

EDITORIAL

Manipulating allergen genes

The impact of molecular biology on allergen immunochemistry is best illustrated by the fact that within the past 2 years an impressive array of allergens has been cloned and sequenced. The list, now up to 15 in all, includes allergens derived from mites; grass, weed and tree pollens; the domestic cat; and vespid venom (not all have yet been published) [1–9]. As a result of these studies, the primary structure of the respective allergen molecules is known, and in several cases the degree of structural homology between families of antigenically related allergens has been established, e.g. the Group I mite allergens and the *Amb a* I homologues of ragweed pollen. Similarity searches of DNA or amino acid sequence databanks have given insights into the biological function of several allergens, e.g. mite Group I (thiol proteases) and birch pollen allergen I (plant pathogenesis resistance protein). Indeed, where no sequence similarities have been found (e.g. *Der p* II, *Fel d* I, *Amb a* I), the allergen function remains tantalizingly unknown (raising the interesting prospect that these allergens could have unique functions and/or be members of new protein families).

In terms of allergic disease, the applications of molecular biology to studies of the immune response to allergens is critically important. Now that much of the sequencing work has been done, several groups of investigators are focusing on the identification of the specific amino acid residues involved in allergen recognition by B or T lymphocytes. Mites (*Dermatophagoides* sp.) have been the focus for much of this work, and for good reason. On a world-wide basis, they are among the most ubiquitous allergens and there is now strong epidemiological evidence that sensitization and exposure to mite allergens is a highly significant risk factor for the development of asthma [10]. The major allergens of *D. pteronyssinus* (*Der p* I, *Der p* II) and *D. farinae* (*Der f* I, *Der f* II) are immunochemically well defined [11] and panels of monoclonal antibodies have been raised against each group of allergens. All four allergens have been cloned from mite cDNA libraries and T cell clones specific for either the Group I or the Group II allergens have been isolated [1–5, 12, 13]. In this issue, Chua *et al.* report the results of experiments designed to map IgE antibody binding sites on *Der p* II, using peptides expressed from *Der p* II cDNA constructs [14]. Comparison of the properties of the Group I and Group II mite allergens illustrates some of the vagaries of antibody mapping studies. The 25-kD Group I allergens have three disulphide bonds and are readily susceptible to thermal and chemical denaturation [15]. The tertiary structure of

the Group I allergens can be modelled on the X-ray crystal structures of known thiol proteases (papain and actinidin) and a surface-exposed alpha helix (including residues 136–143 of the primary sequence) is predicted to be an important antigenic site. However, at present mapping experiments on the Group I allergens are constrained because the recombinant allergens do not bind well to either monoclonal or human antibodies. However, the 14-kD Group II allergens (also containing three disulphide bonds) are heat-stable and the recombinant allergens have good immunoreactivity, as judged by immunoassay, skin testing and histamine release assays. However, there are very limited data on their three dimensional structure and it is difficult to predict which amino acids to target for investigation of B cell epitopes on Group II molecules. Chua *et al.* [14] tackled the problem in two ways: they sonicated *Der p* II cDNA and generated a lambda gt11 expression library of the random fragments (coding for 20–30 residues peptides); they also cleaved the cDNA at specific sites using restriction endonucleases to generate 'half molecules,' comprising residues 1–69, 69–129 and 42–117, and expressed these long peptides in the pGEX vector, as fusion proteins with glutathione transferase. In each case, human IgE antibodies were used to screen the expressed peptides by plaque immunoassay or dot-blotting. None of the plaques from the random fragment *Der p* II cDNA library bound IgE antibody, whereas plaques containing full length *Der p* II cDNA (encoding 129 amino acids) showed strong reactivity. More surprisingly, peptides comprising half the linear amino acid sequence also showed weak binding to IgE antibodies. Of 57 sera from mite allergic adults or children, only 15 (25%) contained IgE antibodies to one or other of the expressed fusion proteins, whereas almost all the sera (55 out of 57) showed strong IgE antibody binding to native or recombinant *Der p* II. IgE binding to the fusion peptides was in all cases noticeably weaker than to the whole molecule. Of clinical interest is the fact that seven out of eight patients with atopic dermatitis showed reactivity to the peptides, suggesting that these patients have a broader repertoire of IgE anti *Der p* II antibody responses.

While the data in this study may appear to be somewhat negative, the results are significant because they leave little doubt that B cell epitopes on *Der p* II are discontinuous or 'conformational' and they distinguish *Der p* II from several other allergens which appear to contain linear or sequential epitopes (e.g. *Lol p* I, *Chi t* I and *Gad c* I). Another interesting observation was that

Der p II resembles lysozyme, in that it has the same number of amino acids and six cysteine residues at similar positions in the primary sequence. This led the authors to speculate that the antigenic determinants on *Der p* II may be formed by interactions between the amino and carboxy terminal ends of the molecule (as has been well documented for lysozyme), in which case cutting the molecule in half would be expected to destroy the determinants. Testing this hypothesis, and further analysis of *Der p* II B cell epitopes, will require the production of recombinant *Der p* molecules that differ at selected amino acid residues (e.g. by site directed mutagenesis); structural analysis of N-C terminal interactions and disulphide bonding patterns; and, ultimately, determination of the tertiary structure of *Der p* II. Similar strategies will also be applicable to the Group I mite allergens as better expression systems for these allergens are developed.

Although it is too early to tell whether these basic studies will have clinical applications, progress in this field has been rapid and one can expect that within the next 5 years, immunodominant determinants recognized both B cells and T cells on mite and other allergens, will be identified. As a result, our understanding of the mechanisms controlling IgE antibody responses to inhaled proteins will be greatly improved, and it should be possible to develop coherent strategies for modulating or down regulating these responses. The use of synthetic or structurally modified antigenic determinants is one approach to developing new forms of immunotherapy, for which there is increasing interest in the pharmaceutical industry.

References

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