Reactivity of Two Selected Antigens of Neisseria gonorrhoeae

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Two antigen preparations, the soluble antigen and a fraction 1 thereof, isolated in the course of a systematic study of the various antigens of the virulent gonococcus, have been investigated for their ability to serve as antigens for the detection of antibody in patients infected with the gonococcus. The soluble antigen was reactive with 88.2% of the sera from infected females, and fraction 1 was reactive with 71.6% of the sera. Of sera from infected males, only 27.6% reacted with the soluble antigen and only 20.4% with fraction 1. Of sera from individuals presumed free of gonococcal infection, approximately 4% reacted with the soluble antigen; none reacted with fraction 1. This study suggests that these antigens might be adaptable to the detection of human gonococcal antibody, especially in the female.

The systematic study of the various antigens of the gonococcus was undertaken before attempting to develop a serologic test for gonococcal infection. We decided to use complement fixation as one of several research tools to detect immune response against various antigens. This technique can be used to detect immune reactions when other manifestations of antigen-antibody combination, such as precipitation or agglutination, are absent. Complement fixation is particularly valuable in comparing a variety of antigens since either soluble or insoluble antigens may be used (5). The intent in using the complement-fixation procedure was to gain antigen reactivity data which might be used in future work to adapt antigens to serological procedures less cumbersome than the complement-fixation test.

In a previous paper, the isolation of a fraction 1 antigen (F1) from gonococcal soluble antigen (SA) was reported (1). The present paper reports the use of SA and F1 to determine the reactivity of these two antigenic preparations in the human immune response to gonococcal infections.

MATERIALS AND METHODS

Source of sera. All gonococcal patient sera were obtained from a local venereal disease clinic. Blood (10 ml) was drawn from patients who fulfilled the following criteria: Males were diagnosed as having gonococcal infection if they had a typical history of urethritis with dysuria and purulent discharge with typical oxidase-positive colonies of gram-negative diplococci cultured on Thayer-Martin medium (6). Females were diagnosed as having gonococcal infection if cervical cultures on Thayer-Martin medium revealed the presence of typical oxidase-positive colonies of gram-negative diplococci. All cultures were incubated for 18 hr at 37°C with CO2 enrichment.

Blood donors were selected from a group presumed free of gonococcal infection. Sera were procured from 50 males and 58 females between the ages of 18 and 60 years.

Antigen preparation. A strain of virulent Neisseria gonorrhoeae, designated F62 of colonial morphology type 1 (2), was used for the SA and F1 preparation. The bacteria were grown on GC Medium Base (Difco) plus a defined supplement (2). The cultured organisms were harvested into distilled water and passed through a Ribi cell fractionator as previously described (4). The SA was separated from the insoluble cell wall material by centrifugation at 36,000 × g for 30 min, and the SA was lyophilized. The preparation of the F1 is reported in detail elsewhere (1). Briefly, the preparation involves passage of gonococcal SA through Sephadex G-200 followed by collection and concentration of the various fractions. The first half of the first peak eluted had been shown to react with sera from certain patients with gonococcal infection. This antigenic fraction has been designated the F1.

Complement-fixation test. The Laboratory Branch Complement-Fixation test was used throughout this study (7). The antigens were dissolved and diluted in Veronal buffered diluent. Diluted antigens were titrated for the complement-fixation test by initially using goat antiserum against the organism causing gonorrhea. As soon as large quantities of high-titer (1:32 or higher)
human antigonococcal sera were accumulated, pools were used for antigen titrations. The end point of human-serum titrations was taken as the highest dilution showing 30% or less hemolysis.

RESULTS

None of the 106 sera from the blood donor group reacted with the F1. Two of the male sera (4%) and two of the female sera (3.3%) reacted with the SA at titers from 1:2 to 1:8. These reactions are shown in Fig. 1.

The test results of the sera from 98 males and 102 females with gonococcal infection are shown in Fig. 2 and 3. Of the 98 samples of sera from male patients, 27 or 27.6% reacted in the complement-fixation test at a titer of 1:2 or greater with the SA, and 20 or 20.4% reacted with the F1. F1 reactors gave the same or higher titer with the SA. The reactivity of the sera from female patients was higher with both antigens. Ninety of the 102 (88.2%) reacted with the SA, and 73 (71.6%) reacted with the F1 (Fig. 3). All those that reacted with the F1 also reacted with the SA.

DISCUSSION

In a preliminary study of the antigens important in human response to a gonococcal infection we have studied a serological reactivity of gonococcal SA and an extract of the SA. With the sera from the group presumed free of gonococcal infection, there was a small percentage of reactivity at low dilutions with the SA only. These reactions may be due to a cross-reaction with antibodies from other members of the Neisseria genus or other organisms outside the genus. There is also a possibility that reactions are due to an inapparent infection or a previously treated infection with the antibody titer near the end of its decline. Whatever the reason, the number of reactions is small.

The percentage of female or male sera, or both, that reacted with the SA was greater than the percentage that reacted with F1. Approximately one half of those that reacted with SA, but not with F1, did so only at a 1:2 titer; whereas in the remainder, the reactivity occurred at higher dilutions. Although reactivity at a 1:2 dilution may represent experimental error, it may also be due to cross-reactions with antibodies against other organisms; or it may be due to reactivity with antigens other than the F1.

There are several possibilities for the wide discrepancy in the serological detection rate between males and females. First, it may be related to the duration of infection. Since infected males as a group are more symptomatic, they tend to seek treatment earlier. Thus, their decreased exposure time to the gonococcus may account for their lower detectable immune response. Second, infection may be handled differently by the immune mechanism in the two sexes. In the infected male there is generally a purulent urethral discharge, whereas in the asymptomatic infected female the infection usually resides in the cervix. Third, the males may give a greater response against an antigen not
now in our present fractions, or they may respond with an antibody that is not complement fixing, such as IgA.

Even though the complement-fixation test is used only as a research tool in this study, the results can be compared to other reports in the literature (3). The male detection rates were 10%, 50%, 17%, and 21%; and the female detection rates were 26%, 41%, 37%, and 50%. In the present study the male detection rates of 20.4% with F1 and 27.6% with SA, and female detection rates of 71.5% with F1 and 88.2% with SA give a favorable impression of the ability of these antigens to detect gonococcal antibody.

Both the gonococcal SA and F1, thereof, would be very useful antigens to employ in the development of a convenient serologic procedure for the detection of gonococcal antibodies in the case of the asymptomatic female. The SA would have a slight edge over the F1 by showing more reactors. This would be done with the awareness that reactors to the SA might be caused by antibodies to organisms with somewhat similar antigenic makeup. Thus, such reactions would indicate a closer look at the patient. From the detection rates with males, it is evident that if the present antigen cannot be altered to increase the number of reactors, another antigen or other antigens should be sought which would bring this about. With the knowl-

edge that IgA is not fixed by complement, a specific test such as an immunofluorescent procedure using anti-IgA should be investigated to see if the number of infected male reactors could be increased over that obtained with the complement-fixation procedure.

LITERATURE CITED