

ORIGINAL ARTICLE

Allergen structures and epitopes

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Abstract

Human type 1 hypersensitivity diseases such as allergic rhinoconjunctivitis are characterized by allergen-specific IgE antibodies produced in allergic individuals after allergen exposure. IgE antibodies bound to receptors on the surface of effector cells trigger an allergic response by interacting with three-dimensional (conformational) epitopes on the allergen surface. Crystal structures are available for complexes of antibody specifically bound to five allergens, from birch pollen, bee venom, cockroach, cow's milk and timothy grass pollen. The details of the antibody–allergen interaction extending all the way to atomic resolution are available from such complexes. *In vitro* investigations using recombinant monoclonal antibodies and human basophils show that binding affinity is a key to triggering the allergic response. Continued molecular characterization of antibody–allergen interactions is paving the way for the use of recombinant allergens in allergen-specific diagnosis and immunotherapy.

B-cell and T-cell responses have a defining difference in their recognition of antigenic epitopes. An inhaled allergen that crosses the mucosal membrane is taken up by antigen-presenting cells and degraded into peptides. Some of these are presented in the context of major histocompatibility class II molecules on the antigen-presenting cell surface and recognized by T cells. In the case of the T-cell receptor, only the linear amino acid sequence is important for recognition. In contrast, B-cell epitopes are areas on the surface of protein antigens that are recognized by antibodies. The epitopes are normally conformational – that is, they are composed of amino acids that are not in a linear sequence but are brought together by the folding of the amino acid chain. Most IgE epitopes are conformational (1). Some antibodies can bind a linear epitope, but usually with lower affinity, so B cells require a folded allergen for efficient binding of inhaled allergens.

The outcome of IgE-allergen interactions in a sensitized patient occurs when IgE is bound by the high-affinity IgE receptor (FcεRI), for example on mast cells. Allergen binds to the specific IgE on the surface, and cross-linking of high-affinity IgE receptors occurs. This leads to mast cell degranulation, release of mediators like histamine and a cascade of events leading directly to allergic symptoms.

Allergen–antibody interaction

The structural basis of interactions between allergens and the immune system is the formation of the complex

between IgE, allergen and the high-affinity IgE receptor. For this reason, determining the structures of allergens has been an active area of research. To date, more than 30 examples of the structure of known inhaled allergens have been published. However, even if we know the allergen structure, we cannot predict where the antibody-binding epitopes are. To characterize a detailed interaction between an antibody and an allergen, the structure of the complex of the two is needed. In the scientific literature today, only five examples of antibody–allergen interactions are available (2–6): birch pollen Bet v 1 with IgG₁; bee venom Api m 2 with IgG₁; cockroach Bla g 2 with IgG₁; bovine milk Bos d 5 with IgE; and grass pollen Phl p 2 with IgE. The first three are mouse antibodies, and the latter two are human derived antibodies. Notably, all three IgG₁ antibodies inhibited IgE binding to the allergen, indicating at least a partial overlap of the IgG epitopes with IgE epitopes. To obtain structures by X-ray crystallography, all of these studies used Fab fragments (fragment antigen-binding), rather than the full antibody, because Fab fragments are easier to crystallize.

For all five examples of antibody–allergen interactions, the epitopes are conformational. Based on these five examples, some differences are hypothesized between IgE and IgG epitopes. So far, IgG epitopes appear to be convex, and IgE epitopes are planar. An affinity difference might exist between these two different types of binding, which has implications for the allergic reaction. This proposed difference in IgG and

IgE epitopes should be investigated since, at least in tissues, plasma cells go through an IgG-producing stage before they begin producing IgE, so the epitope recognized by IgG and IgE antibodies from the same cell is expected to be the same. One hypothesis to explain this paradox is that affinities of epitope-binding mature, with selection over time for epitopes bound with increased affinity. In any case, we can conclude from the published allergen–antibody structures that generally, shape complementarity is observed between the epitope and the paratope, and this is a major factor in adding energy to the interaction.

An example of a detailed allergen–Fab fragment-specific interaction is shown in Fig. 1, which shows the birch pollen allergen Bet v 1 (2). The paratope on the antibody and the epitope on the allergen have several areas that are in direct contact, and detailed structural analysis of the contacts is possible. The Bet v 1 IgG epitope illustrates an important point: the homologous protein Mal d 1 from apple has an identical fold and for some isoforms possibly identical amino acids in positions important for epitope formation. If so, this could lead to the commonly observed phenomenon of antibody cross-reactivity between these two molecules.

One Bet v 1 epitope amino acid (E45) is buried deep in the paratope of the antibody and is involved in many interactions (Fig. 1). Structural analysis shows close interaction between the antibody and Bet v 1, with the most important interactions through hydrogen bonds. Shape complementarity and many van der Waals interactions are also important. Mutating the Bet v 1 allergen to change E45 to another amino acid residue abolishes binding of the antibody (7). Also, binding of a pool of allergic patient serum showed that binding was decreased by 50% by the mutation. This indicates that a few strong interactions are responsible for antibody–allergen binding.

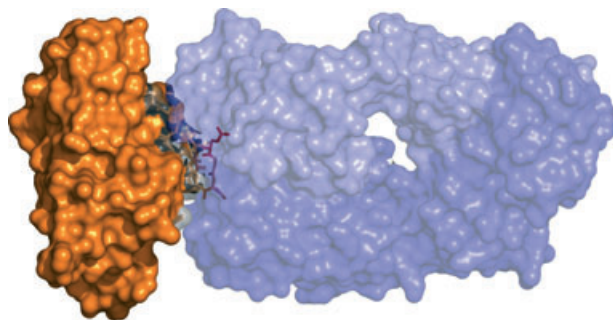


Figure 1 Molecular surface of the complex between Bet v 1 (orange) and the Fab fragment (heavy chain in light blue and light chain in dark blue, transparent) from a specific monoclonal IgG antibody as determined by X-ray crystallography (2). The amino acids from Bet v 1 that are in contact with the antibody are shown in detail. The figure is produced by the author using PyMol. All rights are owned by ALK-Abelló.

Affinity of IgE–allergen interactions influences allergic outcomes

Studying antibody–allergen binding reveals that IgE affinity and clonality are key factors that influence the outcome of IgE–allergen interactions, as shown by Christensen *et al.* (8). This study, like many others in this field, used recombinant antibodies instead of human serum, because the natural IgE response is polyclonal. Christensen *et al.* produced a range of different recombinant IgE antibodies, all directed against Der p 2. Binding affinity was determined as a dissociation constant (K_d) for a range of IgEs with different affinities. Overlap of the epitopes for the different IgE molecules was tested by determining whether more than one IgE could bind to the antigen simultaneously. In this way, a panel of antibodies with different affinities and epitopes was generated.

The clonality effect on basophil degranulation was measured. When three high-affinity IgEs with nonoverlapping epitopes were tested in pairwise combinations, the resulting basophile sensitivities were within a factor of four. However, if basophiles were sensitized using all three antibodies, the sensitivity increased by an order of magnitude. Therefore, clonality of the IgE response is important for the allergic reaction to allergen exposure.

In the next experiment, antibodies of varying affinity, measured as K_d , were used in pairwise combinations in the basophil degranulation tests. An assay with two low-affinity antibodies was found to have a sensitivity three orders of magnitude lower than if two high-affinity antibodies were used. However, even if only one antibody has high affinity, the response was only two- to fivefolds lower than if two high-affinity antibodies were used. Thus, *in vivo*, in the patient, one high-affinity antibody may be sufficient to capture the allergen. Then, even a low-affinity antibody that otherwise would not have much clinical significance could complete the allergen cross-binding and drive the allergic reaction.

In summary, the structures of antibody–allergen complexes reveal the basis for the commonly observed phenomenon of allergen cross-reactivity. This has implications for immunotherapy, if one allergen can give protection against homologous allergens. In addition, work showing the importance of epitope conformation highlights the importance of structural integrity of allergens for the immune response. This must be considered in diagnostic tests, such as allergy skin prick tests. Tests that do not use natively folded allergens risk a high rate of false negatives, because of the inability of IgE to bind. Finally, structural analysis combined with *in vitro* basophil degranulation assays shows how IgE affinity and clonality influence the effector cell response. This work paves the way for constructing recombinant allergens for use in allergen-specific diagnosis and immunotherapy.

Conflict of interest

Kåre Meno is an employee and share holder of ALK-Abelló.

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