Identification of a Gonococcal Antigen Important in the Human Immune Response

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An antigen isolated from Neisseria gonorrhoeae protoplasm reacted well in a flocculation procedure with sera from 86% of the females and 68% of the males infected with gonorrhea; the reactions with sera from presumed noninfected individuals were approximately 12%.

We have recently undertaken the systematic analysis of antigens of Neisseria gonorrhoeae to devise an effective serological system for detection of gonorrhea. During these preliminary studies, we noted a sediment formation in the isolated gonococcal protoplasm which reacted well in a flocculation procedure. This report describes the preparation of this antigen, its reactivity in the flocculation procedure, and a preliminary evaluation of the antigen by using clinically and bacteriologically defined human sera.

The sediment was formed by dissolving lyophilized N. gonorrhoeae protoplasm (1) in 1.0 M NaCl, 0.1 M tris(hydroxymethyl)aminomethane buffer, pH 8.0 (TRBS), and by allowing the solution to stand at 4°C for 48 hr. After the sediment was isolated by centrifugation at 36,000 × g for 30 min, it was washed, centrifuged 8 to 10 times with an excess of 0.85% NaCl (NS), and then lyophilized.

The antigen was prepared as follows. A 50% suspension of sediment in NS was diluted with an equal volume of 1.0% Amidoschwarz stain in glacial acetic acid. This suspension was allowed to stand overnight at 4°C. The stained suspension was washed three times in NS to remove the excess stain and resuspended to a 25% suspension in TRBS. After standing for 48 hr at 4°C, the suspension was washed and centrifuged three times in NS. This final stained and washed antigen was adjusted to a 5% suspension in NS and centrifuged at 500 × g for 10 min to remove the larger particles. The resulting supematant suspension was decanted and centrifuged at 1,000 × g for 10 min to isolate the desired particle size. These particles were resuspended to a 1% solution in NS.

The preliminary evaluation of the flocculation test used the following sera. Sera designated "positive" were from 54 males and 50 females with uncomplicated gonococcal infections documented by culture of N. gonorrhoeae. Sera designated "negative" were from 53 males and 50 females admitted to a local hospital; these patients were not cultured for confirmation as "negative" but were from a presumably low-risk population and did not carry an admitting diagnosis of gonococcal infection. All sera were heated at 56°C for 30 min and clarified by centrifugation.

The tests were performed by placing 0.03 ml of serum on a standard Venereal Disease Research Laboratory slide. One drop of the 1% antigen suspension was added through a 23-gauge, thin-walled needle calibrated to deliver 80 drops/ml. The slide containing antigen and sera was rotated continuously at room temperature at 90 rev/min on a laboratory rotator.

The slides were read through a dissecting microscope at 15× magnification against a dark background and illuminated with an indirect light source. All testing was carried out in a double-blind manner.

The results were classified on the following basis: nonreactive (NR), no change in particle size from negative control; 1+, apparent increase in particle size, but no clumping; 2+, increase in particle size, plus a few small clumps; 3+, formation of floccules, plus smaller clumps; 4+, all particles gathered together into floccules.

A summary of the test results is given in Table 1. Sera that were read as NR or 1+ were interpreted as serologically nonreactive; those that were graded 2+, 3+, or 4+ were considered serologically reactive. Of the designated "positive" females, 86% were serologically reactive; of the designated "positive" males, 68.6% were serologically reactive; and of the presumed "negative" females and males, 12.0% and 11.3%, respectively, were serologically reactive.

The flocculation reactions with sera from fe-
TABLE 1. Results of flocculation test, using "B" antigen

<table>
<thead>
<tr>
<th>Clinical category</th>
<th>Serological results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td>&quot;Positive&quot;</td>
<td></td>
</tr>
<tr>
<td>54 male</td>
<td>68.6</td>
</tr>
<tr>
<td>50 female</td>
<td>86.0</td>
</tr>
<tr>
<td>&quot;Negative&quot;</td>
<td></td>
</tr>
<tr>
<td>53 male</td>
<td>11.3</td>
</tr>
<tr>
<td>50 female</td>
<td>12.0</td>
</tr>
</tbody>
</table>

* Per cent reactive.

males were very well-defined; all reactive sera were 4+, except for two in the 3+ category. The sera from males, however, were less reactive; of the 37 reactive sera, 10 gave reactions of 2+, and five gave reactions of 3+. Although many serological test systems have been advocated, none have come into routine use, apparently because they lacked sensitivity, specificity, or both. Recently, we reported the isolation of the "A" antigen (3) from gonococcal protoplasm, which appears to be important in the complement-fixation procedure when tested with human sera (2), whereas the sediment antigen, now reported as the "B" antigen, appears to be important in a flocculation procedure. The high detection rate (86%) of infected females, together with the ease of test performance, suggests that a technique utilizing this antigen might be used as a screening test for inapparent gonococcal infections; those patients giving positive reactions could be further evaluated to determine actual presence of infection.

At present, work is underway to study the specificity of the antigen in greater detail, to further evaluate and standardize the test procedure, and to assess the stability of the antigen preparation. It is hoped that by use of this and other refined antigens a better understanding of the human immune response to gonococcal infections may be obtained.

LITERATURE CITED