

## COMMENTARY

# Correlating IgE reactivity with three-dimensional structure

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This Commentary discusses the work of Neudecker et al. in this issue of the *Biochemical Journal* in which site-directed mutagenesis and NMR spectroscopy have been used to analyse in detail the IgE-binding capacity of two cross-reactive allergens: Apg1.0101 from celery (*Apium graveolens*) and Pru av 1 from cherry (*Prunus avium*), which are both members of the pathogenesis-related allergen family. The study, showing that the IgE-binding epitopes are highly patient specific, will have a

profound impact on our understanding of conformational IgE-binding epitopes, raising serious questions about the therapeutic usefulness of conventional site-directed-mutagenic approaches for the production of hypo-allergenic protein variants.

**Key words:** allergy, cross-reactivity, IgE, recombinant allergen, three-dimensional structure.

## INTRODUCTION

Allergic reactions represent, without doubt, an increasing healthcare problem in industrialized countries, where an increasing incidence of IgE-mediated allergies, such as rhinitis, conjunctivitis, bronchial asthma and atopic eczema, causes a healthcare burden to society in the order of billions of U.S. dollars [1]. A common hallmark of these diseases is the production of allergen-specific IgE raised against normally harmless environmental antigens. In sensitized atopic individuals, allergen re-exposure induces cross-linking of high-affinity Fc $\epsilon$ RI receptor-bound IgE on effector cells and thus immediate release of anaphylactogenic mediators [2]. Although the molecular mechanisms leading to allergic reactions are quite well understood [3], our knowledge about the repertoire of molecular structures involved in the pathogenesis of allergic reactions is still fragmentary. This is mainly due to the ability of the immune system of atopic individuals to mount IgE immune responses to a wide variety of molecular structures. Among these, cross-reactive structures present in different apparently unrelated allergenic sources are mainly responsible for the well-known phenomenon of polysensitization, which is frequently encountered in clinical practice.

## ANTIGENS, ANTIBODY RESPONSES AND CROSS-REACTIVITY

Although antigens can be defined easily by the amino acid sequence providing the primary structure of a given protein, which can in many cases be post-translationally modified, we have much more difficulty in defining allergenicity. Few, if any, structural features are currently known to be common for allergens in general, even though most allergens can be grouped into smaller numbers of cross-reactive structural classes. It is, however, likely that features other than structural ones are more relevant for the allergenicity of a protein, such as solubility, stability, size and pathway of uptake and presentation. Every protein can be allergenic if presentation of the antigen through the MHC class II pathway induces a switch in B-cells, leading to the production of antigen-specific IgE antibodies concomitantly with the establishment of memory B-cells able to mount a fast IgE response after re-exposure to the antigen. Therefore investigation of the

switch mechanisms might be more important in reaching an understanding of the establishment of allergic reactions than investigation of the structure of allergens. In contrast with allergenicity, cross-reactivity is largely determined by structural aspects [4]. Antigens are cross-reactive only if they share structural features able to generate epitopes recognized by the CDRs (complementary determining regions) of a monoclonal antibody or of by a set of antibodies generated during a polyclonal response.

## ABOUT B-CELL EPITOPES

Unfortunately the literature is widespread with misconceptions regarding the term 'epitope', which are derived from different experimental set-ups following disparate aims. Immunization with linear peptides can be very useful for producing antibodies to identify denatured or unfolded molecules. It must be emphasized that these antibodies do not generally recognize the same epitopes recognized by antibodies elicited during natural exposure to correctly folded antigens, but are restricted to short linear sequences within the unfolded protein. These anti-peptide antibodies obtained by forced immunization do not have any biological significance, and results from such epitope-mapping studies, including those obtained by screening of phage-surface-displayed short-peptide molecular libraries, can be extremely misleading. In addition to anti-peptide antibodies, an array of different functional methods to measure some aspects of antigen-antibody interaction have been used in the attempt to define B-cell epitopes, including adsorption of serum with antigen fragments, competition tests with monoclonal antibodies, use of modified antigens obtained by site-directed mutagenesis and short overlapping peptides covering the whole sequence of a given antigen. Some of these methods have been partially successful in the identification of crucial residues or general regions recognized by an antibody, but none have yet allowed the complete definition of a B-cell epitope. The only method that can determine the complete structure of a B-cell epitope is preparation of a complex of a monoclonal antibody Fab fragment with its antigen, crystallization of this complex and determination of its structure, resolving those determinants recognized by the CDRs of the antibody on the native protein. Unfortunately, the only structural

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information about allergen Fab crystallization complexes derives from the co-crystallization of Bet v 1, the major birch (*Betula verrucosa*) pollen allergen, with a mouse monoclonal IgG 1 Fab fragment in a model system [5]. However, even this model epitope covering a buried surface of 931 Å<sup>2</sup> must be classified as discontinuous.

### B-CELL EPITOPES AND CROSS-REACTIVITY

To predict potential cross-reactivity between proteins, it is informative to compare protein folds. In the absence of similarity in folding, protein cross-reactivity is virtually excluded. All IgE cross-reactive allergens described so far have been found to reflect shared features at the level of both primary and tertiary structure. Whereas all cross-reactive proteins have a similar fold, the reverse is not true: proteins with a similar fold are not necessarily cross-reactive. This is mainly due to the fact that protein folding is liberal with respect to amino acid substitutions for many positions in the sequence. Such substitutions may markedly affect the protein outer surface or directly involve contact residues important for the antigen-antibody interaction, thus reducing or abolishing antibody reactivity. The work presented in this issue of the *Biochemical Journal* by Neudecker and co-workers [6] clearly shows that the introduction of a proline residue at position 112 of Pru av 1, the major allergen of cherry, results in the disruption of the native tertiary structure of the molecule and thus an almost complete loss of IgE reactivity. Since the whole surface of a molecule is antigenic during a polyclonal response resulting from natural exposure [7], the single point mutation Ser<sup>112</sup> → Pro disrupting the native tertiary structure of Pru av 1 has a dramatic effect on the allergenicity of the whole protein. This observation is of crucial importance for the future development of safe vaccines for allergen-specific immunotherapy.

### IgE BINDING, ALLERGY VACCINES AND NEW FORMS OF IMMUNOTHERAPY

Beside the extreme complexity of many allergenic sources, such as mites, foods and fungi, and the difficulties related to the preparation of standardized extracts for a safe immunotherapy, the only treatment able to cure allergy, the major problem encountered during desensitization is the high risk of anaphylactic reactions. Obviously, the availability of single hypo-allergenic variants for the most important allergens present in each allergenic source would be a great help for rapid progress in the field. However, site-

directed mutagenesis aimed at eliminating IgE-binding epitopes, although conceptually sound, is unlikely to help us in this regard. We are dealing with hundreds of different allergen structures and, more importantly, with an unknown, but certainly much larger, number of highly patient-specific IgE-binding epitopes. Therefore from a practical point of view, although potentially useful in single specific cases, site-directed mutagenesis will not be the method of choice for the development of allergy vaccines. What we need are more generalized cost-efficient approaches to generate molecules useful for the treatment of large number of allergic individuals without risk of anaphylaxis. We will only reach this goal if we are able to produce vaccines able to elicit strong protective immune responses independent of the patient-specific IgE-binding characteristics. As demonstrated by Neudecker et al. [6], in the single case of an allergen from the pathogenesis-related proteins, hypo-allergenic variants can be more easily and cost-efficiently produced by irreversibly preventing the folding process of an allergen, than by time-consuming mutation of residues that are assumed to be involved in the formation of IgE-binding epitopes. Such unfolded proteins, easily produced by single mutations introduced based on structural considerations, will retain almost, if not all, T-cell epitopes needed to provide B-cells with the necessary help to mount protective responses against allergens, and there is no reason to assume that this simple and efficient approach should not work in clinical practice.

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