used to screen a phage peptide display library.¹ Epitopes were mapped to the Pru p 3 surface by analyzing the electrostatic properties of the mimotopes. IgE from sera of peanut allergic individuals specific for the natural peanut 2S albumin Ara h 2 was used to isolate mimotopes which were all mapped to one specific area suggesting an immunodominant conformational epitope.²

Grafting of surface areas of allergens aims to recreate individual IgE epitopes on homologous proteins with low to no IgE binding. Homologous sequence fragments are replaced in the recipient protein by sequences from the allergen. Alternatively, spatially adjacent amino acid residues, but distant in the primary sequence, are mutated in the recipient protein according to the residues of the allergen. The resulting chimeras allow the evaluation of patient-specific epitope profiles. A conformational IgE epitope of Ber e 1, the allergenic Brazil nut 2S albumin, was mapped using chimeras based on the sunflower 2S albumin SFA-8.³ A variant of norcoclaurine synthase (NCS), a non-IgE-binding PR-10 protein, was used to produce nonallergenic NCS-soybean Gly m 4 chimeras.⁴ IgE binding of sera from soybean allergic individuals clustered in six major areas of the NCS-Gly m 4 chimeras.

Mouse monoclonal antibodies raised against an allergen can be screened for their ability (a) to recognize conformational epitopes by

not reacting with the denatured allergen and (b) to inhibit patients' IgE binding. To fine-map the epitope, the allergen sequence can be altered by mutagenesis or chimeric proteins of the allergen and a homologous nonallergenic protein can be produced. Loss of reactivity of the altered allergen with the antibody indicates that the right residues have been targeted. Ana o 2, an allergenic cashew nut hexameric bicupin, is homologous to the soybean allergen Gly m 6. The conformational epitope on Ana o 2 of a mouse mAb which competes with patients' IgE was fine-mapped by screening Ana o 2-Gly m 6 chimeras.⁵ In addition, alanine mutations were applied. Key residues of the epitope were identified near the N-terminus of the acidic subunit of Ana o 2.

Amide hydrogen/deuterium exchange (HDX) coupled with proteolysis and mass spectrometry (MS) is a powerful method to study protein-protein interactions. In HDX labeling, allergens are exposed to a deuterated solvent resulting in the exchange of protons by deuterium in the amide backbone of the protein. The deuterated allergen is then bound to a monoclonal antibody followed by replacing accessible deuterium by hydrogen. The antibody-allergen complex is subjected to proteolysis. Protein fragments that were protected by the antibody and contain deuterons are detected by their increased molecular weight by mass spectrometry, thus indicating the binding sites of the antibody. Epitope mapping of the almond bicupin Pru du 6 was performed by HDX-MS with a monoclonal antibody (mAb) that competes with patients' IgE.⁶ The epitope was further mapped by analyzing antibody binding to chimeras of Pru du 6 and the homologous soybean allergen Gly m 6.

Heteronuclear single quantum coherence (HSQC) experiments are applied in nuclear magnetic resonance (NMR) spectroscopy of ¹⁵N-labeled proteins to provide the correlation between amide nitrogen atoms and protons. Each amide yields a peak that is assigned to a particular amino acid residue. HSQC detects residues affected by binding of an antibody which results in a chemical shift of peaks indicating residues that are part of the epitope or perturbed by changes in the local protein conformation. Unambiguous assignment of an epitope is not always possible. Parvalbumin-specific single-chain antibodies were isolated from a phage library by sequential biopanning against cod (Gad m 1), carp, and trout parvalbumins.⁷ One single-chain antibody was able to inhibit binding of IgE from fish-allergic patients to all three parvalbumins. HSQC-NMR analysis of the Gad m 1 antibody complex showed participation of residues conserved among these parvalbumins.

X-ray crystallography of an allergen-antibody complex allows the most precise identification of conformational epitopes. Sophisticated algorithms and high technical expertise are required to analyze

TABLE 1 Technologies for the mapping of conformational IgE epitopes

Method	Advantages	Disadvantages	Examples
Phage peptide display	Mimotopes can be isolated quickly, polyclonal antibodies can be used.	Off-target mimotopes may be isolated, mimotopes need to be experimentally validated, epitopes need to be located by sophisticated computer algorithms.	Pru p 3 (peach nonspecific lipid-transfer protein) ¹ Ara h 2 (peanut 2S albumin) ²
Grafting of allergen surface areas unto nonallergenic homologous proteins	Polyclonal IgE can be used, evaluation of patient-specific epitope profiles and monitoring of AIT possible.	Not all chimeras are easily expressed, not all chimeras are soluble, fine-tuning requires production of large numbers of chimeras.	Ber e 1 (Brazil nut 2S albumin) ³ Gly m 4 (soybean Bet v 1 homologue) ⁴
Monoclonal antibodies combined with production of chimeric proteins and site-directed mutagenesis	No crystal structures are required.	Labor-intensive and time-consuming, false positive results may occur when the loss of antibody reactivity is due to changes in the structure outside of the epitope.	Ana o 2 (cashew hexameric bicupin) ⁵
Amide hydrogen/deuterium exchange mass spectrometry (HDX-MS)	Isotope-labeling of allergen is not required, no protein size limitations.	Resolution is limited by the size of the peptide fragments that can be produced.	Pru du 6 (almond hexameric bicupin) ⁶
Heteronuclear single quantum coherence nuclear magnetic resonance (HSQC-NMR) spectroscopy of allergen-antibody complexes	Production of cocrystals are not required, chemicals needed to isotope label the allergen are relatively inexpensive.	Requires production of isotope-labeled allergen, protein size limitation is ~30 kDa, unambiguous assignment of epitopes is not always possible.	Gad m 1 (cod β -parvalbumin) ⁷
X-ray crystallography of allergen-antibody complexes	Produces highest possible resolution of epitopes.	Requires the production of cocrystals, data acquisition may take a considerable amount of time or may be impossible, requires high technical skill.	Bos d 5 (cow's milk β -lactoglobulin) ⁸
Computational methods	Antibody-allergen cocrystals are not required.	Structures of homologous allergens are necessary, only limited validation of methods possible at present.	Bos d 5 (cow's milk β -lactoglobulin) ⁹ Phl p 5 (group 5/6 timothy grass pollen allergen) ⁹

crystals and interpret data. At present, the structure of only one cocrystal of two IgE Fab fragments in complex with dimeric β -lactoglobulin (Bos d 5) is available.⁸ The IgE-binding site was exclusively located in one Bos d 5 monomer encompassing an area of 890 Å².

Computational methodologies in combination with experimental data offer an alternative to predict epitopes. The software tool SPADE requires structures of at least two homologous allergens and their IgE cross-reactivity data.⁹ SPADE was evaluated using the x-ray structures of Bos d 5 and Phl p 2 complexed with IgE. The epitopes of Bos d 5 and Phl p 2 were localized with 33% and 57% accuracy, respectively. More experimental data are required to further validate SPADE or other in silico prediction methods.

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CONFLICTS OF INTEREST

The author declares that he has no conflicts of interest.

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