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REVIEW

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Can alternative epitope mapping approaches increase the impact of B-cell epitopes in food allergy diagnostics?

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Summary

In vitro allergy diagnostics are currently based on the detection of specific IgE binding on intact allergens or a mixture thereof. This approach has drawbacks as it may yield false-negative and/or false-positive results. Thus, we reviewed the impact of known B-cell epitopes of food allergens to predict transience or persistence, tolerance or allergy and the severity of an allergic reaction and to examine new epitope mapping strategies meant to improve serum-based allergy diagnostics. Recent epitope mapping approaches have been worthwhile in epitope identification and may increase the specificity of allergy diagnostics by using epitopes predominately recognized by allergic patients in some cases. However, these approaches did not lead to discrimination between clinically relevant and irrelevant epitopes so far, since the polyclonal serum IgE-binding epitope spectrum seems to be too individual, independent of the disease status of the patients. New epitope mapping strategies are necessary to overcome these obstacles. The use of patient-derived monoclonal antibodies instead of patient sera for functional characterization of clinically relevant and irrelevant epitope combinations, distinguished by their ability to induce degranulation, might be a promising approach to gain more insight into the allergic reaction and to improve serum-based allergy diagnostics.

KEYWORDS
B-cell epitopes, epitope mapping, food allergy, monoclonal antibodies

1 | INTRODUCTION

Food allergy is currently diagnosed by careful history, food challenges, skin prick test (SPT), and measurement of specific IgE (sIgE). The double-blind placebo-controlled food challenge (DBPCFC) is the gold standard, but is a costly and burdensome procedure. Both SPT and sIgE measurement, using entire foods or single allergenic components, are hampered by false-positive test outcomes.[1] This might be related to the presence of both clinically relevant and irrelevant antibodies as it has been shown for serum-based diagnostics of anti-neutrophilic cytoplasmic antibody-associated vasculitis.[2] Serum-based measurement might be improved by defining the epitope specificity, affinity, critical amino acids, and antibody isotypes relevant for allergy.

Extensive research has been carried out, especially for peanut[3-10] and cow's milk,[11-16] to identify the IgE and IgG_4 epitopes of food allergens. Linear epitopes, composed of a continuous amino acid sequence, have been detected by screening patient sera on sequential overlapping peptide libraries or allergen fragments. Conformational epitopes, composed of sterically closed amino acids upon folding, were characterized by phage display technique or mass spectrometry partly in combination with B-cell epitope prediction web

tools or software, although the use of these techniques still has to be proven in future studies.[6,17-21]

So far, it is impossible to discriminate between clinically relevant and irrelevant epitopes or combinations using current approaches and to use these differences as diagnostic or prognostic markers. This review will discuss current knowledge, based on a relatively small number of studies investigating linear as well as conformational epitopes and comparing allergic and tolerant patients, and will propose alternative approaches for epitope mapping, focussing on epitope specificity and how this might impact serum-based allergy diagnostics.

2 | REQUIREMENTS FOR EFFECTOR CELL DEGRANULATION BY FcεRI CROSS-LINKING

The major requirement for degranulation is the cross-linking of at least two FcεRI receptors. Cross-linking will only be feasible if two FcεRI receptors are spaced apart by 50-240 Å. This range has been defined by using artificial allergens and hence might be somewhat smaller or larger for native allergens.[22–24] Consequently, the distance of two functional IgE epitopes within one combination is restricted to the required distance of two FcεRI receptors. As an example, possible IgE epitope combinations of Ara h 2.0201, based on a 3D model built with the SWISS-MODEL web portal, are shown in Figure 1.[25–29] Residue distances greater than 35 Å, calculated with Chimera,[30] were considered as functional epitope combinations, highlighted on the 3D structure using Schrödinger Release 2018-1 (Maestro, Schrödinger, LLC, New York, NY, 2018). Additionally, at least one epitope of the combination must be recognized with high affinity since the cross-linking has to take place for at least 100 seconds. Moreover, at least 1000 cross-links of FcεRI receptors on the surface of one effector cell have to take place.[31-33] If all these requirements for FcεRI cross-linking are met, the extent of degranulation is regulated by sIgE concentration, affinity, the ratio of allergen sIgE antibodies compared to total IgE, and the specificity and number of epitopes recognized.[34] These requirements suggest that certain epitope/antibody combinations are only present in allergic and not in tolerant patients. However, basophils from 10% to 20% of the general population do not respond at all due to low expression of syk and/or SHIP-1 resulting in the inhibition of intracellular signalling.[35-38] Thus, different expression levels might regulate the extent of degranulation.

3 | FOOD ALLERGENS AS ANTIGENS—INFLUENCE OF PROCESSING AND DIGESTION

Food allergens are modified by processing during industrial manufacturing or food preparation.[39] After processing, a fraction of these antigens will enter the buccal mucosa unaltered and the remainder will be reprocessed by human digestion before entering the tissue and the bloodstream.[40-42]

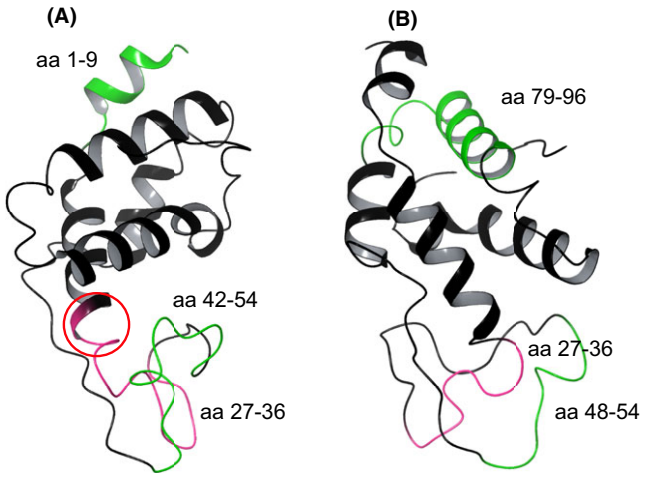

FIGURE 1 Determination of potentially relevant epitope combinations of the major peanut allergen Ara h 2. The 3D structure of Ara h 2 was obtained by SWISS-MODEL, and residues distances were determined by creating a residue distance map. Since the greatest distance was smaller than 50 Å, distances between 40 and 45 Å were considered as distances more likely to form relevant epitope combinations (highlighted in green). Distances between 35 and 40 Å were considered as possible epitope combinations but less likely (highlighted in pink). Predicted conformational epitopes by identified mimotopes are surrounded by red circles. A, One possible relevant epitope combination (40-45 Å) regarding the used model is between the described epitope aa 1-9 and the epitope aa 42-54 highlighted in green. Additionally, a combination with the same epitope aa 1-9 and the epitope aa 27-36 highlighted in pink is less likely (35-40 Å). B, Another possible relevant epitope combination (40-45 Å) regarding the used model is between the described epitope aa 79-96 and the epitope aa 48-54 highlighted in green. Additionally, a combination with the same epitope aa 79-96 and the epitope aa 27-36 highlighted in pink is less likely (35-40 Å).

They can be modified during industrial manufacturing, which can lead to aggregation or modification of polysaccharide structures or appropriate amino acid residues. Certainly, only a portion of the same allergen is affected. One example is the roasting of peanuts, which usually leads, by Maillard reaction, to the modification of amino acids, especially the hydroxylation of prolines. In the study of Bernard et al,[43,44] the modification of the proline residue located in the DPYSP^{OH}S motif of Ara h 2 (aa 49-54 + 61-66 for Ara h 2.0201 and aa 49-54 for Ara h 2.0101) caused an increase in allergenicity. The same increase in allergenicity has been observed for the peanut allergen Ara h 1.[43] Processing effects vary, so for example, allergenicity of the hen's egg allergen ovalbumin is decreased upon heat treatment, as shown by patients reacting to raw hen's egg, but not to cooked ones.[45] Modification by heating has also been observed for the cow's milk, with allergens α- and ß-lactalbumin tending to aggregate upon heat treatment although caseins stay unaffected. However, testing *in vitro*-processed cow's milk allergens showed inconsistent results between *in vitro* degranulation and the clinical history of the patients, often showing tolerance to heated milk.[46] Such observations of allergenicity changes require further research, to define allergen features that lead to increase or decrease.

Knowledge of these features might help to predict the potential allergenicity of proteins even though different conditions of one processing method will have a great impact.

After ingestion, a part of the allergen enters the buccal mucosa and subsequently the bloodstream without being digested; however, as demonstrated in studies with peanut, gastric processing seems to further enhance the uptake and degranulation.[40,42] The influence of digestion on food allergens has been estimated in several studies and is dependent on the allergen structure. Stable proteins, including Ara h 2 and ovalbumin, remain unaffected by low pH and proteolysis,[47] whereas Ara h 1 and 3, more labile proteins, are fragmented by pepsin. Upon entering the gut, peptides derived from digestion tend to aggregate due to the basic pH in the gut,[48-50] which may lead either to shielding of previously accessible epitopes or to the development of new, presumably conformational epitopes. In short, industrial manufacturing, in combination with intestinal processing as well as matrix effects (not discussed here), influences the allergenicity of food proteins potentially by changing epitope profiles even though aggregation can also affect the solubility of the allergen. Precipitated and non-soluble allergen can falsely pretend no IgE binding in *in vitro* studies.

4 | IDENTIFICATION OF LINEAR AND CONFORMATIONAL FOOD ALLERGEN B-CELL EPITOPES

Linear epitopes of several food allergens have been identified, mostly by overlapping peptide libraries, allergen fragments, or phage display peptide libraries.[9,12,15,51,52] These approaches were partly coupled with B-cell epitope prediction software or webtools like ABCPred, BepiPred 1.0, and DNASTAR Protean.[21,53] Moreover, in the studies of Zheng *et al* and Chen *et al*, the predicted B-cell epitopes were experimentally confirmed with high accordance.[17,54] However, no discrimination can be made between epitopes recognized by IgE, IgG, and IgA probably important in the allergy context. So far, prediction models cannot be used without experimental data to result in more accurate diagnosis or immunotherapy since current prediction models do not consider the special requirements of the allergic reaction described above. In comparison with linear epitopes, the detection of conformational epitopes requires more sophisticated techniques, like X-ray crystallography, mass spectrometry, or phage display libraries.[55] These phage display libraries can consist of peptides deriving from the allergen of interest or of random peptides. By using random libraries, peptides recognized by allergen-specific antibodies have been shown to consist of sequences mimicking a continuous or discontinuous epitope of the allergen; such peptides are called mimotopes and can be mapped on the 3D structure of the allergen by predictive webtools like EpiSearch.[10,56] A large drawback of these approaches is their failure to detect alterations in allergens caused by post-translational modifications or processing, and specific antibodies can only be detected against single epitopes and never in combination with others. Additionally, the assignment of mimotopes to surface patches of an allergen is solely based on *in silico* approaches and thus hampers the reliability of the outcome. Although mimotope mapping has been performed for a few allergens with conclusive functional results,[10,57,58] it still has to be in context with inhibition and mutation studies using the full-length protein. The general limitation is the requirement of a high-resolution structure for the allergen of interest, constraining a broad application of this approach. Admittedly, mass spectrometry can be used to investigate the influence of post-translational modification using native proteins, and X-ray crystallography to detect epitope combinations. Co-crystallization studies have been performed with murine monoclonal IgG antibodies being able to reduce binding of human polyclonal IgE.[59-62] Continuatively, co-crystallization has been carried out using monoclonal IgE antibodies generated by combinatorial heavy and light chain libraries of allergic patients. However, it has not been proven whether these antibodies also occur naturally.[63,64] Information from these studies can help in understanding the features being responsible for allergenicity and in defining critical amino acids more precisely. This knowledge can support the creation of more accurate serum-based diagnostics by modifying critical amino acids recognized by clinically non-relevant IgE antibodies. Moreover, it will give the opportunity to develop hypoallergenic variants for immunotherapy and better (IgE) epitope prediction tools.[65] However, the co-crystallization of polyclonal serum antibodies bound to the allergen of interest is an almost insuperable bottleneck, making X-ray crystallography a more theoretical approach for conformational epitope mapping. These obstacles might be overcome by human-derived monoclonal (IgE) antibodies.

5 | DISCRIMINATION BETWEEN PERSISTENCE AND TRANSIENCE BY MEANS OF IgE-BINDING EPITOPES

Most cow's milk allergic children outgrow their allergy by 3-4 years of age, although 15% remain allergic. In comparison, HEA arises later in childhood and 34% of these children will retain a persistent allergy.[66] Persistence has been studied through analysis of the epitope recognition pattern in patient sera. In CMA, persistent allergy was clearly associated with multiple IgE-binding epitopes on α_{S1}-, α_{S2}-, κ-casein, α-, and ß-lactalbumin as these were not recognized by IgE antibodies of children with transient allergy. However, the recognized epitopes do not coincide in different studies.[12,15,67-69] In HEA, four linear IgE-binding epitopes of ovomucoid have been associated with persistent allergy since they were not recognized by IgE antibodies of transient allergic children.[70,71] In short, these data favour the use of epitopes to predict persistence and to discriminate between transience and persistence more precisely.

6 | DISCRIMINATION BETWEEN ALLERGY AND TOLERANCE BY MEANS OF IgE-BINDING EPITOPES

For allergies less likely to be outgrown, identification of epitopes/antibodies discriminating between tolerance and allergy is essential to

avoid unnecessary food elimination therapies. In cases of peanut allergy, tolerant patients appeared to recognize the same IgE-binding epitope spectrum on Ara h 2 (linear epitopes) as allergic patients, although individual allergic patients recognized a higher number of different epitopes with higher IgE titres.[72] By means of the two key marker epitopes Ara h 2_10 (aa 28-42) and Ara h 2_18 (aa 52-66), allergic patients were correctly diagnosed with a sensitivity of 70% and a specificity of 60%. Sensitivity and specificity rose to, respectively, 90% and 95% by adding the IgE-binding epitopes Ara h 1_16 (aa 46-60) and Ara h3_140 (aa 418-432), which were recognized by few allergic patients.[72] In comparison, the sensitivity and specificity of intact Ara h 2 were defined as, respectively, 60%-100% and 60%-96%, showing no advantage using these two key marker epitopes.[73] Meanwhile, the conformational IgE-binding epitope pattern of Gly m 4 showed no correlation with clinical reactivity at all.[74] The impact of linear or conformational epitopes remains restricted in terms of ruling out tolerance or confirming allergy, as tolerant patients recognize the same epitope spectrum, although less frequently. In summary, to date, no clear discrimination between allergic and tolerant patients is feasible based on the detection of epitopes.

7 | SEVERITY PREDICTION OF AN ALLERGIC REACTION BY MEANS OF IgE-BINDING EPITOPES

Several studies have been performed to predict the severity of an allergic reaction by using explicit or multiple IgE-binding epitopes of one or multiple allergenic components. To assess whether the severity of allergic reactions towards peanut is related to the type and number of epitopes recognized, patients with different symptoms (ranging from mild to severe and a sIgE level >14 kU/L) have been studied.[9] Patients suffering from severe symptoms recognized fewer linear epitopes than patients with mild symptoms, suggesting that specific epitopes may be more relevant than the quantity of epitopes recognized. This may relate to the requirements for effector cell degranulation or suggest a greater relevance for conformational epitopes. However, conformational B-cell epitopes did not contribute to severe symptoms as assessed by mimotopes mapped to the surface of Ara h 2 and 6.[10] In CMA, the difference in IgE-binding epitope recognition pattern was analysed by including patients allergic to all forms of milk, patients tolerating heated milk, and patients with an outgrown allergy. Patients reactive to all forms of milk had a more diverse IgE- and IgG_4-epitope recognition pattern, comparable to a study of Sackesen *et al*[75] in which patients with persistent CMA reacted to processed milk and patient with transient tolerated processed milk. The IgE-binding pattern of patients non-reactive to heated milk was comparable to the recognition pattern of patients who had outgrown their allergy, although the IgG_4-binding pattern was comparable to patients being allergic to all forms of milk. Patients tolerating heated milk had the lowest $sIgG_4$ level and possessed low-affinity IgE antibodies as the tolerant group did. In contrast, when investigated using a competition assay, the allergic group exhibited low and high-affinity antibodies.[76] Low IgG_4 levels in heated milk tolerating patients suggest a direct class switch from μ to ε without a mature germinal centre and thus, less somatic hypermutations and affinity maturation. High-affinity antibodies might be generated by sequential class switching from μ to $\gamma 4$ and to a lesser extent by a subsequent class switch to ε.[77] In several studies, auxiliary $sIgG_4$ levels were similar between tolerant and allergic patients.[78,79] In conclusion, severity prediction can be made regarding tolerance of processed or non-processed food since transient patients do tolerate processed food and patients with persistent CMA do not; recognizing specific epitopes not recognized by transient patients. Thus, a more specified dietary advice might be given based on recognized epitopes.

However, the prediction of symptom severity is not yet feasible and due to a range of host-related factors will probably remain difficult.[80,81] Maximum release and the sensitivity of mast cells and basophils are dependent on intrinsic factors such as the regular exposure to an antigen that influences the production of a histamine-releasing factor interacting with surface-bound IgE. This interaction might lead to a higher extent of degranulation and/or spontaneously release without antigen exposure.[82] Additionally, different miRNAs have been implicated in up- or downregulation of genes involved in key signalling of mast cells (inhibition by miR-155 and miR-223; enhancement by miR-142-3p and miR-221).[83-86] Moreover, polymorphism of mediator degrading enzymes can influence host-related mediator tolerance thresholds by increasing or decreasing enzyme levels or metabolic capacity. Polymorphism of the histamine-degrading enzyme N-methyltransferase was associated with a higher risk of asthma, and patients showing SNP His645Asp on diamine oxidase (DAO) metabolizing histamines were prone to develop an allergic reaction at lower sIgE levels.[87-89] Further, the polymorphism of platelet-activating factor acetyl hydrolase Thr198 and Val397 leads to lower substrate affinity prolonging the activity of platelet-activating factor,[90] correlated positively with the severity of systemic reactions and anaphylaxis.[91,92] Finally, mast cell priming might be stimulated by medicines like beta-blockers and ACE inhibitors, increasing the severity of an allergic reaction.[93]

8 | IMPACT OF IgG-BINDING EPITOPES IN ALLERGY PREDICTION

Investigations of the impact of epitopes in allergy prognostic and diagnostic have been mostly based on IgE-binding epitopes, although antibodies of different isotypes, mainly IgG_4, seem to play a role in tolerance induction or retaining tolerance. In several studies, IgG_4 increases coincided with decreases in sIgE, after outgrowing allergy or successful allergen immunotherapy (AIT).[78,79,94] No statistically significant association with IgG_4 epitopes comparable to IgE epitope pattern was found regarding persistence and transience, or severity of symptoms for the investigated food allergens peanut and cow's milk.[6,12,72,76,95,96] Moreover, conflictive results were obtained regarding the overlap between IgE- and IgG4-binding patterns. Caubet *et al* have been shown that transience is associated with similar IgE- and

IgG4-binding pattern while Savilahti *et al* found similar IgE- and IgG4-binding pattern in patients with transient and persistent CMA.[69,97] In contrast, ratios of IgE to IgG_4 could, in some studies, distinguish between peanut tolerant and allergic patients in children and adults[98,99] supporting the hypothesis that specific IgG_4 can block IgE binding to definite epitopes. In a study with peanut-sensitized children divided by tolerance and allergy, mast cell (LAD2 cells) and basophil degranulation induced by sera from peanut allergic children were inhibited by pre-incubation of the allergen with sera from peanut tolerant children prior to stimulation. However, degranulation was only partially restored using IgG_4-depleted sera.[99] These results can have several explanations:

1. sterical hindrance of pre-bound IgE antibodies from peanut tolerant children
2. blocking of IgE binding from allergic children by pre-bound-specific IgG_4
3. the binding of antigen by specific IgG bound by the FcγRIIb (CD32b) receptor on the surface of basophils or mast cells inhibiting degranulation by co-aggregation of the FcεRI and FcγRIIb[100]
4. or a mixture thereof.

For further investigation, the stimulation allergen can be pre-incubated with different well-characterized (epitope specificity, affinity) monoclonal IgG and IgE antibodies from peanut tolerant patients or a mixture thereof instead of polyclonal sera.

9 | IMPACT OF IgE- AND IgG-BINDING EPITOPES IN FOOD ALLERGY IMMUNOTHERAPY MONITORING

In contrast to allergy prediction, the impact of IgG-, especially IgG_4-, binding epitopes in AIT monitoring has been investigated more intensively. In general, patients, irrespective of the AIT outcome, showed an increase in allergen-specific IgG_4 levels as well as a temporary increase in IL-10 secreting FoxP3-positive Treg cells controlling a class switch to IgG_4 and a decrease in sIgE levels.[101-104] In a peanut AIT trial, newly formed IgG_4 antibodies had specificities partly identical to identified IgE-binding epitopes. Simultaneously, the total sIgE decreased, although, as also shown in CMA AIT trials, new IgE specificities were developed.[94] Additionally, patients who discontinued AIT due to adverse reactions showed an increase in quantities and affinity of epitope sIgE paired with a greater diversity of recognized IgE- and IgG4-binding epitopes with little overlap.[105] Based on IgE-binding pattern prior starting AIT, a prediction model for safety (=number of adverse reaction while AIT) and efficacy (=time required to achieve desensitization) was developed. The model included two sets of 16 IgE-binding regions of caseins to be associated with safety and efficacy.[106]

In further milk and peanut AIT trials, the induction of a hyporesponsive state of basophils was observed. This state was additionally applicable for hen's egg-sensitized patients in a population of peanut allergic patients.[107,108] A possible explanation is the IgG epitope-specific co-aggregation of the inhibitory FcγRIIb (CD23b) and FcεRI receptor potentially inhibits the PI3k pathway by SHIP-1 activation and subsequently loss of syk expression.[109] This might also explain the risk reduction in high-risk infants to develop a peanut allergy by early and regular introduction of peanut consumption as shown in the LEAP and LEAP-On study. Infants in the consumption group, compared to the avoidance group, showed higher specific IgG_4 levels and IgG_4/IgE ratios.[110,111] IgG_4-binding epitopes do not seem to have a high impact in allergy prediction but do seem to be useful in food AIT monitoring. However, further investigation on IgG- and IgE-binding epitope pattern in patients with a positive and negative outcome must be performed.

10 | LIMITATIONS OF THESE APPROACHES

For most IgE-binding epitopes, a limited number of studies were performed to pinpoint epitopes to be used as diagnostic markers. These attempts were only partly successful as allergy and tolerance still cannot be predicted by using IgE B-cell epitopes. A fundamental limitation is related to the use of polyclonal sera containing a mixture of antibodies with multiple isotypes recognizing diagnostically relevant and irrelevant epitopes. This heterogeneous antibody repertoire in serum was confirmed by mutagenesis studies showing multiple amino acids to be critical for one epitope in CMA.[112,113] Another essential limitation is the restricted focus on IgE-binding epitopes since the role of IgG- or IgA-binding epitopes is almost unknown. Thus, for improving serum-based food allergy diagnostics, human monoclonal antibodies instead of polyclonal patient sera might help to define clinically relevant epitopes and to investigate the role of antibodies with different isotypes, as explained below.

11 | DIFFERENT EPITOPE MAPPING APPROACHES MAY INCREASE THE CONTRIBUTION OF ALLERGEN-SPECIFIC B-CELL EPITOPES

To overcome the limitations described in the previous paragraphs, requirements for degranulation should be considered. Since cross-linking of at least two FcεRI receptors is crucial, an allergic subject must feature IgE antibodies recognizing two epitopes with a distinct distance on the cell surface, of which at least one with high affinity.[22] In contrast, two IgE antibodies, with low affinity recognizing a relevant epitope combination, or one IgE-antibody recognizing one epitope of a relevant epitope combination may occur in a tolerant patient without causing degranulation. Current IgE serum-based diagnostics cannot discriminate between high- and low-affinity antibodies, impeding the discrimination between relevant and irrelevant epitopes or combinations. Instead of polyclonal sera, a more distinct

characterization of specific epitopes might be feasible by using human monoclonal antibodies. This approach may also give precise information about epitope specificity and affinity (illustrated in Figure 2). Furthermore, co-crystallization of an epitope-specific monoclonal antibody or a ScFv with an allergen of interest is significantly more likely to be successful compared to the use of polyclonal sera, which offers a chance to detect authentic conformational epitopes.

Since IgE-producing B-cells in blood are scarce, the first studies with IgE monoclonal antibodies were performed with murine allergen-specific antibodies, originally IgG but subsequently heterologously expressed as IgE antibodies.[34] In addition, human monoclonal IgE antibodies were obtained by construction of phage display ScFv hybrid libraries with allergic donor-derived epsilon heavy chain and synthetic variable regions for the light chain.[114,115] However, these approaches did not address the whole allergen-specific antibody repertoire of one subject. In studies by Hoh *et al* and Patil *et al*,[116,117] a part of the antibody repertoire was analysed by single cell sequencing of the specific B-cell receptor. The lack of sequenced receptors derived from IgE B-cells can be explained by the small number of PBMCs used in these studies (1-10*10^6 cells/experiment), whereas the frequency of Ara h 1- or Ara h 2-specific B-cells was determined to be around 0.01% of the B-cell fraction in allergic patients.[117] Due to this low frequency, we recommend the use of a large volume of blood derived from allergic or tolerant donors to increase the number of circulating allergen-specific B-cells. These B-cells can be single cell sorted and expanded *in vitro*, or the mRNA can be isolated directly.[118] Moreover, specific B-cell clones can be obtained by immortalization and limited dilution, but this approach may cause *in vitro* class switching or lead to selection due to the expansion of the fittest clones. Additionally, we propose, in contrast to Hecker *et al*,[115] the isolation of all allergen-specific B-cells irrespective of the isotype produced as the role

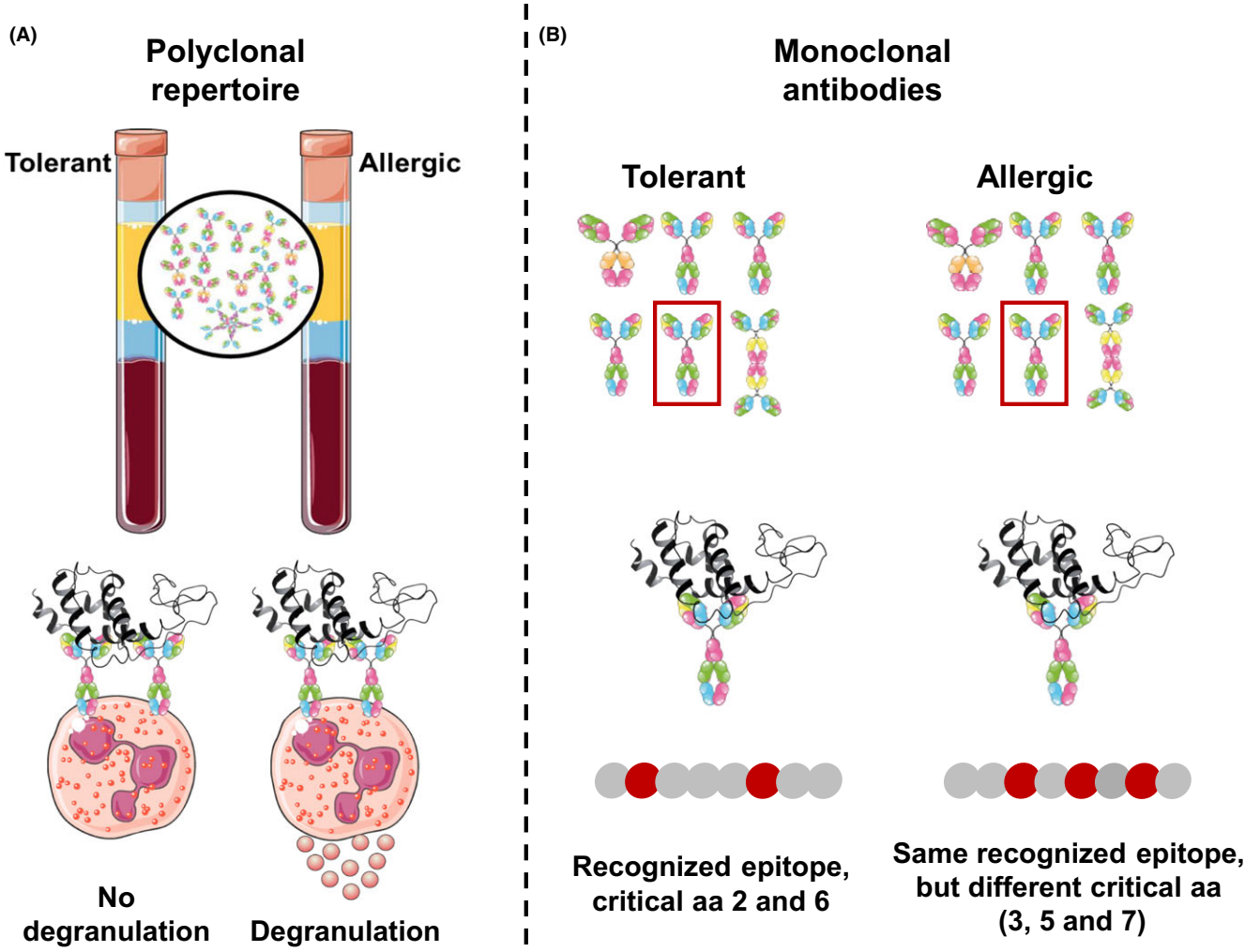

FIGURE 2 Defining differences in epitope-binding pattern of tolerant but sensitized and allergic patients are hampered by similar appearing polyclonal antibody repertoires (parts of the graphic from Servier Medical Art by Servier- http://smart.servier.com/). A, The polyclonal repertoires in sera of tolerant but sensitized and allergic patients seem to be similar but only antibodies out of the repertoire from allergic patients are able to induce an allergic reaction. B, The use of monoclonal antibodies enables a more extensive characterization of individual antibodies responsible for the allergic reaction. Tolerant as well as allergic patients can possess antibodies with similar epitope specificities but with different affinity.

of different allergen-specific isotypes remains unclear. Finally, we recommend the inclusion of tolerant and allergic patients sensitized to the same allergen for the generation of specific monoclonal antibodies. Monoclonal antibody repertoires within these two groups may allow the discovery of differences in epitope specificity and affinity. To ensure the capture of B-cells producing antibodies with comparable affinity and epitope specificity as the polyclonal serum repertoire, the plasma of the patients should be saved and analysed for their epitope recognition spectrum and affinity. In addition, competition assays with monoclonal antibodies and serum antibodies can be performed to detect differences in affinity.

Potentially, these findings can contribute not only to the improvement of food allergy diagnostics but also monitoring of allergy immunotherapy. This epitope mapping strategy will be restricted by the absence of short and long-living plasma cells in the blood as well as the still discussed presence of IgE memory B-cells.[77,119-122] If re-stimulated IgG memory B-cells are the source of class-switched IgE-producing cells, instead of IgE memory B-cells, the sequence of IgG B-cell receptors can be heterologously expressed as IgE antibodies and functionally characterized as IgG and IgE antibodies. Based on this hypothesis by Aalberse and Platts-Mills, IgE B-cells class-switched from IgG memory cells are not able to survive in the germinal centre where affinity maturation takes place and the variable region of the IgE B-cell receptor do not undergo further somatic hypermutations.[77] Another aspect to take into account is the expression of the low-affinity FcεRII (CD23) on non-allergen specific translational B-cells in the blood stream.[123,124] The cells can bind allergen-specific IgE antibodies leading to the selection of B-cells irrelevant for allergy. Non-specific antibodies can be excluded through a specificity check, such as performing an ELISA coated with the allergen of interest.

Another approach to enlarging the impact of B-cell epitopes in food allergy diagnostics might be the development of improved computational epitope prediction. To exceed the abilities of already available prediction software or webtools for B-cell epitopes, computational approaches for predicting allergenic functionality must meet, as described above, the special requirements for degranulation, for example, the defined distance between two epitopes. Before using these distances as a basis for prediction, they should be estimated by natural and not, as now, by artificial allergens. Furthermore, new algorithms must consider the potential oligomerization of allergenic proteins, since this can increase the number of possible epitope combinations for triggering effector cell degranulation.

12 | CONCLUSION

The use of monoclonal antibodies might be a powerful tool to define the allergen-specific antibody repertoire of tolerant and allergic patients more precisely in terms of epitope specificity, affinity, and feasible epitope combinations. This will produce more knowledge about the reasons some sensitized patients can tolerate an allergen with no symptoms while others will experience a (severe) allergic reaction. Differences in antibody repertoires can lead to improved sIgE measurement by modifying critical amino acids recognized by clinically irrelevant antibodies and thus preventing false-positive test results and avoiding burdensome food challenges. In addition, the characterization of allergen-specific antibody repertoires during immunotherapy may lead to a better understanding of the underlying mechanism.

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CONFLICT OF INTEREST

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