

Significance of IgE-binding epitopes in allergic disease

Key words: IgE, IgE-binding epitopes, cow's milk allergy, allergen processing, allergen structure, pollen allergens

It is a well-established concept that the allergic response and subsequent inflammation are induced by an allergen that cross-links at least 2 IgE antibodies bound to the high-affinity IgE receptor on presensitized effector cells such as mast cells and basophils. The interaction between an allergen and an IgE antibody is highly specific whereby the antibody with its variable site recognizes a region on the allergen called the *IgE-binding epitope*. In the century of recombinant technology and protein sequence acquisition, it was debated whether IgE epitopes and their sequence pattern would be the key feature of allergens. Because it is still very difficult to isolate intact human monoclonal allergen-specific IgE antibodies, epitope analysis has been restricted to the use of polyclonal sera of patients with allergy.¹ Therefore the main strategy to identify the epitopes is the fragmentation of allergens either enzymatically,^{2,3} synthetically,^{4,5} or by different recombinant technologies.⁶⁻¹⁰ The binding pattern of the IgE antibodies from patient sera to the allergen fragments allowed the localization of binding regions. Only a small number of defined sequential IgE epitopes on a single molecule could be identified, for example, on grass pollen, allergen Lol p5.¹¹ The greater part of epitopes turned out to be conformational.^{12,13} Birch pollen allergen Bet v1 lost its IgE-binding capacity when divided in 2 fragments, a C-terminal and an N-terminal part.¹⁴ Der p2, a major mite allergen, had no allergenic activity after deletion of a structurally stabilizing disulfide bond.¹⁵ Furthermore, the disruption of a sequential epitope on grass pollen allergen Phl p5b, by site-directed mutagenesis, did not lead to a substantial loss of IgE-binding capacity toward the holoallergen.¹² Instead, only a deletion of a region responsible for the stability of the 3-dimensional structure of the allergen

Abbreviation used

CMA: Cow's milk allergy

induced a significant reduction of both IgE-binding capacity and allergenic activity. The same phenomenon was seen with the mite allergen Der f2, which showed no more allergenic activity when the compact structure was destroyed by genetic engineering.¹⁶ Thus the structural integrity of an allergen seemed to be an important prerequisite for binding of polyclonal IgE antibodies and the induction of an allergic response.

When some of the identified IgE-binding regions were projected onto the 3-dimensional structures of allergens, it not only turned out that these regions were found on the surface of the molecule, which could be expected,¹⁷ but also revealed that the complete surface was nearly covered by possible IgE-binding epitopes.¹⁸ With regard to how many IgE antibodies might be able to bind an allergen at the same time, data from a cocrystallization experiment of birch pollen allergen Bet v1 with a mouse monoclonal antibody showed that in spatial terms a maximum of 2 to 3 antibodies would be able to bind to the complete protein.¹⁹ In conclusion, although the surface of an allergen is covered by many epitopes, only a small proportion of IgE antibodies would be able to bind to the molecule at the same time. It is also interesting to note that the spatial distribution or density of epitopes on an antigen can influence the affinity of binding by different antibody subclasses.²⁰ It is an open question whether IgE antibodies need a certain epitope density for their binding. Experiments showed that stable fragments of a certain length of the grass pollen allergen Phl p5b had increased allergenic activity in contrast to the parental molecule, suggesting that an optimal spatial distribution of epitopes on the allergen fragments seemed to be functionally important.²¹ Furthermore, one must realize that the structure of IgE, while binding to its constant region of the high-affinity receptor FcεRI on mast cells or basophils, seems to be rather flexible.²² It is suggested that the Fc part of the IgE antibody binds to the receptor in a banana-like shape, whereas both IgE Fc and FcεRIα chain bind in a 1:1 stoichiometry.²³ Consequently, the Fab fragment, with its antigen-recognition site, can float around enough to adjust its position and bind the antigen. On the other hand, there could be a given distance from one variable site to another that has to be bridged by the cross-linking allergen not only because the IgE molecule is bigger than the normal allergen but also because it

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is fixed to the receptor, which itself will be distributed in a defined manner over the cell surface. The postulated distance between 2 IgE antibodies binding an allergen has not been calculated so far. Taken together, these facts indicate that the best possible allergen will be a biochemically stable protein with a compact structure and IgE-binding epitope distribution on the molecule surface with an optimal density.

It is an open question whether these observations are also true for food allergens. In this issue of *The Journal*, Chatchatee et al (p 379) determined the IgE- and IgG-binding epitopes on α_{S1} -casein, a major allergen from cow's milk. By synthesizing 96 synthetic decapeptides overlapping the entire molecule, they identified 9 IgE- and 6 IgG-binding regions. Two of the IgE-binding regions were recognized only in patients whose cow's milk allergy (CMA) persisted until the age of 9 years, whereas in children who were likely to outgrow their CMA, no IgE antibodies binding to these 2 regions could be identified. First, these IgE-binding regions may become helpful tools for the course of CMA in children, although first the predictive value of these peptides has to be determined in a prospective study. This would be very important because it has been shown that long-lasting food sensitization during the first 2 years of life is related to later allergic airway disease,²⁴ and early differentiation of patients may influence preventive and therapeutic measures. Second, the authors confirm their own data demonstrating that the presence of IgE antibodies directed against linear as opposed to conformational epitopes is a predictor of persistent allergic disease. Obviously, protracted food hypersensitivity is associated with the production of relevant amounts of IgE antibodies binding to linear epitopes. The problem is that the process underlying this phenomenon is not clarified. It is interesting to speculate whether the production of IgE antibodies directed against certain IgE-binding epitopes is regulated by the different mechanisms of processing and resorption of the allergens by the mucosal layer. Although inhalants such as grass pollen allergens are relatively stable on the nasal and bronchial mucosa, they are readily degraded in the gastrointestinal tract.²¹ In contrast, food allergens from peanuts remain intact when treated with stomach juice.²⁵ Thus patients with persistent CMA recognizing predominantly linear epitopes may encounter the allergen through a different mucosal barrier than children who outgrow their CMA, or else the processing of the allergen is different between the 2 groups. How this would be related to a different course of disease is undecided. Furthermore, Chatchatee et al found that not all IgE-binding regions were recognized by IgG antibodies. They argue that this may be explained by different switch regulation of antibody production for IgE and IgG. In this case, the switch from IgM to IgE might have come directly without an intermediary step for production of IgG heavy chain for this specific clone. But it could also be a sign of different routes of allergen presentation to the immune system, as was shown for the induction of novel antibodies during immunotherapy with inhalant allergen extracts (grass pollen).²⁶

The identification of IgE-binding epitopes until now has not answered the question of what makes an antigen an allergen, but in the future it may help to differentiate between patient groups and disease courses.

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