

EDITORIAL

Grass under scrutiny

The first view of grass allergens is one of overwhelming complexity with each of the species showing a large number of IgE binding entities particularly when examined by IgE Western blotting. There are, however, several factors, including the relative success found for desensitization, that indicate these problems are not insurmountable. Firstly, the species of grass pollen is often confined by geographic and seasonal factors; and secondly, the allergens of different species cross react antigenically and have chemical similarities. A third factor to be emphasized here is that many of the allergens within a species show amino acid sequence homology and can share T and B cell epitopes and that many of the IgE-binding bands found in some electrophoretic techniques are isoallergens or post translational modifications. The publications by Matthieson and Lowenstein [1,2] in this issue describe the purification and properties of what are apparently the major grass allergens, the group V allergens. These components have in the past been obscured by heterogeneity in the group I allergens. Now serological and molecular analysis have revealed them to be a defined group of molecules that are more reactive with IgE than their more abundant group I counterparts. For instance, using a pooled serum the RAST inhibition achieved with Phl p V to an immobilized timothy extract was 75%, and in another example 15 out of 20 patients allergic to rye reacted strongly to *Lol p V*, compared with eight for *Lol p I*.

One may ask whether this is saving the best till last, or can more major allergens be expected. The results in these studies and others with CRIE show that the major IgE-binding specificities can be accounted for by the group V and group I allergens, and the heterogeneity of these allergens in SDS-PAGE can account for many of the IgE-binding bands in Western blotting [2]. The most important allergens are then the group I and group V allergens and a few less important groups such as groups II-IV described for rye [3]. Minor reactivities to cytochrome C (e.g. *Cyn d X* and *Poa p X*) and some less well described allergens are sporadic. Although it seems unlikely the current immunochemistry will reveal new major allergens it is possible that new technologies may reveal important reactivities of components which are unstable in extracts or present in too small a quantity for reliable analysis. The recently reported Kentucky Blue grass *Poa p* cDNA clones which produce a product reacting at high frequency with IgE in allergic serum may be in this category [4].

Although the purified group V timothy allergen *Phl p V*

produces a single immunoprecipitin by CRIE and a sequencable product [1], SDS-PAGE revealed considerable heterogeneity with a major band at mol. wt 28 kD, another at 32 kD, and several possible aggregation and degradation products. The strong possibility that the 32- and 28-kD components have a different but related primary structure is indicated by the finding that N terminal sequencing revealed two possible amino acids for residues 1, 3, 6, 9 in the first 25 residues. When rye grass DNA is probed by Southern hybridization with cDNA clone which appears to encode *Lol p V*, to be discussed below, it shows multiple banding also suggesting the presence of several related genes. The amino acid sequencing has also revealed a significant role for amino acid modifications in that six of the first 25 residues were found to be modified. The presence of group V has in extracts of a variety of temperate pollens been examined with a monospecific and anti *Phl p V* and eight out of 10 pollens showed strong reactivity to the antiserum in bands corresponding to regions with high IgE-binding activity [2]. The pattern was similar to the timothy extracts indicating as high degree of conservation in antigenic and molecular properties. The eight positive pollens were from the subfamily *Pooideae* which include rye, timothy and Kentucky Blue grass. Reactivity to the more distantly related grasses Bermuda *Cyrodon dactylon* and reed *Phragmites communis* has not been detected although these contain components which bind IgE cross reactivities from other grasses and a group I entity reactive with anti-*Lol p I* antibodies.

Just as the importance of group V allergens has been established the sequence of cDNA clone coding for *Lol p V* or a molecule closely related to *Lol p V* has now been reported [5]. The clone was isolated by its ability to produce a protein reactive with monoclonal antibody raised by Smart *et al.* originally thought to be against *Lol p I*. Its target now corresponds to *Lol p V*. The N-terminal residues of the sequence have high homology with *Phl p V* N-terminal sequence and the alanine content is high 23% in keeping with the N-terminal sequence of *Phl p V*. It appears the hydroxyproline residues of *Phl p V* are retained in rye as unmodified proline given the presence of a repeated TPA (one-letter amino acid code) motif which would correspond to the T (Mod) A repeats. Hydroxyproline present in collagen has also been found in plants. The product expressed by the clone had IgE binding activity and was reactive with 90% of allergic sera. This gives clonal proof that a single gene product has such a high degree of reactivity and provides an IgE-binding

molecule which can be manipulated to fragments or by site directed mutagenesis for those interested in determining the epitopes. The binding activity in the recombinant molecule has in fact been found to be confined to a C terminal segment. Experiments to determine the percent of IgE-binding reactivity retained by this molecule and the affinity of interaction need to be conducted. The *Lol p V* like molecule has been localized to the starch granules and *Lol p I* to the cytosol [5]. The sequencing activity has, however, not only been confined to rye. Sequences of cDNA clones encoding a similar molecule, or group of molecules, with several highly homologous regions including the N-terminal have been reported for Kentucky Blue grass [6]. Perhaps they also are group V allergens rather than the tentative designation of a new group IX. Whatever the relationship with the group V allergens it is clear the clones of rye and Kentucky Blue grass have extensive homology throughout these sequences.

With the excitement of the group V allergens it must be remembered the group I allergens exemplified by *Lol p I* described by Marsh *et al.* [3] are still important. The interactions of *Lol p I* and the related but serologically non cross reactive and less important *Lol p II* and *Lol p III* are of interest [7]. The *Lol p II* and *Lol p III* allergens share a homologous sequence to that found in the vicinity of the IgE-binding region described by Esch and Klapper [9]. Since responses to all three are linked with the class II HLA-DR3 MHC products it is probable they share T cell epitopes and a possible sequences has been identified. The complete amino acid sequence of *Lol p II* and *Lol p III* were determined by Ansari some time ago. A cDNA sequence has now been obtained for *Lol p I* and its 100 C-terminal residues show high homology with *Lol p II* and *III* [10,11].

The impact of these reports is that it appears that most of the IgE binding to grass pollens is to the group V (or related molecules) and group I allergens. Not only this but also the molecular structures of the components have become available. It should be noted the progress has come from the combined use of immunochemistry, monoclonal antibodies and recombinant DNA technology. Essentially protein isolation allows the antigenic characterization of the allergen and the other techniques are powerful tools for definition and submolecular investigation. It is important that the significance of these advances be recognized and followed up. For the immunochemists and molecular biologists this means the provision of monoclonal antibodies and sequences of these allergens in the major grass species. In a recent report by a rye grass working party, six candidate reference extracts were examined for a submission as a WHO reference standard [12]. Two aspects warrant

comment. Firstly, although several laboratories examined the allergens present, it was not possible at that time to measure and state the concentration of *Lol p I*, *Lol p II*, etc, in each extract. Indeed there was no attempt to list and qualify the allergens in the IUIS nomenclature. It is understood that this is being prepared for a further submission. Secondly, some of the grass extracts lacked certain allergens. Given that even using well defined allergens avoidance and desensitization regimes are protracted procedures, there seems little excuse for starting off with substandard reagents.

Now that what appears to be the two major allergen groups of grass have identified and sequenced, more concerted attempts to produce new generation desensitizing agents can be made. Much can be done to replace the course of repeated injections and increase safety and efficacy. With grass there is the advantage that the current procedure already enjoys considerable success and that many of the allergens are related. Also data from ragweed clearly demonstrates desensitization to the purified allergens can be effective for rhinitis, perhaps by decreasing the inflammatory load. An obvious starting-point will be to investigate the use of T cell epitopes to alter the ability of these cells to regulate immunoglobulin production and other elements of allergic disease such as eosinophilia. Indeed, perhaps the first area to benefit from improved and purified allergen preparations will be the implementation of more extensive T cell investigations. The recent developments in lymphokine biology have not only increased our understanding of the function of different lymphokines greatly, but have also provided simplified and more meaningful assay procedures such as quantitating mediator released by ELISA or radioimmunoassay.

References

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W. R. THOMAS

*Division of Molecular Biology,
Western Australian Research Institute for Child Health,
Princess Margaret Hospital,
Roberts Road, Subiaco,
Western Australia 6008.*

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