In Shape – The Art of Mapping Conformational Epitopes

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In type I allergy, IgE antibodies directed against specific epitopes on foreign proteins or glycoproteins are produced during the process of sensitization. These IgE antibodies are further bound to effector cells like mast cells and basophils, and their crosslink through the corresponding allergens is essential for the initiation of allergic symptoms. The vast majority of allergens contain conformational B cell epitopes, comprising amino acids that are in close vicinity only in the folded protein whereas they are noncontinuous in the linear sequence. Resulting from this three-dimensional architecture, conformational epitopes are difficult to characterize.

However, it is of great importance for the better understanding of immune responses and for the design of effective active and passive treatment strategies to gain also precise knowledge of the interacting surface between IgE antibodies and their corresponding epitopes.

The most direct way of identifying conformational epitopes is still the determination of the three-dimensional structure of the antibody-allergen complex by X-ray crystallography [1, 2]. But there are limitations in the performance as the procedure is very time consuming and needs a large amount of purified protein complexes. Another method is presented in the article of Gieras et al. [3] showing that peptide-specific monoclonal antibodies (mAb) can also map conformational IgE epitopes. An al-

ternative approach is the usage of filamentous phage libraries that display random sets of peptides. Panning of these libraries with the antibody of interest will select peptides that are involved in antibody-antigen interaction and that may mimic conformational B cell epitopes.

The article of Tiwari et al. [4] in this issue describes the mapping of a conformational epitope on the major cockroach allergen Bla g 2. This mapping was performed by the panning of a 12-mer phage display peptide library (i.e. Ph.D. library purchased from the New England Biolabs) with the mAb 7C11 [5] that is described as a surrogate for human IgE. For this reason, a modified version of the panning protocol was established to select in a more stringent way for high-affinity binding phages. Only two displayed peptides could be identified that share very high degrees of amino acid identities as they differed in only one amino acid. The isolated phages displayed peptides with similar specificity and binding strength. Peptides were further matched on the surface of the three-dimensional structure of Bla g 2 (PDB ID: 1YG9) with the help of the computational algorithm EpiSearch. In the present study, mapping of the two peptides led to the isolation of four overlapping surface patches that share four amino acids. The four patches identified 17 out of 18 experimentally determined residues; this corresponds to a discovery of 94%. The predicted conformational epitope

in the current study was finally validated by comparison with the genuine epitope presented by the already published co-crystal of Bla g 2 and mAb 7C11 [5].

The strength of this article is the selection of two peptides that – via the identification of four high scoring patches – discovered in the end 94% of all experimentally determined residues. The selection of these peptides was performed by a modified phage display screening method that included an additional elution step to get rid of weakly bound phages as well as a 10-fold reduction step in mAb 7C11 concentration from one panning round to the next. These very stringent isolation conditions select for high-affinity binders, therefore accepting low panning output. The current approach uses EpiSearch for mapping isolated peptides, a program that is described to show the advantages of being quite flexible and time-saving in comparison to other computational algorithms. EpiSearch was already applied for epitope mapping in six unrelated experimental data sets; in these cases it correctly mapped the location of conformational epitopes and the identified highest scoring patches covered in most cases more than 50% of the experimentally determined residues [6]. The authors of the current paper could even report an improvement in prediction of participating amino acid residues compared to the described former studies although, quite surprisingly, it was shown that the EpiSearch program is able to map 17 out of 18 amino acids from the genuine epitope with the help of two 12-mer peptides.

Nevertheless, there are also some weak points within the paper. The isolated peptides that were described here for the very first time were characterized only in their phage-bound version. The production of soluble peptides would offer tools to precisely study their interaction with the specific antibody not only for binding specificity and inhibition potential but also for affinity in Biacore analysis. Additionally, it would have been beneficial to isolate and map more than the two described peptides. Therefore, more extensive screening or screening of a more diverse library would be essential.

The identification of conformational epitopes via screening of random peptide libraries and further their mapping with the use of the EpiSearch program as described in this article seem to be useful in terms of defining their correct localization. These days much effort is put on the development of new fast and precise strategies dealing with epitope mapping. An example for a recent tool is the software SPADE that is the first approach predicting IgE epitopes by combining structural and cross-reactivity data and that compared to other prediction tools yields highest specificities [7].

However, with currently available technologies, the only rational method of identifying all amino acid residues from a conformational – ‘in shape’ – epitope is measuring the crystal structure of an allergen in complex with the corresponding antibody.

References

- 1 Niemi M, Jylha S, Laukkanen ML, Soderlund H, Makinen-Kiljunen S, Kallio JM, Hakulinen N, Haahtela T, Takkinen K, Rouvinen J: Molecular interactions between a recombinant IgE antibody and the beta-lactoglobulin allergen. *Structure* 2007;15:1413–1421.
- 2 Padavattan S, Flicker S, Schirmer T, Madritsch C, Randow S, Reese G, Vieths S, Lupinek C, Ebner C, Valenta R, Markovic-Housley Z: High-affinity IgE recognition of a conformational epitope of the major respiratory allergen Phl p 2 as revealed by X-ray crystallography. *J Immunol* 2009;182:2141–2151.
- 3 Gieras A, Cejka P, Blatt K, Focke-Tejkl M, Linhart B, Flicker S, Stoecklinger A, Marth K, Drescher A, Thalhamer J, Valent P, Majdic O, Valenta R: Mapping of conformational IgE epitopes with peptide-specific monoclonal antibodies reveals simultaneous binding of different IgE antibodies to a surface patch on the major birch pollen allergen, Bet v 1. *J Immunol* 2011;186:5333–5344.
- 4 Tiwari R, Negi SS, Braun B, Braun W, Pomes A, Chapman MD, Goldblum RM, Miodoro-Horiuti T: Validation of a phage display and computational algorithm by mapping a conformational epitope of Bla g 2. *Int Arch Allergy Immunol* 2012;157:327–334.
- 5 Li M, Gustchina A, Alexandratos J, Wlodawer A, Wunschmann S, Kepley CL, Chapman MD, Pomes A: Crystal structure of a dimerized cockroach allergen Bla g 2 complexed with a monoclonal antibody. *J Biol Chem* 2008;283:22806–22814.
- 6 Negi SS, Braun W: Automated detection of conformational epitopes using phage display peptide sequences. *Bioinform Biol Insights* 2009;3:71–81.
- 7 Dall’Antonia F, Gieras A, Devanaboyina SC, Valenta R, Keller W: Prediction of IgE-binding epitopes by means of allergen surface comparison and correlation to cross-reactivity. *J Allergy Clin Immunol* 2011;128:872–879.