Immunological Studies with an M-Deficient Histoplasmin Skin-Test Antigen

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Previous studies have shown that a single skin test to histoplasmin may induce complement-fixing antibodies or M precipitins (or both) to histoplasmin in histoplasmin-sensitive, but serologically negative, individuals. Ideally a skin-test antigen should be one which detects hypersensitivity without stimulating humoral antibodies. Histoplasmin skin-test antigens presently used contain both H and M antigens. The present study was undertaken to evaluate an histoplasmin skin-test antigen deficient in the M component but containing the H antigen. Thirty histoplasmin-hypersensitive subjects were bled prior to administration of the experimental skin-test antigen and at various time intervals thereafter. Only six of the thirty hypersensitive subjects showed serological responses. The sera of the six, however, only showed weak precipitin reactions, five showed M bands, and only one showed an M and an H band. None showed complement-fixation titers with either the yeast or mycelial antigens of Histoplasma capsulatum. Our data suggest that the use of a skin-test antigen purified to contain only H component would detect histoplasmin hypersensitivity without inducing antibodies and would eliminate false-positive serological reactions caused by the M component.

Previous studies (3) have shown that a single skin test with histoplasmin may induce complement-fixing antibodies or M precipitins (or both) to histoplasmin in histoplasmin-sensitive, but serologically negative, individuals. Such studies by various investigators indicate that the incidence of this response may range from 3 to 58% in healthy hypersensitive subjects. Ideally, a skin-test antigen should be one that detects hypersensitivity without stimulating humoral antibodies. Production of such a histoplasmin would reduce the number of false-positive reactions noted in the serodiagnosis of histoplasmosis. It has been suggested (4) that histoplasmin skin-test antigens that contained only the H component might react in such a manner. The present study was undertaken to evaluate a histoplasmin skin-test antigen that possessed the H component but little or no M component.

MATERIALS AND METHODS

Antigens. The M-deficient histoplasmin was produced by the method described by Wiggins and Schubert (6). Antigen was obtained from 12 different Histoplasma capsulatum isolates after 6 months of growth in synthetic asparagine medium at 25 C. The growth from each culture was treated with Merthiolate (1:10,000) and filtered through Whatman no. 3 filter paper. The filtrates were then screened for H and M components. Filtrates containing only the H component or traces of the M component were pooled. The collected antigen was Seitz filtered and checked for sterility.

Proper concentration of the experimental skin-test antigen was attained by dilution with a phenolized phosphate buffer solution, pH 7.38 (5). The other histoplasmin skin-test antigens used were 1:100 diluted National Communicable Disease Center (NCDC) antigen lot H-42 and 1:100 diluted Parke, Davis & Co. antigen lot C1-521.

Skin tests. The patients were skin-tested and examined by one examiner (R.T.). An 0.1-ml amount of diluted histoplasmin was injected intradermally into the volar surface of the left forearm 4 to 6 inches (10 to 15 cm) below the elbow. The tests were read 48 hr later, and the size of the largest diameter of induration was recorded. A reaction was considered positive when the induration measured 5 mm or more.

Bleeding procedure. Each subject was bled on four different occasions; the first was just prior to administration of the skin test (zero time). The second was 2 days later, immediately after reading the results of the skin test. The third and fourth blood samples were taken 15 and 30 days, respectively, after the initial bleeding. All sera were preserved with Merthiolate (1:10,000) and forwarded to the Mycology Section of NCDC. The sera were stored at ~20 C before and after testing. Each serum was tested for complement-fixing antibodies and H and M precipitins.
Serological tests. Complement fixation and immunodiffusion tests were carried out as described in an earlier report (3). Antigens were titrated by the agar-gel method. Two parallel rows of six wells each were cut into the agar on 2- by 3-inch (5.08 by 7.62 cm) slides. Each well was 3 mm in diameter and was separated from adjacent wells by a distance of 7 mm.

RESULTS

Preliminary experiments were performed with the M-deficient histoplasmin to determine the concentration of antigen which would elicit a skin-test reaction in sensitized subjects comparable to the one elicited by the standard H-42 histoplasmin. These studies indicated that 0.1 ml of a 3.5:1,000 diluted M-deficient antigen produced reactions equivalent to those obtained with the H-42 standard. To further verify this, extensive tests were performed on 87 patients free of pulmonary disease. Each subject received both antigens, one in each arm. Figure 1 is a scattergram showing the correlation of indurations between the two antigens in these subjects. The points are approximately equally distributed on both sides of the line of equivalence, indicating no consistent difference in the antigenicity of the two histoplasmins. Furthermore, by using a pared r test \( P = 0.05 \), the average difference (0.06) between indurations produced by the two antigens was not statistically significant, i.e., not significantly different from zero.

Forty prebled subjects were skin tested with the 0.35% M-deficient histoplasmin, and their antibody responses were monitored. Thirty of these subjects were hypersensitive, demonstrating areas of induration ranging from 5 to 20 mm. Of these 30 reactors, none demonstrated complement-fixing antibodies to histoplasmin and only 6 (20%) demonstrated precipitins. The sera of five of these six patients contained precipitins to the M antigen only. The sera of the sixth patient had precipitins to both the H and M antigens. Table 1 summarizes the serological data accumulated from 114 hypersensitive and serologically negative subjects who had received a single skin test with H-42 histoplasmin, 19 similar subjects who were tested with the Parke, Davis & Co. histoplasmin, and 30 hypersensitive subjects who were tested with the M-deficient antigen. Our data indicate that the M-deficient skin-test antigen, in contrast to the two conventional antigens, elicited no complement-fixing antibodies within the 30-day period after administration of the skin test. The M-deficient antigen did elicit M precipitins, but the precipitin bands produced were noticeably weaker than those elicited by the conventional histoplasmin skin-test antigens.

<table>
<thead>
<tr>
<th>Type histoplasmin</th>
<th>No. of hypersensitive subjects</th>
<th>No. (% of subjects with antibodies)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CF</td>
<td>Precipitins</td>
</tr>
<tr>
<td>H-42</td>
<td>114</td>
<td>10 (9) 17 (15)</td>
</tr>
<tr>
<td>Parke, Davis &amp; Co.</td>
<td>19</td>
<td>2 (11) 7 (37)</td>
</tr>
<tr>
<td>M-deficient</td>
<td>30</td>
<td>0 (0) 6 (20)</td>
</tr>
</tbody>
</table>

a Antibodies to histoplasmin.

TABLE 1. Antibody responses detected in the sera of hypersensitive individuals within 30 days after receiving a single histoplasmin skin test

TABLE 2. Agar-gel titers for H and M components in the histoplasmin skin-test antigens

<table>
<thead>
<tr>
<th>Histoplasmin</th>
<th>Titer against sera containing precipitins</th>
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<tbody>
<tr>
<td></td>
<td>Anti-H</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>H-42</td>
<td>1:64</td>
</tr>
<tr>
<td>Parke, Davis &amp; Co.</td>
<td>1:128</td>
</tr>
<tr>
<td>M-deficient</td>
<td>1:64</td>
</tr>
</tbody>
</table>
Further studies were performed to determine more accurately the relative amounts of H and M antigens in the three histoplasmins used. Various dilutions of 10 times concentrated histoplasmin skin-test antigens were run in immunodiffusion tests against various dilutions of anti-H, anti-M, and anti-H and M sera to determine the highest dilution of antigen reactive with the highest dilution of antisera. The agar-gel titers obtained for H and M precipitogens in these antigens are shown in Table 2. Apparently, all of the skin-test antigens studied contained approximately the same level of H antigen. However, the M-deficient antigen contained 5 to 10 times less M antigen than did the standard H-42 and Parke, Davis & Co. histoplasmins.

The results indicate that M-deficient histoplasmin skin-test antigens elicit hypersensitive reactions in H. capsulatum-infected individuals without inducing complement-fixing antibodies in their sera. The skin-test phenomenon appears mainly to be a product of reaction with the H antigen, since diminution of the M component failed to affect the skin-test potency of the M-deficient antigen. (Fig. 1).

Antigen analysis indicates that the M-deficient histoplasmin contained five to ten times less M antigen than the standard H-42 and Parke, Davis & Co. histoplasmin antigens (Table 2). The M antigen concentration was sufficiently low to eliminate false positives in the complement-fixation test but not in the agar-gel test. Although M bands were produced with the sera of individuals who had been skin tested with the M-deficient histoplasmin, they were very faint. (Table 1).

The experimental antigen, despite traces of the M component, was superior to conventional skin-test antigens in that it did not stimulate formation of histoplasmin antibodies detectable by the complement-fixation test. Recent reports (1, 2) indicate that the histoplasmin antigens H and M may be effectively separated by ion-exchange chromatography. Our data suggest that the use of a skin-testing antigen purified to contain only H component would be ideal for the detection of histoplasmin hypersensitivity without inducing antibodies, and would eliminate false-positive serological reactions caused by the M component.

ACKNOWLEDGMENTS

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LITERATURE CITED