Allescheria boydii and Aspergillus fumigatus Skin Test Antigens

LEWIS K. MAY, RALPH A. KNIGHT, AND H. WILLIAM HARRIS

Department of Medicine, Woman's Medical College of Pennsylvania, Philadelphia, Pennsylvania

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ABSTRACT

MAY, LEWIS K. (Woman's Medical College of Pennsylvania, Philadelphia), RALPH A. KNIGHT, AND H. WILLIAM HARRIS. Allescheria boydii and Aspergillus fumigatus skin test antigens. J. Bacteriol. 91:2155–2157. 1966.—Protein and polysaccharide fractions were extracted from culture filtrates of Allescheria boydii and Aspergillus fumigatus by the methods of Seibert and of Heidelberger, and injected intradermally into guinea pigs previously infected with these fungi. The diameter of erythema and induration was determined at 8, 24, and 48 hr. The protein and polysaccharide antigens yielded specific skin reactions in homologously infected guinea pigs. Erythema appeared at 8 hr with both the protein and polysaccharide antigens. At this time, the polysaccharide skin tests showed erythema and a central blanched wheal. A similar wheal was not observed with the protein. The erythema of the polysaccharide reaction began fading at 24 hr, whereas the protein reaction remained unchanged through 48 hr with both antigens. In guinea pigs, the area of erythema was more constant and thus easier to measure than was induration.

Evidence for a high prevalence of subclinical human histoplasmosis and coccidioidomycosis resulted from epidemiological studies with the use of appropriate skin tests. The incidence of subclinical human infection with Aspergillus and Allescheria species is unknown, since antigens appropriate for epidemiological studies are not available. Palmer, Edwards, and Alffather (6) detected a geographic area, including parts of Kansas, Oklahoma, Texas, and Louisiana, in which nonspecific histoplasmin hypersensitivity was prevalent. They believed this to have resulted from subclinical infection with an unknown organism antigenically related to Histoplasma capsulatum. Reports by Pepys et al. (7) and by Campbell and Clayton (2) on the clinical and immunological aspects of human pulmonary aspergillosis have generated interest in the possibility that this infection might be more prevalent than has been recognized heretofore.

With the belief that skin test antigens derived from Aspergillus and Allescheria species might be useful in epidemiological studies, this investigation was designed to produce protein and polysaccharide antigens from these fungus strains and to evaluate the delayed hypersensitivity reactions obtained by intradermal injections of these antigens in experimentally infected guinea pigs.

MATERIALS AND METHODS

Preparation of antigens. Aspergillus fumigatus (culture 5256-Emmons) and Allescheria boydii (culture USPHS 2775) were used. The organisms were seeded to low-form flasks containing 1 liter of the Bureau of Animal Industry Tuberculin Medium. The seeded flasks were placed on a slowly moving mechanical shaker, incubated at 37°C until growth ceased, and then autoclaved. The mycelial matt was separated by filtration, and the culture filtrate was used for chemical extractions.

Inorganic ions were dialyzed from the culture filtrates, and the dialysates were concentrated to approximately one-tenth volume by pervaporation. An attempt to remove polysaccharide fractions was made by use of the ethyl alcohol precipitation method of Heidelberger et al. (4). Protein fractions were prepared by use of the multiple ammonium sulfate precipitation method of Seibert (8). The products were lyophilized and weighed. The nitrogen and reducing sugar content of each fraction was determined by use of the Micro-Kjeldahl and Anthrone methods, respectively.

Inoculation of animals. Cultures of A. boydii and A. fumigatus for animal inoculation were seeded into tubes containing 10 ml of Sabouraud broth and a few sterile glass beads, and incubated at 37°C for 4 days. Twice daily during the incubation period, the cultures
were thoroughly agitated on a mixer to disrupt the mycelial mat. After incubation the cultures were centrifuged, washed three times with saline, and resuspended. Each suspension contained 2.5 × 10^8 to 5.0 × 10^8 viable units per ml.

Albino guinea pigs were randomly divided into three groups. Each of the nine animals in group I was injected intraperitoneally with 1.0 ml of the A. boydii suspension every 2 weeks for a total of three injections. The 11 animals in group II received A. fumigatus on an identical schedule, and 10 control guinea pigs (group III) received 1.0 ml of sterile saline intraperitoneally on the same schedule.

Two weeks after the last intraperitoneal injection, all guinea pigs were skin-tested with 10 and 25 µg/0.1 ml of the A. boydii and A. fumigatus protein and impure polysaccharide products.

Specificity of the A. boydii and A. fumigatus antigens was investigated in groups of four to six guinea pigs infected with Cryptococcus neoformans, H. capsulatum, or Candida albicans and subsequently skin-tested with the A. boydii and A. fumigatus antigens.

In all experiments, the diameter of erythema and induration produced by each antigen was measured in millimeters at 8, 24, and 48 hr. Erythema rather than induration was used for the quantitative assessment of skin reactivity, because the boundaries of induration were indefinite in many animals.

**RESULTS**

Chemical analyses of antigens. Table 1 indicates the nitrogen and carbohydrate content of the A. boydii and A. fumigatus fractions. It is obvious that the "polysaccharide" fractions contained considerable amounts of nitrogenous material.

Skin test results. Table 2 illustrates the results of intradermal skin tests with A. boydii and A. fumigatus antigens in guinea pigs infected with these organisms and in control animals.

The 10 and 25 µg/0.1 ml skin test doses produced skin reactions of comparable size. Usually, the "polysaccharide" fractions produced erythema and a centrally blanched wheal by 8 hr, both of which tended to fade by 24 hr. The protein fractions produced maximal erythema in 24 hr, and a centrally blanched wheal was not observed.

Table 3 indicates that the A. boydii and A. fumigatus antigens produced no cross-reactions in animals infected with these fungi, or with C. neoformans, C. albicans, or H. capsulatum. No reactions were observed in noninfected controls.

**DISCUSSION**

The purpose of this study was to attempt the isolation of material suitable for use as skin test antigens in studies of hypersensitivity to A. boydii and A. fumigatus.
The protein fractions contained small quantities of reducing sugars (measured as glucose). This was not unexpected, since Affronti (1) found similar amounts of carbohydrate in his purified protein fractions of mycobacteria.

All attempts to obtain a nitrogen-free carbohydrate fraction of the A. fumigatus antigen were unsuccessful. This protein may be intimately associated with, or bound to, the polysaccharide fraction of the organism. Similar extraction methods employed by Knight and Marcus (5) with H. capsulatum and Blastomyces dermatitidis yielded fractions containing 4% or less protein.

Skin reactions in some guinea pigs were characterized by an indistinct boundary of induration; hence, the skin test reactions were better evaluated by measuring erythema. Edwards et al. (3) found erythema to be more accurate than induration in assessing skin hypersensitivity of guinea pigs infected with anonymous mycobacteria and tested with homologous antigens. In humans, however, skin tests with these mycobacterial antigens elicited reactions comparable to those with standard purified protein derivative (PPD) tests for tuberculosis, and these tests were best evaluated by measuring induration.

The antigens developed in this investigation appear to be specific, but to have a low level of sensitivity in guinea pigs. Their use as diagnostic aids in individuals known, or suspected, to harbor infections by these fungi is worthy of investigation. In addition, test trials in large groups of normal individuals may be warranted to secure evidence of subclinical infections.

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Literature Cited


