

EDITORIAL

Allergenic crossreactivity of fungi with emphasis on yeasts: strategies for further study

There are few phases of man's existence into which fungi do not enter, for good or ill and, if prevalence and abundance are taken into account, fungi must be considered one of the most successful forms of life. As Christensen [1] has said of fungi: 'If they have not yet exactly inherited the earth, they do eventually inherit a goodly portion of the living things on the earth, as well as the goods and products made from these living things.' All but a very few fungi reproduce by means of spores and most of us are unaware of the vast invisible cargo of fungal spores carried in the air. *Ustilago zeae* for example, which causes smut of corn has been calculated to produce 50 trillion spores from an acre of corn only moderately infected with the fungus. All these spores from just one acre, distributed at 10 spores per cubic foot, would cover 34 cubic miles of air. Spores from 100 million acres, roughly the amount of corn sown in the United States in the 1960s, would, at the same density, contaminate 3.4 billion cubic miles of air [1].

Given the ubiquitous nature of fungi, the number and diversity of species, their composition and the vast numbers of tiny, buoyant spores they frequently produce, it is not surprising that 'mould allergies' occur commonly and widely. Fungal spores range in size from about 1–300 µm and this means that many spores can penetrate the airways of man as far as the bronchioles. This small size and the sheer quantity of spores produced in the vicinity of man probably contribute significantly to the involvement of some species in human allergic disease. *Alternaria* for example, a very common fungus recognized as an important source of allergic sensitivity [2,3], grows on plants, grass cuttings, fallen leaves and straw and produces prodigious numbers of spores after only a few days growth. The spores are extremely buoyant and easily become airborne creating vast invisible clouds said to amount to tons of spores such that the air both indoors and outdoors is rarely free of *Alternaria*. *Penicillium*, another fungus commonly encountered in everyday life, produces hundreds of millions of spores 2–5 µm in diameter in only a few days so there is great potential for these, and especially other Deuteromycetes (Fungi Imperfecti) [4], to enter the airways and provoke reactions.

In the on-going quest for allergen identification, characterization and standardization and for the elucidation of allergenic crossreactions, the number and diversity of fungal species, their variability in response

to growth conditions, differences between physical forms and strain variations, all contribute toward making the study of fungal allergens more difficult than, for example, comparable studies on pollens. In addition, given that the identity of fungal species involved in immediate allergic reactions is still poorly researched and that identification and characterization of the full spectrum of allergens from most species has barely begun, it is to be expected that details of allergenic crossreactivity between fungi is still rudimentary.

Subjects with allergies to fungi often demonstrate broad patterns of positive skin and serum IgE antibody reactivities to a range of different fungi (3,5). For example, in a study of 76 subjects classified as allergic to fungi on the basis of case histories and skin-test reactions to a panel of different fungal extracts (Baldo & Baker, unpublished), most of the subjects showed positive serum IgE antibody reactions to several of the 17 different species included in the study. The fungi examined were: *Alternaria alternata*, *Aspergillus fumigatus*, *Aureobasidium pullulans*, *Botrytis cinerea*, *Candida albicans*, *Cladosporium herbarum*, *Curvularia lunata*, *Epicoccum purpurascens*, *Fusarium moniliforme*, *Helminthosporium halodes*, *Mucor racemosus*, *Penicillium notatum*, *Phoma betae*, *Rhizopus nigricans*, *Saccharomyces cerevisiae*, *Stemphylium botryosum* and *Trichoderma viride*. Forty-seven of the 76 sera were positive to 14–17 of the species, 21 sera reacted to 6–13 species and eight sera proved positive to 1–5 species. Results such as these could be interpreted in two ways—either that many fungi show allergenic crossreactivity or allergic subjects have independent parallel sensitivities to individual species.

The latter view is taken by Salvaggio and Aukrust [3] but our findings so far suggest extensive crossreactions between species. Even at first sight, it seems unlikely that subjects will develop up to 17 different individual and separate allergic sensitivities. The limited studies on crossreactivity so far undertaken have demonstrated crossreactivity but the number and diversity of species examined has been limited [5–9].

Having highlighted both the prevalence of fungi as an allergen source, the difficulties inherent in the study of fungal allergy and emphasized our current ignorance of fungal allergens, some constructive effort should be made to briefly review strategies and experimental approaches that can help to rapidly alleviate this situation.

Application of immunoaffinity techniques

Immunoabsorption and elution of antibodies provides a simple and direct method of establishing whether serological reactivity to a wide range of allergens is due to crossreactivity or separate sensitizations to the different allergen sources. Native or denatured allergen extracts, purified allergens or cloned allergens, can be immobilized onto solid supports such as CNBr-activated paper or nitrocellulose, or excised from a gel following electrophoresis, and used to immunoabsorb antibodies from the sera of allergic subjects [10]. Adsorbed antibodies can then be eluted from the allergen-solid phase complex and used to probe blotted proteins from other allergen sources to investigate crossreactivities or the eluted antibodies can be used in direct-binding immunoassays. This immunoaffinity approach may be used to investigate inter- or intraspecies crossreactivity of allergens [11] and the efficiency and discriminatory power of the method lends itself to delineating allergenic crossreactions between fungi where multiple species recognition is frequently involved.

Molecular cloning approaches

The application of recombinant DNA technology to allergen studies [12,13] has dramatically improved our ability to rapidly determine primary structures, detect amino acid polymorphisms and identify families of isoallergens, identify B and T cell determinants, engineer peptides with selected determinants, produce allergens in 'large' quantities for laboratory and clinical use and define the molecular basis of crossreactivities.

Expressed cloned fungal proteins, selected with patients' sera containing crossreacting IgE antibodies, may be used to screen cDNA libraries of crossreacting fungi by direct DNA hybridization [12]. Crossreactivity may also exist at the T cell level and recombinant IgE-binding clones are often the only feasible sources of pure allergenic proteins that can be used to stimulate and clone T cells. cDNA clones isolated from cDNA libraries of crossreacting fungi may be expressed and tested for their capacity to stimulate T cell clones reactive with the defined allergen. This approach may help to determine if T cell stimulation by different fungal extracts is due to a family of proteins sharing a common T cell determinant(s).

Enzymes as allergens

We can only assume that fungi, or any other allergen source, do not contain proteins for the specific purpose of provoking allergic reactions and allergenic proteins from any source may be made up of many different

structural and functional proteins. It does not seem surprising therefore that some allergens will be enzymes and, in fact, one might be surprised if at least some were not. At the level of primary structure, enzymes, as a broad group, show all of the diversity that one would expect to see in any large selection of different unrelated proteins but the property they have in common, viz. catalytic activity, has ensured that many different enzymes from a wide variety of sources have been isolated and studied for many years. This is of potential importance to those interested in identifying and characterizing allergens although there have been few attempts so far by researchers to exploit the available knowledge supplied by enzymologists over the years.

In 1988, Baldo and Baker [14] reported that the enzyme enolase from bakers' yeast *Saccharomyces cerevisiae* was a major allergenic component and that both *S. cerevisiae* extract and purified *S. cerevisiae* enolase showed allergenic crossreactivity with a *Candida albicans* extract. It was also pointed out that, unlike most allergens, bakers' yeast enolase is well characterized and is therefore 'a potentially important molecule for structure-activity studies directed at defining the physicochemical basis of allergenicity'. *C. albicans* enolase is reported to be a useful marker antigen for invasive candidiasis [15] and recent immunoblotting studies on the sera of 57 allergic patients demonstrated antigenic crossreactivity and 82% amino acid sequence homology between an IgE-binding 46 kDa *C. albicans* band and *S. cerevisiae* enolase [16]. The 46 kDa protein had previously been shown by Savolainen *et al.* [17] to be a major *C. albicans* allergen in a study of 28 asthmatic children each with positive skin and RAST scores for *C. albicans* extract. Subsequently, the same group identified the 46 kDa protein as the most important IgE-binding component in a group of 156 subjects with atopic dermatitis [18] and demonstrated parallel IgE antibody reactivity between this protein and *S. cerevisiae* enolase [19]. In this issue, Ito *et al.* [20] confirmed the reported crossreaction and present results which suggest that *C. albicans* enolase contains several IgE-binding determinants some of which crossreact with *S. cerevisiae* enolase.

The amino acid sequences of *S. cerevisiae* [21,22] and *C. albicans* [23–25] enolases are known as is the crystal structure of the former enzyme [26–28]. Both proteins are therefore prime candidates for defining B and T cell allergenic determinants and with the current interest in 'yeast' enolases, this should not be long in coming.

Another enzyme, alcohol dehydrogenase, was recently shown to be a major allergen of *C. albicans* [29] and this finding again draws attention to the possibility of other characterized enzymes being contributors to the

allergenicity of fungi. Once more, bakers' yeast is likely to provide the best prototype for study since the economic and commercial importance of this species has led to a more thorough examination of its enzyme constituents than almost any other fungal species. In addition to enolase, alcohol dehydrogenase, aldehyde dehydrogenase, carboxypeptidase Y, cytochrome C, glucose-6-phosphate dehydrogenase, alpha-glucosidase, hexokinase, invertase, L-lactic dehydrogenase, 3-phosphoglyceric phosphokinase, proteinase A, pyruvate decarboxylase, transaldolase and transketolase from *S. cerevisiae* are all available in pure form and/or are well characterized so their possible role as allergens can readily be investigated. Identification of any of the pure enzymes as allergens opens the possibility of using the pure proteins to selectively detect crossreacting allergenic enzymes in other species of fungi.

Fungal polysaccharides as allergens

There are numerous reports on the carbohydrate nature of many fungal allergens [30] but the chemical nature of many fungal carbohydrates and their involvement in allergy have been studied little. Mannans occur widely in fungi [31–35] and may be important crossreacting allergenic components in yeasts and so-called imperfect yeasts. In studies designed to differentiate between protein- and polysaccharide-induced reactions, *C. albicans* mannans were found to cause immediate allergic reactions in both man and monkeys [36,37]. Similarly, the dermatophyte *Trichophyton mentagrophytes* produced both immediate and delayed reactions in man and guinea-pigs but degradation experiments showed that the carbohydrate portion was involved in provoking the immediate reactions [38]. Recently, it was suggested that polysaccharides may be responsible for allergenic crossreactivity between *Pityrosporum ovale* and *C. albicans* [39].

Fungi, more than pollens, mites and other allergen sources, seem to provide examples of carbohydrate allergens but unequivocal evidence of the allergenicity of pure polysaccharides has been hard to obtain. Efforts should be made to investigate this question and characterized mannans from yeasts offer perhaps the best starting point. Although IgE antibodies reactive with *C. albicans* mannan and polysaccharide from *Aspergillus fumigatus* have been detected in the sera of subjects allergic to these fungi [40], it is often difficult to exclude the involvement of contaminating proteins or attached peptide. At the level of allergenic crossreactivity of fungi, the involvement of carbohydrate allergens must be considered to still be an open question.

Fungal C-substances

'C-substance' is the name originally given to a component of *Streptococcus pneumoniae* that precipitates with C-reactive protein (CRP) in the presence of Ca^{2+} [41]. Antigens that precipitate with CRP are widely distributed in nature especially in fungi [42,43]. *S. pneumoniae* C-substance is a ribitol teichoic acid [44] polymer and although C-substances from different sources are usually different [42–45] they all react with the CRP combining sites via phosphorylcholine (or a closely related choline derivative) determinants and are precipitated by anti-phosphorylcholine-specific antibodies [45].

C-substances have been detected in a wide range of fungi including *Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *A. niger* and *A. terreus*, *Cladosporium herbarum* and *C. werneckii*, *Epidermophyton floccosum*, *Fusarium* spp., *Mucor racemosus*, *Paecilomyces variotii*, *Penicillium notatum*, *Trichophyton mentagrophytes*, *T. rubrum* and *T. schoenleinii* and C-substances from some parasites and the house dust mite have been shown to be allergenic [43]. With crossreactivity in mind, the possibility of the occurrence of allergenic fungal C-substances and of the involvement of choline or phosphorylcholine residues in the allergenic determinants is intriguing and suggests one possible basis for wide-ranging allergenic crossreactivity between fungi.

The fungi are a vast, diverse and complex group of organisms and they present a considerable challenge to the practicing allergist and laboratory investigator seeking to identify allergy provoking species, refine diagnosis, identify and characterize allergenic constituents, introduce safe immunotherapy and standardize diagnostic and therapeutic solutions. Knowledge of allergenic crossreactions between species at both the clinical and immunochemical levels will reduce the apparent complexity of the above tasks and, at the levels of diagnosis and allergen avoidance, be of early benefit to the patient. At the same time, an accumulation of new laboratory data on fungal allergens is likely to significantly add scientifically to our fundamental knowledge of protein structures, homologies and functions and to our immunochemical understanding of polysaccharide and other carbohydrate-containing antigens.

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