Isolation of a Purified Skin Test Antigen from Blastomyces dermatitidis Yeast-Phase Cell Wall

MICHAEL V. LANCASTER* and RONALD F. SPROUSE†

Department of Veterinary Microbiology and Department of Medical Microbiology, University of Missouri, Columbia, Missouri 65201

Received for publication 4 May 1976

Preparative polyacrylamide gel electrophoresis was used to isolate individual components of an alkaline-soluble–water-soluble fraction of the cell wall of Blastomyces dermatitidis yeast phase. One component isolated demonstrated exceptional specificity and reactivity when tested on guinea pigs infected with B. dermatitidis. This component displayed no cross-reactivity when tested on animals infected with Histoplasma capsulatum. The significance of isolation of a purified, specific antigen is discussed.

The requirement for a reliable immunological tool useful in epidemiological surveillance, serodiagnosis, and diagnosis of blastomycosis long has been obvious. Past attempts to purify antigenic components generally have been limited to analysis of crude mycelial filtrates of Blastomyces dermatitidis (4, 6, 7). However, Cox and Larsh (1) recently have succeeded in extracting a partially purified cell wall extract (designated ASWS PM-10) from the yeast phase of B. dermatitidis with increased reactivity and specificity in guinea pigs sensitized with mercapto-methyl-killed yeast cells of B. dermatitidis. Although this alkaline-soluble–water-soluble (ASWS) extract is only partially purified, its skin test reactivity appears to be consistent from preparation to preparation and consequently warrants additional examination.

Preparative-scale polyacrylamide electrophoresis was judged a suitable separation technique for obtaining purified components from the ASWS extract since the extract had already been characterized by analytical polyacrylamide disc electrophoresis (2). Thus, the objective of this study was to isolate purified components from the ASWS extract of B. dermatitidis yeast cell walls by preparative-scale polyacrylamide electrophoresis and to evaluate the skin test reactivity and specificity of those components.

MATERIALS AND METHODS

Antigen fractionation. The ASWS antigen was prepared from B. dermatitidis by the procedure described by Cox and Larsh (1). Once the ASWS antigen had been prepared, it was applied directly to a preparative disc electrophoresis system. A 5% polyacrylamide gel containing tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 8.9, 0.014 M) was photopolymerized in a preparative electrophoresis column (2.5 by 15 cm). Tris(hydroxymethyl)aminomethane-glycine buffer (pH 8.9, 0.015 M) was used for electrode and elution buffers. ASWS antigen (1.2 ml, 10 mg/ml) was layered on the gel surface. The anode of a constant-current power supply was connected to the lower chamber, and the cathode was connected to the upper buffer chamber. Elution buffer (pH 8.9) was pumped across the lower end of the gel at 15 ml/h. Power was then applied to the column (1,000 V at 10 mA; 10 W), and electrophoresis was continued for 16 h. Column effluent was monitored by ultraviolet absorbance at 280 nm and was collected in a fraction collector. Consecutive fractions showing 280-nm absorbance were pooled, dialyzed against distilled water, and lyophilized. The fractions collected were subsequently analyzed by analytical disc electrophoresis according to the method of Davis (3). Gels were stained with amido black 10B in 7% acetic acid and destained in 7% acetic acid. Stained zones were recorded with a densitometer.

Skin testing of pooled fractions. Pooled electrophoretic fractions of the ASWS extract were assayed for skin test reactivity by intradermal inoculation (approximately 5 μg of protein dissolved in 0.1 ml of sterile saline) of guinea pigs infected with either B. dermatitidis or Histoplasma capsulatum (5). All pooled fractions were tested on Blastomyces-infected pigs, Histoplasma-infected pigs, and noninfected pigs. Blastomycin KCB-26 (1:100) was applied to Blastomyces-infected pigs as positive controls. Induration was recorded at 24 and 48 h after inoculation. Induration readings of 48 h were used for statistical calculations and for interpretation of results.

RESULTS

Figure 1 represents the elution profile of the preparative electrophoresis and separation of
ASWS antigen. Four peaks absorbing at 280 nm were apparent. All four eluted from the column within 8 h of initiation of electrophoresis. Figure 2 represents densitometer tracing of the analytical disc electrophoresis of the pooled fractions and unfractonated ASWS antigen. The unfractonated ASWS tracing shows at least four peaks, designated f1, f2, f3, and f4, with Rf values of 0.99, 0.77, 0.53, and 0.11, respectively. Densitometer tracings of the pooled fractions demonstrate identity of those fractions to the four zones in the unfractonated ASWS. In addition, no cross-contamination is apparent in any of the pooled fractions.

Table 1 represents the results of skin testing the pooled fractions. Controls were essentially negative, with only one animal reacting to ASWS and ASWS f4. Inspection of the data in Table 1 suggests ASWS f4 to be a superior antigen to the ASWS unfractonated antigen on a weight-to-weight basis. The cross-reactivity of ASWS appears to be present in f1, f3, and f4 but absent in f2. Data presented in Table 2 further support the superiority of ASWS f4. Whereas ASWS reacted in 73% (8/11) of the Blastomyces-infected animals, it also reacted in 2 of 10 Histoplasma-infected animals. ASWS f4 reacted in 100% (11/11) of the Blastomyces-infected group while reacting on none of the Histoplasma group. Further, the range of induration size for ASWS f4 in the Blastomyces-infected group was 9 to 11 mm. Statistical analysis indicated no significant difference (P > 0.05) in average indurations among the ASWS fractions tested on Blastomyces-infected animals. ASWS f1, f2, and f3 showed no significant differences for each antigen between the reactions elicited on Blastomyces-infected and Histoplasma-infected animals. Both unfractonated ASWS and ASWS f4

![Fig. 1. Elution pattern of Blastomyces cell wall fraction ASWS PM-10 from preparative polyacrylamide gel electrophoresis.](image)

![Fig. 2. Densitometer tracings of Blastomyces cell wall ASWS PM-10 fractions from preparative electrophoresis. Origin is designated by O. Rf values of four components are shown.](image)

**Table 1. Results of skin testing Blastomyces cell wall fractions**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Concentration (mg of protein/0.1 ml)</th>
<th>Control*</th>
<th>Blastomyces infected</th>
<th>Histoplasma infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>KCB-26</td>
<td>1:100 dilution</td>
<td>0.0</td>
<td>9.4</td>
<td>NT†</td>
</tr>
<tr>
<td>HKC-5</td>
<td>1:100 dilution</td>
<td>0.0</td>
<td>NT</td>
<td>12.2</td>
</tr>
<tr>
<td>ASWS</td>
<td>5.0</td>
<td>1.2</td>
<td>8.6</td>
<td>2.2</td>
</tr>
<tr>
<td>ASWS f1</td>
<td>5.0</td>
<td>0.0</td>
<td>5.4</td>
<td>2.4</td>
</tr>
<tr>
<td>ASWS f2</td>
<td>5.0</td>
<td>0.0</td>
<td>7.2</td>
<td>5.2</td>
</tr>
<tr>
<td>ASWS f3</td>
<td>5.0</td>
<td>1.2</td>
<td>7.8</td>
<td>7.2</td>
</tr>
<tr>
<td>ASWS f4</td>
<td>5.0</td>
<td>0.0</td>
<td>9.6</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* Measurement represents mean induration.
† Nine guinea pigs were tested.
‡ Eleven guinea pigs were tested.
§ Ten guinea pigs were tested.
¢ NT, Not tested.

**Table 2. Number and percentage of reactors to Blastomyces cell wall fractions**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>No. (% of reactors at 48 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASWS</td>
<td>1/9 (11) 8/11 (73) 2/10 (20)</td>
</tr>
<tr>
<td>ASWS f1</td>
<td>0/9 (0) 6/11 (55) 4/10 (40)</td>
</tr>
<tr>
<td>ASWS f2</td>
<td>0/9 (0) 8/11 (73) 6/10 (60)</td>
</tr>
<tr>
<td>ASWS f3</td>
<td>1/9 (11) 9/11 (82) 9/10 (90)</td>
</tr>
<tr>
<td>ASWS f4</td>
<td>0/9 (0) 11/11 (100) 0/10 (0)</td>
</tr>
</tbody>
</table>
elicited reactions that were significantly larger in the Blastomyces-infected group than in the Histoplasma-infected group.

DISCUSSION

This study has presented evidence that a single component isolated from the cell wall of the yeast phase of B. dermatitidis is both reactive and specific when used as a skin test agent to differentiate Blastomyces infections from Histoplasma infections in guinea pigs. The component was shown superior to unfractonated ASWS with regard to specificity and reactivity and was at least as good as KCB-26.

Comparison of our results with those presented by Cox and Larsh (1) reveal similarity in specificity of the ASWS antigen. In both studies the antigen reacted in about 80% of the Blastomyces-infected animals and 10% of the Histoplasma-infected animals. However, maximal reaction occurred at 24 h in the Cox and Larsh study and at 48 h in our study. In the earlier study, 100 μg of material was required, whereas our study used only 5 μg of protein for each skin test. Both differences may be attributed to the difference in methods used to sensitize the guinea pigs. Cox and Larsh used guinea pigs that had been sensitized to Blastomyces and Histoplasma yeast, whereas this study used animals that were infected and represented active blastomycosis and histoplasmosis.

One might expect antigens prepared from whole yeast cells to exhibit a high degree of specificity when tested on animals sensitized with killed homologous yeast cells as compared with animals infected with hyphal-phase cells. Sensitization of the test animals with yeast cells precludes initial exposure to the pathogen's infective particles and subsequent morphological stages as the organism converts in vivo to the yeast phase. Such changes in morphology could, conceivably, be important sources of antigenic stimulation. Further, since infected animals carry the organism for extended periods, greater sensitivity to skin testing would be expected in infected animals. Cox and Larsh produced an antigen that was more specific and consistent than earlier blastomycins. The use of animals sensitized with homologous organisms rather than chronically infected with hyphal-phase particles could lead to higher specificity than shown by blastomycin. In addition, sensitization could be expected to lead to relatively low sensitivity, as demonstrated by the quantity of antigen required to elicit responses.

Testing of the crude ASWS fraction on in-

fected guinea pigs (Tables 1 and 2) demonstrated similar specificity and reactivity to that shown on sensitized pigs. However, only 5 μg was required on infected pigs, whereas 50 μg was required on sensitized pigs. The discrepancy in sensitivity could be attributed to either antigen purity or animal sensitivity, or both.

Purification of the components of ASWS antigen led to an antigen that reacted on 100% of our Blastomyces-infected test group, with no cross-reaction on the Histoplasma test group. The relatively small size (10 to 11 animals/group) may be responsible for these high percentages, but we feel that even in larger groups the figures would not vary significantly. ASWS f, produced reactions on the Blastomyces group that varied only from 9 to 11 mm in size. The lack of variation of ASWS f, while other fractions varied from 0 to 15 mm on the same guinea pigs, adds support to the observed high reactivity rate.

Studies should be undertaken to establish the relation of ASWS f, reactivity in infected guinea pigs representing active disease to that in sensitized guinea pigs representing inactive or resolved disease. In addition, the reliability of ASWS f, as a skin test antigen for human use should be evaluated. If such investigations lead to positive results, the ASWS f, could be an important investigative tool in the detection and diagnosis of blastomycosis.

ACKNOWLEDGMENTS

This work was supported by Public Health Service research grant AI-09614 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED