

Characterization of Immunologically Important Antigens and Allergens of *Aspergillus fumigatus*

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Abstract. Using a variety of immunochemical methods, including quantitative immuno-electrophoretic techniques, combined with gel filtration and iso-electric focusing, and production of monospecific antisera for identification and affinity purification, 4 major components of *Aspergillus fumigatus* have now been partially characterized. Numbering of these was derived from a reference allergic bronchopulmonary aspergillosis (ABPA) self-crossed radio-immuno-electrophoresis pattern of reactivity.

Two major intracellular/cytoplasmic, concanavalin A (Con A)-binding antigens, Ag 7 and Ag 13, of molecular weights 150-200 and 70 kilodaltons (kD), respectively, were confirmed to be of importance for both ABPA and aspergilloma in specific sandwich enzyme-linked immunosorbent assays. A rapidly released component, Ag 5, of molecular weight 35 kD, proved both antigenic and allergenic, with aspergilloma patients having especially high-titre IgG antibodies. The major allergenic component Ag 3, of molecular weight 24 kD by gel filtration and 18 kD by SDS-PAGE was, like Ag 5, relatively heat-labile and non-Con-A-binding. Interestingly, T cell clones have been identified which respond primarily to an 18-kD fraction.

Introduction

Aspergillus fumigatus is associated with several pulmonary diseases in which the diagnosis may be confirmed by the demonstration of specific IgE and/or IgG antibodies. Antigenic extracts, whether from culture filtrate or mycelial sources, are complex mixtures of components which may vary quantitatively from batch to batch. Some components may also be so widely cross-reacting that antibodies to them may not be disease-related. This study, therefore, has attempted to identify and characterize components of major immunological importance with regard to both IgG and IgE antibody reactivities of patients, in particular with allergic bronchopulmonary aspergillosis (ABPA) and aspergilloma, and also to *A. fumigatus*-reactive T cell clones from ABPA and control individuals.

Antigen/Allergen Identification

The most important or 'major' antigenic or allergenic components, i.e. components to which the ma-

jority (>60%) of patients had IgG or IgE antibodies, were identified using ABPA sera in self-crossed radio-immuno-electrophoresis tests. 15 IgG- and IgE-binding components were identified when an ABPA serum pool was used and a numbered pattern of reactivity was recognized (fig. 1). By comparing 32 individual ABPA serum responses to the reference pattern, Ag 7 and Ag 13 were identified as major antigens and Ag 3 in particular as a major allergen [Longbottom, 1983].

Characterization of Major Components

Separation on Sephacryl S-200 of a protein-enriched precipitate was monitored by fused rocket radio-immuno-electrophoresis. This showed the presence of an antigenic/allergenic component in the first fractions eluted (molecular weight, MW, 150-200 kilodaltons, kD) and a highly allergenic component in fractions of MW 24 kD.

Analysis of the first fraction identified its main component to be Ag 7. This was further characterized as a heat-stable (100°C, 5 min) acidic glycoprotein

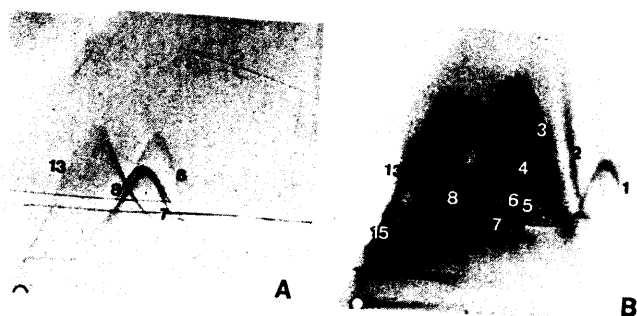


Fig. 1. Reference self-crossed radio-immuno-electrophoresis pattern of reactivity for ABPA sera. *A. fumigatus* antigen was electrophoresed (first dimension) and then into gel incorporating the ABPA 'serum pool' (second dimension). A Protein-stained plate. B Corresponding autoradiograph. Note (1) the presence of Ag 7 and Ag 13 in A, and the associated radio-active uptake on Ag 7 in B, and (2) the intensity of radioactive uptake on Ag 3 in B.

which binds to concanavalin A (Con A; suggesting the presence of α -D-manno- or α -D-glucopyranoside end residues) apparently composed of 36-kD subunits (dissociated by SDS) [Harvey and Longbottom, 1986a].

The second major antigenic component, Ag 13, which was identified as the previously recognized C antigen [Tran Van Ky et al., 1966] with a monospecific antiserum kindly supplied by Dr DWR MacKenzie (PHLS Collindale), proved to be a relatively heat-labile (75°C, 15 min), Con-A-binding glycoprotein with MW 70 kD [Harvey and Longbottom, 1987].

Specific sandwich enzyme-linked immunosorbent assays (ELISAs) developed to detect IgG antibodies to both Ag 7 and Ag 13 confirmed their potential importance for diagnosis of both ABPA and aspergilloma patients [Harvey and Longbottom, 1986b].

A rapidly released antigenic/allergenic component, Ag 5, was identified by its ability to induce an initial early antibody response in rabbits immunized with *A. fumigatus* 'germlings' (germinated conidia). This component was relatively heat-labile (56°C, 30 min), did not bind to ConA and had an MW of approximately 35 kD [Taylor and Longbottom, 1988]. The surface nature of this component was confirmed in fluorescent antibody and ELISA studies. It is considered of importance for a possible involvement in the triggering of immunological (immediate-type) reactions in patients.

The major allergen, Ag 3, was identified as the 24-kD allergenic component of the gel fractionation. Further studies showed it to be very heat-labile, non-Con-A-binding (therefore lacking α -D-mannoside or glucoside terminal residues) [Longbottom, 1986] and on sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis and electroblotting to have an MW of 18 kD. Using particles derived from nitrocellulose blots of SDS gels, an *A. fumigatus*-reactive T cell clone has also been shown to react predominantly with an 18-kD component [in preparation].

In conclusion from the mosaic of antigenic/allergenic components present in extracts of *A. fumigatus*, four components of major importance for ABPA and aspergilloma patients have been characterized.

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