

Received Date : 15-Apr-2016

Revised Date : 14-Jun-2016

Accepted Date : 21-Jun-2016

Article type : Review

Phage display peptide libraries in molecular allergology: from epitope mapping to mimotope based immunotherapy

Jernej Luzar, Borut Štrukelj, Mojca Lunder*

Chair of Pharmaceutical Biology, Faculty of Pharmacy, University of Ljubljana

*corresponding author

e-mail: mojca.lunder@ffa.uni-lj.si

address: Aškerčeva 7, 1000 Ljubljana, Slovenia

telephone: +386 1 47 69 570

Key words: immunotherapy, mimotopes, phage display peptide libraries

Abstract

Identification of allergen epitopes is a key component in proper understanding of the pathogenesis of type I allergies, for understanding cross-reactivity and for development of mimotope immunotherapeutics. Phage particles have garnered recognition in the field of molecular allergology due to their value not only in competitive immunoscreening of peptide libraries but also as immunogenic carriers of allergen mimotopes. They integrate epitope discovery technology and immunization functions into a single platform. This article provides an overview of allergen mimotopes identified through the phage display technique. We discuss the contribution of phage display peptide libraries in determining

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/all.12965

This article is protected by copyright. All rights reserved.

dominant B-cell epitopes of allergens, in developing of mimotope immunotherapy, in the understanding of cross-reactivity and in determining IgE epitope profiles of individual patients to improve diagnostics and individualize immunotherapy. We also discuss the advantages and pitfalls of the methodology used to identify and validate the mimotopes.

Introduction

With a current estimate of more than 150 million patients and a prediction of more than 300 million in Europe in the next decade, allergies constitute a public health concern of pandemic proportions requiring immediate action from scientific community (1). Identification of allergen epitopes is a key component in proper understanding of the pathogenesis of type I allergies, for understanding cross-reactivity and for developing mimotope immunotherapeutics. The term mimotope was first coined by Mario Geysen in 1986 (2), it was used to describe peptides that mimic epitopes. Such peptides mimic binding sites of the protein and are able to compete with the native protein for antibody binding. Phage display technology, coupled with in vitro selection, enables rapid identification and optimization of peptides or proteins based on their structural or functional properties (3). By means of phage displayed peptide libraries structures that mimic natural epitopes, such as conformational B-cell epitopes, can be defined (4-7). Peptides displayed on the phage and which are recognized by an anti-allergen antibody do not necessarily resemble the natural allergen B-cell epitopes; they mimic the physicochemical properties of the amino acids and are therefore mimotopes (Fig. 1) (8). Phage particles have garnered recognition in the field of molecular allergology due to their value not only in competitive immunoscreening of peptide libraries but also as immunogenic carriers of allergen mimotopes (Fig. 2). They integrate epitope discovery technology and immunization functions into a single platform (9).

In this work we review allergen mimotopes identified through screening peptide libraries displayed on bacteriophages. The methodology contributes to identification of dominant allergen epitopes and consequently to the development of mimotope immunotherapy. Moreover determining IgE epitope profiles of individual patients contributes to our understanding of cross reactivity and leads toward improved diagnostics and individualized immunotherapy. Several advantages and pitfalls of the methodology used for identification

and validation of mimotopes are also discussed.

Determining allergen's dominant epitopes

Major allergens contain a few prominent B-cell epitopes (10). Both conformational and linear epitopes are responsible for allergic reactions. Determining linear epitopes is useful for food allergens that undergo digestion or food processing. For inhaled allergens, conformational epitopes appear to be the primary targets of IgE response (11). While linear epitopes can be determined by analyzing IgE binding to overlapping peptides derived from the primary sequence (12), identification of conformational epitopes requires more elaborated methods, such as X-ray crystallography of antibody–antigen complexes or site-directed mutagenesis (13). A promising alternative to these methods is screening libraries of random oligopeptides such as biological phage-displayed libraries (14, 15) or chemical, “one-bead-one-compound” libraries (16) that allow selection of epitope mimicking peptides, regardless of whether or not the epitope is linear or conformational or even non-protein in nature (17). In combination with subsequent computer-based mapping of the identified peptides to the three-dimensional structure of allergen, IgE epitopes can be revealed (18).

Various lengths of cyclic and linear oligopeptides have been used for screening, from 7-mer to 15-mer. Even though epitopes are suggested to consist of at least 8 amino acids, energy calculations imply that a smaller subset of 5–6 amino acids is the key contributor to the binding between antibody and epitope (19-21). Some authors propose that only four or five amino acids residues are sufficient to comprise linear epitopes (22). Therefore, it has been suggested that choosing shorter peptides for immunoscreening favors the selection of those peptides containing the essential amino acids contributing to the binding between IgE molecules and epitopes, and deselects those which could simply be a result of non-specific binding (23).

The selection of target for immunoscreening is another important aspect when using oligopeptide libraries. The target can facilitate or hinder identification of dominant IgE epitopes. Targeting allergen-specific monoclonal IgG allows identification of a single consensus sequence or motif that may represent a dominant epitope, however conserved epitope specificity between human IgE and the target monoclonal should be confirmed. One of the early attempts to define dominant

conformational epitopes by peptide libraries displayed on phages was performed in 1998 for the birch pollen major allergen Bet v 1 (24). Two Bet v 1 specific murine monoclonal antibodies were screened with a constrained and a linear peptide library. However, retrieved peptides were not recognized by IgE from birch pollen allergic patients' sera which was also reactive with Bet v 1, indicating that the retrieved peptides did not mimic the IgE epitopes. Further studies confirmed that the monoclonal antibodies used had different epitope specificity compared to Bet v 1-affinity purified human IgE (25). On the other hand, targeting monoclonal antibodies is not unavoidably unsuccessful. Human monoclonal antibodies obtained from the memory B-cells of patients with dust mite allergy were screened to identify mimetics of dust mite allergens Der p 1 and Der p 2-dominant epitopes. Mimotope characteristics of selected peptides were reinforced by specific binding to IgG and IgE from the sera of allergic patients compared to control myeloma IgG (26). Affinity purified allergen-specific polyclonal IgG antibodies from immunized animals have also been successfully used to identify dominant IgE epitopes of the crustacean panallergen arginine kinase (27) and the major cat allergen Fel d 1 (28). As with the use of monoclonal antibodies, additional tests are needed to confirm the conserved epitope specificity between IgE epitopes of allergic patients and IgG of immunized animals. Polyclonal fractions (IgE or IgG isotype) of immunoglobulins from patients have also been frequently used as a target. By targeting the whole polyclonal IgE fraction from an individual patient, a unique pattern of mimotopes for each patient can be revealed (23, 29, 30). However, to extract a dominant epitope from such complex patterns requires an advanced bioinformatics approach, as demonstrated by Christiansen *et al.* in the process of epitope identification of a prominent peanut allergen Ara h 1 (30). Targeting affinity purified, allergen-specific IgE or IgG from the sera of allergic patients should more straightforwardly lead to selection of dominant peptide mimotopes for which high amount of paratopes exists. In this manner dominant IgE epitopes of Bet v 1 (25), timothy grass pollen allergen Phl p 5a (31), carp parvalbumin (32) and peach allergen Pru p 3 (33) were identified.

Confirmation of conserved epitope specificity between human IgE and the target antibody is essential. Proposing dominant IgE epitopes merely through computational surface mapping (34) does not provide reliable evidence. Even when obtained by targeting allergen-specific IgE, the proposed dominant epitope should be additionally validated. Competition tests of selected

peptides, e.g. synthetic peptides or those displayed on phages, with allergen for binding sites on target antibodies and IgE from patients should corroborate peptide mimotope nature. However, due to conformational inhomogeneity of peptides in solution, sequence-identical synthetic peptides might be ineffective in blocking antibody – allergen interaction, as was the case in the study of Jensen-Jarolim *et al.* (24). Ultimately, immunization with mimotopes of dominant IgE epitopes should elicit IgG responses to intact allergens and substantiate their immunotherapeutic potential.

Mimotopes of allergens for immunotherapy

Allergen-specific immunotherapy by the administration of increasing doses of allergen extracts remains the single curative approach to allergic diseases. Its potential to induce specific immune tolerance and yield a long term disease modifying effect is ascribed to the down-regulation or modulation of the allergen-specific Th2 response and the induction of allergen-specific blocking antibodies (35). However, crude allergen extracts do not guarantee complete avoidance of anaphylactic side effects. They contain variable allergen content and are often cumbersome to standardize. Moreover, they contain allergenic as well as a variety of nonallergenic components giving rise to new IgE reactivities to previously non-IgE binding allergen components (36). Progressing to specific isolated or recombinant allergen molecules as a whole, avoids the drawbacks of crude extracts; however, it is still not possible to predict which epitopes will be targeted by resulting IgG. The IgG antibodies may modulate the binding of specific IgE to allergen in both directions. They may enhance or inhibit the interaction depending on their own epitope specificity (37, 38). T-cell epitopes, linear peptides derived from allergen primary structure have been considered as an approach to specific immunotherapy and have been used in clinical trials (39) in contrast to B-cell epitopes which to the present date, have not been used on allergic patients. However due to the length of these peptides (10-30 amino acids) the risk of at least two B-cell epitopes being present and consequently possibility of IgE crosslinking remains (39). Therefore, it is an attractive concept to use isolated B-cell epitopes capable of inducing IgG response directed to an IgE epitope. The “blocking” IgG response should prevent the IgE - allergen interaction without sensitizing effector cells such as mast cells or basophils via FcεRI. Selection of suitable target for screening peptide libraries and an appropriate assay to confirm epitope specificity is again essential to determine mimotopes with immunotherapeutic potential.

In the study of Jensen-Jarolim *et al.* oral immunization of mice with a mimotope displayed on phages in multiple copies yielded IgG response to the natural allergen (24). However, the mimotope had different epitope specificity compared to affinity purified patients' IgE and therefore induced IgG directed to a Bet v 1 epitope distinct from the IgE epitope, which enhanced allergic skin reactivity in a murine model (40, 41). The authors interpreted the enhancement as IgG-induced stabilization of a conformational state in which certain IgE epitopes are more readily accessible. Using affinity purified IgE from birch pollen allergic patients as target, the same group (25) selected a different mimotope. Displayed on phage it was again used to immunize BALB/c mice and this time development of a blocking IgG response that should accompany specific immunotherapy, was achieved.

To establish the immunotherapeutic potential of a selected mimotope it should initially be capable of binding to IgE isolated from the sera of patients with a particular allergy or able to inhibit IgE binding to the natural allergen. Additionally the capacity to induce IgG response to their corresponding allergen epitopes should be demonstrated in immunization trials. In this manner, the therapeutic potential of individual mimotopes of grass pollen Phl p 5 was studied. Using Phl p 5a-specific IgE from patients' sera as target, Hantusch *et al.* identified 15 candidates from peptide phage library (31). Three peptides (F1, F29 and H32) displayed on phages with the strongest capabilities for blocking the interaction between specific IgE and Phl p 5a were used to immunize Biozzi mice and two of them (F1 and F29) indeed induced antibodies recognizing the original antigen.

Additional studies on the therapeutic advantages of B-cell epitopes devoid of allergen-specific T-cell epitopes were carried out by Wallmann *et al.* in which peptide F1 was administered by gene gun or intradermally as a cDNA construct with phage coat protein pIII to BALB/c mice. Immunization elicited Phl p 5-specific antibody response, without triggering allergen-specific T-lymphocytes (42). Therapeutic efficacy of peptide H32 was also further tested. The peptide was chemically coupled at a high density to keyhole limpet hemocyanin and as such induced Phl p 5a-reactive IgG in naïve BALB/c mice. Its previous lack of immunogenicity in Biozzi mice was attributed to low copy number of peptides displayed on phage protein pIII. Moreover, anti-inflammatory effects of the mimotope vaccine were observed in the memory mouse model of

acute asthma. The authors presumed the absence of allergen-specific T-cell epitopes might have contributed to this (43).

As observed in the above studies peptide mimotopes are usually connected to other peptides and proteins, since synthetic peptides are often not immunogenic enough. Allergen mimotope immunizations have been performed using immunogenic carriers such as keyhole limpet haemocyanin (43), streptococcal albumin binding protein (44), phage coat proteins as a part of gene vaccine (42) and also using phage particles directly from libraries (25, 40, 41).

Bacteriophage particles as immunogenic carriers of allergen mimotopes allow for variation in peptide copy number on their coat proteins. This is important since the strength of anti-peptide response depends on immunogen peptide copy number. Moreover, filamentous phage carriers were also shown to elicit more focused anti peptide response compared to traditional carrier proteins such as ovalbumin (45). They are well-documented as immunogenic carriers for recombinant peptides (46-52). They have been administered orally (46), subcutaneously (50), intraperitoneally (45), intramuscularly (53), intravenously (54) and intradermally (55). They have also been also used in Phase I/II human immunization studies (56). Filamentous phages are at least as immunogenic as traditional carrier proteins such as albumin or keyhole limpet haemocyanin and have relatively few endogenous B-cell epitopes to divert the antibody response from its intended target (57).

The low cost of production of phage particles, their high degree of stability and their potential for high valency display of peptides makes them attractive as immunogenic carriers. On the other hand, the bacterial production and preparation heterogeneity at present limits their application in human vaccines, despite being apparently safe and well tolerated in animals and people (9).

Investigating cross-reactivity mechanisms

Mimotope peptides are useful for studying potential cross-reactivity between homologous proteins. With phage display libraries structure determination of major allergens is not a prerequisite for analyzing surface exposed areas of the allergen and for mapping conformational epitopes. Therefore, it represents an important tool in cross-reactivity investigations. Predictions

of cross-reactivity can be made based on the homology of mimotope peptides pointing towards homology of surface exposed residues on allergen three-dimensional structure. As cross-reactive antigens not only share similar amino acid sequence but also structural homology, the generation of such mimotopes could be useful for the development of immunotherapy targeting epitopes within a clinically cross-reactive allergen group.

A high degree of cross-reactivity resulting from shared IgE-binding epitopes is a characteristic of taxonomically related grasses (58). Suphioglu *et al.* used a murine monoclonal antibody that recognizes major allergens of a number of clinically significant grass pollens as target for screening peptide library displayed on phages (59). The peptide, which occurred with the highest frequency, was able to inhibit binding of both target monoclonal antibody and human IgE from patients' sera to eight different grass pollen extracts. The authors concluded that the selected mimotope peptide represents a common epitope among related allergens of different grass pollens.

Cross-reactivity is also a familiar phenomenon between allergens from pollens and food allergens. Plant profilins, a highly conserved panallergen family (amino acid sequence identities over 70% between most pairs of members), which is present in almost every plant source investigated seem to be one of the main responsible agents of cross-sensitization between plant foods and pollens (60). Cross reactions among distantly related species are likely to be a consequence of the presence of common epitopes. Along these lines the first attempt to compare epitopes of profilins using phage display libraries was performed by Leitner *et al.* in 1998. Mimotopes of profilins from birch pollen, mugwort pollen and celery tuber were selected on affinity purified immunoglobulin fraction from patient sera. The prevalently selected peptide mimotope reduced binding of IgE from patients' sera to birch pollen as well as to mugwort and celery tuber profilin. The authors proposed it mimics a common conformational IgE epitope for these examined profilins (61). Similarly, epitopes of Cuc m 2, model plant food profilin, were compared to those of timothy grass, birch pollen and human I profilin through mimotope peptides selected on IgE fraction from pooled sera of melon allergic patients, of which a majority also reacted with timothy pollen profilin (Phl p 2) and birch pollen profilin (Bet v 2). Localization of mimotope peptides onto the surface of Cuc m 2 and the two pollen profilins (Phl

p 2 and Bet v 2) led to homologous surface residues; greater differences were obtained for human I profilin, in agreement with high and low cross-reactivity, respectively. Due to the absence of Bet v 2 in the living environment of the patients, cross-reactivity with the allergen was explained by the presence of common epitopes (62).

In birch pollen-allergic patients associated food allergy is also often mediated by Bet v 1-homologous proteins from food. Since divergent patterns of cross-reactivity and clinically relevant allergic reactions to food occur in the individual patients, Mittag *et al.* (63) investigated and compared patient individual IgE-epitope patterns on Bet v 1 and the homologous food proteins Gly m 4 (soy), Ara h 8 (peanut) and Pru av 1 (cherry). The peptides targeting the IgE fraction of each individual patient were selected by specific elution with each of the respective allergens. Individual patterns of epitope regions on each allergen were found for individual sera of five patients; however, three antigenic areas that are likely to be IgE-binding epitopes common to all or most of the patients were defined. This was consistent with the extensive cross-reactivity against the four proteins that was found in cross-competitive ELISA. Results obtained for Bet v 1 showed a higher degree of similarity with Pru av 1 than with Gly m 4 and Ara h 8. Correspondingly, there is higher degree of sequence identity between Bet v 1 and Pru av 1 than with legume homologues.

As shown by Mittag *et al.* (63), not only common epitopes of homologous allergens, but also individual IgE epitope patterns are important. Peptide mimotopes from phage displayed libraries enable discovery of IgE epitope specificity and diversity in individual patients as well.

Determining individual IgE epitope profiles

Information on IgE epitope specificity and diversity of individual patients may support the development of patient tailored immunotherapy and diagnostic tools for predicting cross-reactivity and allergenic potential of novel foods for individual patients. Indeed, different IgE epitope patterns have been found to be associated with the allergic phenotype, where e.g. epitope specificity correlated with persistency and diversity correlated with severity (64).

Bogh *et al.* identified and compared individual epitope patterns of intact and digested Ara h 1, the first identified major peanut allergen. Heterogeneity of the Ara h 1-specific IgE antibody

Accepted Article
response among patients was demonstrated by individual patterns of selected sequences for each patient. However, some were shared by two or more patients. From individual patterns the authors deduced five important IgE binding epitopes on Ara h 1 by mapping selected peptides on the 3D model of Ara h 1 (23). In contrast to most previous studies of food allergens (65, 66), phage display technology allowed identification of conformational epitopes. By comparing the mimotope patterns obtained in elution with intact or digested Ara h 1, which were similar regardless of the elution condition, the authors substantiated their previous results that the digests of Ara h 1 aggregate and could in this way retain allergenicity (67).

It has been suggested that coincident of IgE and IgG₄ binding epitopes and increased affinity of specific IgG₄ antibodies is associated with the development of tolerance, by specific IgG₄ blocking specific IgE antibody binding to the allergen (68, 69). Along these lines Bogh *et al.* (29) additionally determined conformational IgG₄ epitope patterns of three allergic patients with the greatest IgE epitope pattern similarity from previous study (23). In the study of Bogh *et al.* individual distinct IgG₄ epitope recognition patterns emerged and appeared more heterogeneous compared to IgE epitope patterns. Additionally, IgE epitope-mimics had much higher affinity in phage ELISA than IgG₄ epitope-mimics, suggesting the existence of Ara h 1-specific IgE antibody clones able to bind with higher affinity than any clones in the Ara h 1-specific IgG₄ antibody repertoire. The study demonstrates IgE and IgG₄ epitope patterns and affinities of individual patients could be obtained and compared through mimotope peptides from phage display libraries. In combination with bioinformatics this approach could provide a diagnostic and prognostic insight in the course of immunotherapy.

The full strength of phage display technology combined with high throughput sequencing and bioinformatics approach was recently demonstrated by Christiansen *et al.* and led from patient specific IgE mimotope motifs towards the discovery of dominant epitope on Ara h 1 (30). Peptides were selected on IgE from Ara h 1 reactive patients and IgE from control sera, separately for each patient or control. The output of selection and high-throughput sequencing was a great variety of peptides (average per sample after filtering process was 161072 (SD=82792)). The bioinformatics pipeline following was based on clustering and contrasting with control samples and led to identification of a relevant peptide motif. Peptides contributing

to the motif were aligned to the primary sequence of Ara h 1 around position 136. The authenticity of the dominant epitope was confirmed also with serum reactivity towards Ara h 1 on peptide micro-arrays. The identified epitope was also included in the previously described epitope hotspot of Ara h 1 (65), however no similarity was found with conformational epitopes identified by conventional phage display described above (23).

The approach of Christiansen *et al.* also highlighted and efficiently circumvented two major issues of phage displayed peptide libraries. When dealing with complex biological samples such as human sera, the first issue is selection of unspecific peptides or target unrelated peptides (70). Christiansen *et al.* noticed the most frequent peptides in the patients samples were also the most frequent in the control samples, despite small general overlap between the two groups, which indicated that they were target unrelated. In fact, on average 6 out of 10 most frequent peptides in each selection round were also observed in the control samples. The clustering approach in the bioinformatics pipeline very efficiently eliminated target unrelated peptides and peptide sequences that clustered with similar peptides obtained in control samples (30). When dealing with smaller number of peptides identification and elimination from individual patterns can be achieved by comparisons to target unrelated peptides using a database such as SAROTUP (71).

The second issue that emerged in the study of Christiansen *et al.* (30) is the low frequency of true target binders which makes them difficult to detect by limited Sanger sequencing, especially from screening of complex mixtures such as immunoglobulins. Consequently, and as mentioned previously, strong additional evidence is required to identify a conformational epitope mimic beyond simple *in silico* mapping to allergen surface.

Conclusion

Oligopeptide libraries displayed on phage particles allow determination of dominant epitopes or epitope profiles of individual patients, regardless if the epitopes are linear or conformational or even non-protein. One important aspect is the choice of target for immunoscreening. In particular, when targeting monoclonal IgG or polyclonal IgG from immunized animals, conserved epitope specificity between allergic patients' IgE and the target antibodies should be confirmed. Mimotope peptides of dominant epitopes capable of inducing IgG response directed

to an IgE epitope have immunotherapeutic potential and filamentous phages themselves are promising immunogenic carriers for these peptides. On the other hand, epitope profiles can be useful for studying potential cross-reactivity between homologous proteins, for designing recombinants for immunotherapy, in a predictive manner for assessing allergenic potential of novel foods and genetically modified foods. Additionally, epitope profiles can be used as predictive biomarkers for persistency and severity of an allergic disease.

Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

Jernej Luzar and Mojca Lunder made significant contribution to acquisition and interpretation of the data. Mojca Lunder drafted the article. Borut Štrukelj critically revised the article. All authors approved the final manuscript.

Figure 1 Mimotopes recognized by an anti-allergen antibody paratopes mimic the physicochemical properties of the amino acids, which compose the natural allergen B-cell epitopes.

Figure 2 Phage particles displaying oligopeptide libraries enable identification of allergen mimotopes (A), peptide mimetics of epitopes' physicochemical properties, through competitive immunoscreening (B). They are also suitable immunogenic carriers (C) with possible role in mimotope immunotherapy (D).

References

1. Calderon MA, Demoly P, Gerth van Wijk R, Bousquet J, Sheikh A, Frew A, et al. EAACI: A European Declaration on Immunotherapy. Designing the future of allergen specific immunotherapy. *Clin Transl Allergy* 2012;**2**:20.
2. Geysen HM, Rodda SJ, Mason TJ. A-Priori Delineation of a Peptide Which Mimics a Discontinuous Antigenic Determinant. *Molecular Immunology* 1986;**23**:709-715.
3. Gray BP, Brown KC. Combinatorial peptide libraries: mining for cell-binding peptides. *Chem Rev* 2014;**114**:1020-1081.
4. Vodnik M, Molek P, Strukelj B, Lunder M. Peptides binding to the hunger hormone ghrelin.

Horm Metab Res 2013;**45**:372-377.

5. Zager U, Lunder M, Bozic B. Using Phage Display in Autoimmunity Research. *Acta Chimica Slovenica* 2011;**58**:742-754.
6. Devlin JJ, Panganiban LC, Devlin PE. Random peptide libraries: a source of specific protein binding molecules. *Science* 1990;**249**:404-406.
7. Moreau V, Granier C, Villard S, Laune D, Molina F. Discontinuous epitope prediction based on mimotope analysis. *Bioinformatics* 2006;**22**:1088-1095.
8. Pali-Scholl I, Jensen-Jarolim E. Biopanning for the characterization of allergen mimotopes. *Methods Mol Med* 2008;**138**:271-283.
9. Henry KA, Arbabi-Ghahroudi M, Scott JK. Beyond phage display: non-traditional applications of the filamentous bacteriophage as a vaccine carrier, therapeutic biologic, and bioconjugation scaffold. *Front Microbiol* 2015;**6**:755.
10. Vrtala S, Ball T, Spitzauer S, Pandjaitan B, Suphioglu C, Knox B, et al. Immunization with purified natural and recombinant allergens induces mouse IgG1 antibodies that recognize similar epitopes as human IgE and inhibit the human IgE-allergen interaction and allergen-induced basophil degranulation. *J Immunol* 1998;**160**:6137-6144.
11. Pomes A. Relevant B cell epitopes in allergic disease. *Int Arch Allergy Immunol* 2010;**152**:1-11.
12. Reese G, Ayuso R, Leong-Kee SM, Plante MJ, Lehrer SB. Characterization and identification of allergen epitopes: recombinant peptide libraries and synthetic, overlapping peptides. *J Chromatogr B Biomed Sci Appl* 2001;**756**:157-163.
13. Dall'antonia F, Pavkov-Keller T, Zangger K, Keller W. Structure of allergens and structure based epitope predictions. *Methods* 2014;**66**:3-21.
14. Riemer A, Scheiner O, Jensen-Jarolim E. Allergen mimotopes. *Methods* 2004;**32**:321-327.
15. Davies JM, O'Hehir R E, Suphioglu C. Use of phage display technology to investigate allergen-antibody interactions. *J Allergy Clin Immunol* 2000;**105**:1085-1092.
16. Leung NY, Wai CY, Ho MH, Liu R, Lam KS, Wang JJ, et al. Screening and identification of mimotopes of the major shrimp allergen tropomyosin using one-bead-one-compound peptide libraries. *Cell Mol Immunol* 2015.
17. Tang ZM, Jiang WC, Chang PP, Fang M, Rong Y, Hu LH. Mimotope selection of blood group A antigen from a phage display 15-mer peptide library. *Transplant Immunology* 2013;**29**:39-42.
18. Roggen EL. Recent developments with B-cell epitope identification for predictive studies. *J Immunotoxicol* 2006;**3**:137-149.
19. Bannon GA, Ogawa T. Evaluation of available IgE-binding epitope data and its utility in bioinformatics. *Mol Nutr Food Res* 2006;**50**:638-644.
20. Van Regenmortel MHV. Mapping Epitope Structure and Activity: From One-Dimensional Prediction to Four-Dimensional Description of Antigenic Specificity. *Methods* 1996;**9**:465-472.
21. Laver WG, Air GM, Webster RG, Smith-Gill SJ. Epitopes on protein antigens: misconceptions and realities. *Cell* 1990;**61**:553-556.
22. Tanabe S, Arai S, Yanagihara Y, Mita H, Takahashi K, Watanabe M. A major wheat allergen has a Gln-Gln-Gln-Pro-Pro motif identified as an IgE-binding epitope. *Biochem Biophys Res Commun* 1996;**219**:290-293.
23. Bogh KL, Nielsen H, Madsen CB, Mills EN, Rigby N, Eiwegger T, et al. IgE epitopes of intact and digested Ara h 1: a comparative study in humans and rats. *Mol Immunol*

2012;**51**:337-346.

24. Jensen-Jarolim E, Leitner A, Kalchhauser H, Zurcher A, Ganglberger E, Bohle B, et al. Peptide mimotopes displayed by phage inhibit antibody binding to Bet v 1, the major birch pollen allergen, and induce specific IgG response in mice. *FASEB J* 1998;**12**:1635-1642.
25. Ganglberger E, Grunberger K, Sponer B, Radauer C, Breiteneder H, Boltz-Nitulescu G, et al. Allergen mimotopes for 3-dimensional epitope search and induction of antibodies inhibiting human IgE. *FASEB J* 2000;**14**:2177-2184.
26. Szalai K, Fuhrmann J, Pavkov T, Scheidl M, Wallmann J, Bramswig KH, et al. Mimotopes identify conformational B-cell epitopes on the two major house dust mite allergens Der p 1 and Der p 2. *Mol Immunol* 2008;**45**:1308-1317.
27. Yang Y, Cao MJ, Alcocer M, Liu QM, Fei DX, Mao HY, et al. Mapping and characterization of antigenic epitopes of arginine kinase of *Scylla paramamosain*. *Mol Immunol* 2015;**65**:310-320.
28. Luzar J, Molek P, Silar M, Korosec P, Kosnik M, Strukelj B, et al. Identification and characterization of major cat allergen Fel d 1 mimotopes on filamentous phage carriers. *Mol Immunol* 2016;**71**:176-183.
29. Bogh KL, Nielsen H, Eiwegger T, Madsen CB, Mills EN, Rigby NM, et al. IgE versus IgG4 epitopes of the peanut allergen Ara h 1 in patients with severe allergy. *Mol Immunol* 2014;**58**:169-176.
30. Christiansen A, Kringelum JV, Hansen CS, Bogh KL, Sullivan E, Patel J, et al. High-throughput sequencing enhanced phage display enables the identification of patient-specific epitope motifs in serum. *Sci Rep* 2015;**5**:12913.
31. Hantusch B, Krieger S, Untersmayr E, Scholl I, Knittelfelder R, Flicker S, et al. Mapping of conformational IgE epitopes on Phl p 5a by using mimotopes from a phage display library. *J Allergy Clin Immunol* 2004;**114**:1294-1300.
32. Untersmayr E, Szalai K, Riemer AB, Hemmer W, Swoboda I, Hantusch B, et al. Mimotopes identify conformational epitopes on parvalbumin, the major fish allergen. *Mol Immunol* 2006;**43**:1454-1461.
33. Pacios LF, Tordesillas L, Cuesta-Herranz J, Compes E, Sanchez-Monge R, Palacin A, et al. Mimotope mapping as a complementary strategy to define allergen IgE-epitopes: peach Pru p 3 allergen as a model. *Mol Immunol* 2008;**45**:2269-2276.
34. Furmonaviciene R, Tighe PJ, Clark MR, Sewell HF, Shakib F. The use of phage-peptide libraries to define the epitope specificity of a mouse monoclonal anti-Der p 1 antibody representative of a major component of the human immunoglobulin E anti-Der p 1 response. *Clin Exp Allergy* 1999;**29**:1563-1571.
35. Akdis M. Healthy immune response to allergens: T regulatory cells and more. *Curr Opin Immunol* 2006;**18**:738-744.
36. Moverare R, Elfman L, Vesterinen E, Metso T, Haahtela T. Development of new IgE specificities to allergenic components in birch pollen extract during specific immunotherapy studied with immunoblotting and Pharmacia CAP System. *Allergy* 2002;**57**:423-430.
37. Visco V, Dolecek C, Denepoux S, Le Mao J, Guret C, Rousset F, et al. Human IgG monoclonal antibodies that modulate the binding of specific IgE to birch pollen Bet v 1. *J Immunol* 1996;**157**:956-962.
38. Lebecque S, Dolecek C, Laffer S, Visco V, Denepoux S, Pin JJ, et al. Immunologic characterization of monoclonal antibodies that modulate human IgE binding to the major birch pollen allergen Bet v 1. *J Allergy Clin Immunol* 1997;**99**:374-384.

39. Oldfield WL, Larche M, Kay AB. Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. *Lancet* 2002;**360**:47-53.
40. Jensen-Jarolim E, Wiedermann U, Ganglberger E, Zurcher A, Stadler BM, Boltz-Nitulescu G, et al. Allergen mimotopes in food enhance type I allergic reactions in mice. *FASEB J* 1999;**13**:1586-1592.
41. Scholl I, Wiedermann U, Forster-Waldl E, Ganglberger E, Baier K, Boltz-Nitulescu G, et al. Phage-displayed Bet mim 1, a mimotope of the major birch pollen allergen Bet v 1, induces B cell responses to the natural antigen using bystander T cell help. *Clin Exp Allergy* 2002;**32**:1583-1588.
42. Wallmann J, Proell M, Stepanoska T, Hantusch B, Pali-Scholl I, Thalhamer T, et al. A mimotope gene encoding the major IgE epitope of allergen Phl p 5 for epitope-specific immunization. *Immunol Lett* 2009;**122**:68-75.
43. Wallmann J, Epstein MM, Singh P, Brunner R, Szalai K, El-Housseiny L, et al. Mimotope vaccination for therapy of allergic asthma: anti-inflammatory effects in a mouse model. *Clin Exp Allergy* 2010;**40**:650-658.
44. Ganglberger E, Barbara S, Scholl I, Wiedermann U, Baumann S, Hafner C, et al. Monovalent fusion proteins of IgE mimotopes are safe for therapy of type I allergy. *FASEB J* 2001;**15**:2524-2526.
45. van Houten NE, Zwick MB, Menendez A, Scott JK. Filamentous phage as an immunogenic carrier to elicit focused antibody responses against a synthetic peptide. *Vaccine* 2006;**24**:4188-4200.
46. Delmastro P, Meola A, Monaci P, Cortese R, Galfre G. Immunogenicity of filamentous phage displaying peptide mimotopes after oral administration. *Vaccine* 1997;**15**:1276-1285.
47. Bastien N, Trudel M, Simard C. Protective immune responses induced by the immunization of mice with a recombinant bacteriophage displaying an epitope of the human respiratory syncytial virus. *Virology* 1997;**234**:118-122.
48. Frenkel D, Katz O, Solomon B. Immunization against Alzheimer's beta -amyloid plaques via EFRH phage administration. *Proc Natl Acad Sci U S A* 2000;**97**:11455-11459.
49. Galfre G, Monaci P, Nicosia A, Luzzago A, Felici F, Cortese R. Immunization with phage-displayed mimotopes. *Methods Enzymol* 1996;**267**:109-115.
50. Grabowska AM, Jennings R, Laing P, Darsley M, Jameson CL, Swift L, et al. Immunisation with phage displaying peptides representing single epitopes of the glycoprotein G can give rise to partial protective immunity to HSV-2. *Virology* 2000;**269**:47-53.
51. Zuercher AW, Miescher SM, Vogel M, Rudolf MP, Stadler MB, Stadler BM. Oral anti-IgE immunization with epitope-displaying phage. *Eur J Immunol* 2000;**30**:128-135.
52. Manoutcharian K, Gevorkian G, Cano A, Almagro JC. Phage displayed biomolecules as preventive and therapeutic agents. *Curr Pharm Biotechnol* 2001;**2**:217-223.
53. Samoylova TI, Cochran AM, Samoylov AM, Schemera B, Breiteneicher AH, Ditchkoff SS, et al. Phage display allows identification of zona pellucida-binding peptides with species-specific properties: novel approach for development of contraceptive vaccines for wildlife. *J Biotechnol* 2012;**162**:311-318.
54. Vaks L, Benhar I. In vivo characteristics of targeted drug-carrying filamentous bacteriophage nanomedicines. *J Nanobiotechnology* 2011;**9**:58.
55. Roehnisch T, Then C, Nagel W, Blumenthal C, Braciak T, Donzeau M, et al. Chemically linked phage idiotype vaccination in the murine B cell lymphoma 1 model. *J Transl Med*

2013;**11**:267.

56. Roehnisch T, Then C, Nagel W, Blumenthal C, Braciak T, Donzeau M, et al. Phage idiotype vaccination: first phase I/II clinical trial in patients with multiple myeloma. *J Transl Med* 2014;**12**:119.
57. Henry KA, Murira A, van Houten NE, Scott JK. Developing strategies to enhance and focus humoral immune responses using filamentous phage as a model antigen. *Bioeng Bugs* 2011;**2**:275-283.
58. Suphioglu C, Singh MB, Knox RB. Peptide mapping analysis of group I allergens of grass pollens. *Int Arch Allergy Immunol* 1993;**102**:144-151.
59. Suphioglu C, Schappi G, Kenrick J, Levy D, Davies JM, O'Hehir RE. A novel grass pollen allergen mimotope identified by phage display peptide library inhibits allergen-human IgE antibody interaction. *FEBS Lett* 2001;**502**:46-52.
60. Radauer C, Willeroider M, Fuchs H, Hoffmann-Sommergruber K, Thalhamer J, Ferreira F, et al. Cross-reactive and species-specific immunoglobulin E epitopes of plant profilins: an experimental and structure-based analysis. *Clin Exp Allergy* 2006;**36**:920-929.
61. Leitner A, Vogel M, Radauer C, Breiteneder H, Stadler BM, Scheiner O, et al. A mimotope defined by phage display inhibits IgE binding to the plant panallergen profilin. *Eur J Immunol* 1998;**28**:2921-2927.
62. Tordesillas L, Pacios LF, Palacin A, Cuesta-Herranz J, Madero M, Diaz-Perales A. Characterization of IgE epitopes of Cuc m 2, the major melon allergen, and their role in cross-reactivity with pollen profilins. *Clin Exp Allergy* 2010;**40**:174-181.
63. Mittag D, Batori V, Neudecker P, Wiche R, Friis EP, Ballmer-Weber BK, et al. A novel approach for investigation of IgE epitopes and cross-reactivity of Bet v 1 and homologous food allergens in individual patients. *Tissue Antigens* 2005;**66**:494-494.
64. Lin J, Sampson HA. The role of immunoglobulin E-binding epitopes in the characterization of food allergy. *Curr Opin Allergy Clin Immunol* 2009;**9**:357-363.
65. Burks AW, Shin D, Cockrell G, Stanley JS, Helm RM, Bannon GA. Mapping and mutational analysis of the IgE-binding epitopes on Ara h 1, a legume vicilin protein and a major allergen in peanut hypersensitivity. *Eur J Biochem* 1997;**245**:334-339.
66. Shreffler WG, Beyer K, Chu TH, Burks AW, Sampson HA. Microarray immunoassay: association of clinical history, in vitro IgE function, and heterogeneity of allergenic peanut epitopes. *J Allergy Clin Immunol* 2004;**113**:776-782.
67. Bogh KL, Kroghsbo S, Dahl L, Rigby NM, Barkholt V, Mills EN, et al. Digested Ara h 1 has sensitizing capacity in Brown Norway rats. *Clin Exp Allergy* 2009;**39**:1611-1621.
68. Vickery BP, Lin J, Kulis M, Fu Z, Steele PH, Jones SM, et al. Peanut oral immunotherapy modifies IgE and IgG4 responses to major peanut allergens. *J Allergy Clin Immunol* 2013;**131**:128-134 e121-123.
69. James LK, Bowen H, Calvert RA, Dodev TS, Shamji MH, Beavil AJ, et al. Allergen specificity of IgG(4)-expressing B cells in patients with grass pollen allergy undergoing immunotherapy. *J Allergy Clin Immunol* 2012;**130**:663-670 e663.
70. Vodnik M, Zager U, Strukelj B, Lunder M. Phage display: selecting straws instead of a needle from a haystack. *Molecules* 2011;**16**:790-817.
71. Huang J, Ru B, Li S, Lin H, Guo FB. SAROTUP: scanner and reporter of target-unrelated peptides. *J Biomed Biotechnol* 2010;**2010**:101932.



