Isolation of an Antigen of *Neisseria gonorrhoeae* Involved in the Human Immune Response to Gonococcal Infection

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Ion-exchange chromatography was used to isolate a fraction from disrupted gonococci which reacts with sera from patients with gonococcal disease in complement-fixation and gel-diffusion tests. This antigenic fraction was shown to be the same as that previously described as having been isolated by gel filuration. The method reported here has the advantage of greater rapidity of isolation together with some improvement in purity.

The sensitive and specific serological detection of bacterial infections is largely dependent on the use of a suitable antigen in the test system.

Recently, we reported the identification and isolation of a soluble antigen from the disrupted *Neisseria gonorrhoeae*, which reacts with some human sera from patients with a gonococcal (GC) infection (1). This antigen, which we now designate the "A" antigen, has a high molecular weight and is heat-labile. It was originally isolated and characterized by gel filtration of soluble GC material (hereafter referred to as protoplasm) through Sephadex G-200. Complement fixation (CF) tests with this antigen gave promising results in detecting antibodies in patients with a GC infection; therefore, a more rapid way was sought to isolate this antigen in larger quantities.

This paper reports a rapid method of isolating the "A" antigen from protoplasm by using ion-exchange chromatography. By using human and rabbit antisera, the serological reactivity of various fractions was assessed by agar-gel diffusion and CF.

**MATERIALS AND METHODS**

The *N. gonorrhoeae* isolate and culture technique, the preparation of hyperimmune rabbit serum, and the agar-gel diffusion and Sephadex gel filtration techniques were all performed as previously described (1).

Preparation of the protoplasm has been described (2). Briefly, it was prepared by fracturing the cell walls with the Ribi cell fractionator and by separating the insoluble particulate matter (cell walls) from the soluble material (protoplasm) by centrifugation. The protoplasm was lyophilized.

Diethylaminoethyl (DEAE) Sephadex A-25 was swelled, washed, and equilibrated with 0.01 M phosphate buffer (pH 6.5) as suggested by the manufacturer (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) Lyophilized protoplasm was dissolved (40 mg/ml) in 1.0 M NaCl 0.01 M phosphate buffer (pH 6.5). After centrifugation at 37,500 × g for 30 min, the dissolved material was exhaustively dialyzed against 0.05 M NaCl-0.01 M phosphate buffer (pH 6.5). Before application to the ion-exchange column, the material was recentrifuged as before. If this solution and dialysis procedure was not performed, the addition of buffers of increasing molarity to the ion-exchange column caused some precipitation of the material on the column so that later washing of the gel became difficult.

After thorough equilibration and washing of the ion-exchange gel with 0.05 M NaCl-0.01 M phosphate buffer (pH 6.5), 200 mg of the material was applied to the top of a downward-flow column (2.5 by 20 cm). The eluate was monitored at 280 nm with an ultraviolet analyzer and recorder and was collected on an automatic fraction collector. After addition of an eluting buffer, the monitor was observed until a definite peak was observed, followed by a return to the base line. The appropriate tubes were marked, the next buffer was added, and the process was repeated. The NaCl molarity of the buffers in the order added was 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0; each was buffered with 0.01 M phosphate (pH 6.5). The various fractions were concentrated, dialyzed against distilled water, and lyophilized.

Human sera from females aged 24 to 60 were designated "normal" on the basis of no history of previous infection with pathogenic *Neisseria*. The

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human sera, which were designated "immune pool" and which were used for analysis of fractions to determine the location of the "A" antigen in gel-diffusion tests and in the CF tests, were selected from female patients who had culturally positive GC infections at the time the sera were drawn. These sera were selected on the basis of a CF titer of 1:16, with *N. gonorrhoeae* sonicate as antigen.

The Laboratory Branch complement fixation test was used to analyze the various antigenic fractions for their reactivity with sera from patients with GC infections. The method, as previously described (1), consisted of determining the optimal antigen dilution (OAD) of each antigen fraction by using "checkerboard" titrations against the immune pool described above. All antigen fractions were dissolved from the lyophilized state into Veronal-buffered serum at diluent for their reactivity with "checkerboard" titrations.

The highest titer of serum or the human immune pool was used throughout and since all antigen fractions were titered beginning with an initial solution of 1 mg/ml, comparison can be made regarding the relative reactivity of the various fractions. No fractions reacted with normal human sera, and only the fractions eluted at 0.4 M and 0.6 M possessed any CF reactivity with the immune pool (Table 1). These are the same fractions that reacted in the agar-gel-diffusion tests. The 1:64 OAD of the protoplasm as opposed to the 1:32 OAD of the "A" antigen (0.4-M eluate) suggests that material is present in the protoplasm which is lost or modified during ion-exchange chromatography. However, we have been unable to identify any other reactive fractions even though the column was eluted up to 2 M NaCl solution.

**RESULTS**

**Ion-exchange chromatography.** The elution pattern from DEAE-Sephadex A-25 of the protoplasm from F62 type 1 GC isolate is shown in Fig. 1. The "A" antigen eluted predominantly in the 0.4 M peak, but traces also occurred in the 0.6 M peak. Although the shape of the eluting curves varied slightly with different isolates, the antigen(s) always appeared in the same peak(s).

**Agar-gel diffusion.** Fractions containing "A" antigen from both ion-exchange and Sephadex G-200 gel filtration were reacted against hyperimmune rabbit serum and the immune human pool. When the human immune pool was reacted with the DEAE fractions eluted at 0.05 through 1.0 M NaCl, a strong precipitin line was observed with the 0.4 M eluate and a very faint line with the 0.6 M eluate. The line observed with the 0.4-M eluate gave a reaction of complete identity with the line formed against the 0.6-M eluate.

A line of complete identity was also formed between the 0.4-m-eluate and the fraction containing "A" antigen from the Sephadex G-200 gel filtration when reacted against hyperimmune rabbit serum or the human immune pool. Furthermore, the reactions with hyperimmune rabbit serum showed that the 0.4-M eluate from ion-exchange chromatography contained fewer contaminating antigens than the fraction isolated by Sephadex G-200 gel filtration.

**Complement fixation tests.** The analysis of the ion-exchange column fractions by CF is expressed in terms of the OAD of each fraction. Since the same human immune serum pool was used throughout and since all antigen fractions were titered beginning with an initial solution of 1 mg/ml, comparison can be made regarding the relative reactivity of the various fractions. No fractions reacted with normal human sera, and only the fractions eluted at 0.4 M and 0.6 M possessed any CF reactivity with the immune pool (Table 1). These are the same fractions that reacted in the agar-gel-diffusion tests. The 1:64 OAD of the protoplasm as opposed to the 1:32 OAD of the "A" antigen (0.4-M eluate) suggests that material is present in the protoplasm which is lost or modified during ion-exchange chromatography. However, we have been unable to identify any other reactive fractions even though the column was eluted up to 2 M NaCl solution.

**DISCUSSION**

Recently, we reported the isolation of a soluble antigen, now designated the "A" antigen, from the products of GC cellular disruption. This antigen could be prepared in small amounts by Sephadex gel filtration, and further work showed that this antigen reacted well in the CF test with infected human sera. In an attempt to produce this antigen more rapidly and in a purer form, evaluation of isolation by ion-exchange chromatography was undertaken. As shown in this paper,

**TABLE 1. CF analysis of the fractions eluted from DEAE-Sephadex (pH 6.5) by the use of a human immune pool***

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Optimal antigen dilution</th>
<th>Serum titer at that dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protoplasm</td>
<td>1:64</td>
<td>1:64</td>
</tr>
<tr>
<td>Ion-exchange fractions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05 M NaCl</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>0.1 M NaCl</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>0.2 M NaCl</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>0.4 M NaCl</td>
<td>1:32</td>
<td>1:32</td>
</tr>
<tr>
<td>0.6 M NaCl</td>
<td>1:32</td>
<td>1:32</td>
</tr>
<tr>
<td>0.8 M NaCl</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>1.0 M NaCl</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

* Normal human sera were nonreactive against each fraction.

**Fig. 1. Elution pattern from DEAE-Sephadex A-25 of F62 type 1 GC protoplasm; 0.01 M phosphate buffer (pH 6.5). Molarity = moles of NaCl.**
the antigen so isolated appears to be identical to that produced by Sephadex gel filtration and is purer, by the criteria of agar-gel diffusion. A 100-mg amount of protoplasm could be filtered during a typical 3-day run on Sephadex G-200, but 10 times as much material could be isolated in 6 hr from DEAE-Sephadex by utilizing a slightly larger column.

The "A" antigen accounts for much of the CF activity observed in whole protoplasm when titrated with infected human sera. Purification of this antigen in large amounts for further study does not necessitate the complete elution pattern described. Since only the 0.4-M peak is of interest, the material may be applied to the column in a 0.2 M buffer, and the 0.2-M peak may be eluted and discarded. Elution with a 0.4-M buffer then brings down the "A" antigen.

Because the protoplasm gave a higher OAD than did the "A" antigen, using the same immune human pool, the presence of other important antigenic fractions is suggested. Thus far, however, we have not located this additional antigenic activity.

Although we apparently have isolated the same antigen by two methods (i.e., gel filtration and ion-exchange chromatography), we cannot as yet conclude that the antigen giving the gel-precipitin line and the antigen reacting in the CF system are identical. Further purification of the material is needed before such a conclusion can be drawn.

LITERATURE CITED
