

Serum Antibody Response to the 70,000-Molecular-Weight Neisserial Common Antigen in Humans Infected by *Neisseria gonorrhoeae*

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We evaluated the presence of antibodies directed against a 70,000-molecular-weight (70K) common neisserial antigen in sera from patients with first or repeated gonococcal infections and in sera from healthy controls. Sera were taken as soon as possible after the onset of disease, and anti-70K antibodies were detected by Western blot (immunoblot). Results show that significantly fewer patients with gonococcal infection possessed anti-70K antibodies than controls ($P < 0.001$). This suggests a possible role of anti-70K antibodies in natural immunity against *Neisseria gonorrhoeae*.

Winstanley et al. (15) have shown that, compared with healthy controls, sera from patients with gonococcal infection lack cross-reacting bactericidal antibodies for groupable *Neisseria meningitidis*. Although they did not show a fall in bactericidal antibody titers, they postulated that gonococci had absorbed cross-reacting antibodies during the course of the infection. Inversely, we made the hypothesis that preexisting cross-reacting bactericidal antibodies, elicited during natural immunization by other *Neisseria* spp., could confer a certain level of protection against *Neisseria gonorrhoeae* (5).

Mice immunized with live meningococci developed bactericidal antibodies for gonococci (6). When tested on Western blots, the immune sera of these mice reacted preferentially with a 70,000-molecular-weight (70K) antigen of *N. gonorrhoeae* common to 19 out of 19 tested gonococci, 52 out of 53 meningococci, and most nonpathogenic *Neisseria* spp. (1).

Moreover, 70K-specific mice sera were bactericidal for *N. gonorrhoeae* (10). This 70K neisserial antigen is a surface-exposed protein which is antigenically stable after passages in vivo in humans (9) and is immunogenic in humans during the course of natural meningococcal infection and carriage (1). Therefore, we investigated the frequency of the presence or absence of anti-70K antibodies in patients with first or repeated gonococcal infections compared with healthy controls.

Patients and healthy controls. Male patients attending the Venereal Diseases Ward at the Hospital Saint-Louis (Paris, France) for acute urethritis were examined by one of us (P.M.), and a sample of urethral pus was taken. None of the patients had disseminated gonococcal infection or symptoms of immunosuppression. Gonorrhea was diagnosed by direct examination of a Gram-stained smear and culture. The presence of *N. gonorrhoeae* was confirmed by oxidase testing and sugar oxidation. When positive, patients were bled within 7 days (mean, 3 days), and sera were separated and labeled, sent to our laboratory, kept at -20°C , and tested. The information permitting us to classify patients in groups was obtained from clinicians at the end of the survey. Patients who acknowledged no previous history of venereal

disease were considered to have primary gonococcal infections, and only people treated for gonococcal infections in the same hospital ward at least 3 months before were classified as having repeat infections. Healthy controls had no previous history of venereal disease, were chosen from individuals attending the hospital for a systematic pre-nuptial syphilis screening, and were negative for antitreponemal antibodies. All three groups had similar socioeconomic status and were comparable in age.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blots. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blots (immunoblots) were performed as described elsewhere (1). Briefly, gonococci (OD660:1.5) (our reference strain T2) (10) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 12.5% polyacrylamide gels and Western blotted onto nitrocellulose foils (BA 85, 0.45 μm ; Schleicher and Schuell, Dassel, Federal Republic of Germany). Nitrocellulose strips were incubated overnight in sera diluted 1/50 in phosphate-buffered saline, pH 7.4. A 70K-reacting mouse polyclonal serum (10) was used as a control to visualize the migration of the 70K antigen. All procedures for saturation, washing, and immunoenzymatic revelation with anti-human immunoglobulins G, A, and M (Biosys, Compiègne, France) were done as described previously (1). Sera which strongly reacted in the 70K zone were considered positive. Sera which did not react or which gave a very light reaction in that zone and an intense reaction for at least one other major antigen, such as H.8 or the one below 15K (Fig. 1, lane 2), were classified as negative.

As seen in Table 1, all but one serum from healthy controls possessed antibodies against the 70K neisserial common antigen, whereas only 65% and 62% of sera from patients with first or repeated gonococcal infections, respectively, had such antibodies ($P < 0.001$, chi-square test).

Figure 1 shows typical Western blots obtained with sera from patients with first (lane 2) and repeated (lane 3) gonorrheal infections and from a control (lane 4). All sera exhibited antibodies against at least one or several other major gonococcal antigens, such as pili (Fig. 1, a), H.8 antigen (Fig. 1, b), and major outer membrane protein 1 (Fig. 1, c),

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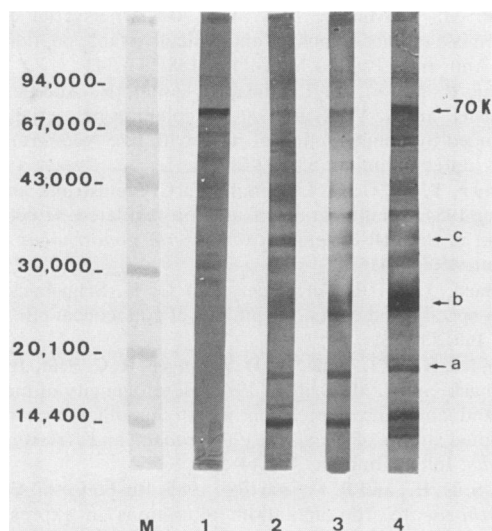


FIG. 1. Western blot analysis of human antibody response against the 70K neisserial common antigen during the course of gonococcal infection. Whole gonococci were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 12.5% polyacrylamide gels and transferred to nitrocellulose foils. The strips were incubated with mouse anti-meningococcal immune serum (lane 1) strongly reactive with the 70K common antigen, with human sera from first (lane 2) or repeated (lane 3) gonococcal infection, or with a control serum (lane 4) from a healthy individual. All but one serum (32 out of 33) from controls strongly reacted in the 70K zone, and 27 out of 40 and 18 out of 29 sera from first and repeated gonococcal infections, respectively, reacted with the 70K antigen. Lane M, Molecular weight markers. Letters to the right of lane 4 indicate positions of bands made by pili (a), H.8 antigen (b), and major outer membrane protein 1 (c).

as well as against several other minor antigens. The frequency of antibodies against the H.8 antigen was not significantly different between each group.

In an earlier paper (1), we showed that meningococcal carriage most often induces anti-70K antibodies in children. We show in the present work that people with first or repeated gonococcal infections frequently lack anti-70K antibodies compared with healthy individuals who have never experienced a gonococcal infection. The high frequency of antibodies against *N. gonorrhoeae* in young adults is likely due to childhood immunization by *N. meningitidis*, a closely related bacterium.

Recently, several antigens, or epitopes, common to most pathogenic *Neisseria* species have been described, such as the H.8 antigen (4), lipopolysaccharide (2, 13), pili (12), immunoglobulin A1 protease (8), and iron-regulated proteins

TABLE 1. Presence of antibodies against the 70K common neisserial antigen in acute sera from male patients with first or repeated gonorrhoeal infections as detected by Western blot

Patients	Total	No. (%) with anti-70K antibodies	Age (yr) \pm SD
First gonorrhoeal infection	40	27 (67.5) ^a	27 \pm 9.3
Control	33	32 (97)	31 \pm 4.9
Repeated gonorrhoeal infection	29	18 (62) ^b	33 \pm 11.2

^a $P < 0.01$, chi-square test.

^b $P < 0.001$, chi-square test.

of 37,000 (11) and 70,000 (14) molecular weight. An antigen common to all pathogenic *Neisseria* strains is of obvious interest for immunoprophylaxis. But the recovery of pre-dating antibodies against such a common antigen would indicate its possible lack of efficiency as a vaccine. Sera from patients who lack antibodies against the 70K antigen reacted with one or several other major antigens, such as H.8, pili, or major outer membrane protein 1. Our belief is that during nasopharyngeal carriage of other *Neisseria* species (peculiarly meningococci) during youth, antibodies elicited against cross-reacting antigens, such as H.8, pili, and protein 1, are more abundant or persist longer (or both) than anti-70K antibodies.

In the present survey, although the difference between patients and controls is statistically highly significant, anti-70K antibodies were recovered in about 60% of patients with gonorrhoea. This could be due to (i) a partial participation of anti-70K antibodies in immunity against gonorrhoea, with other factors permitting the bacteria to develop, (ii) detectable but insufficient titer of anti-70K antibodies, (iii) a transient antigen-specific suppression in acutely infected patients, or (iv) the fact that antibodies were detected in sera taken 2 to 7 days after the onset of the disease and could correspond to a booster effect with insufficient antibody titers when bacteria entered into the host. Testing the sera of these patients prior to their infections would have clarified the last two points. Western blotting allows direct visualization of the antigen to which antibodies are directed but is not quantitative for such antibodies nor does it give indications about their function. A direct assay using purified 70K antigen will greatly help to quantify anti-70K antibodies and better understand their function. This will be a crucial point to elucidate in the future. Moreover, the presence, isotype, and function of anti-70K antibodies on genital mucosal surfaces should be investigated.

However, the significant association (though not necessarily the causality) of first or repeated gonococcal infections with a lower frequency of anti-70K antibodies merits consideration, particularly because the 70K common antigen has been shown to be expressed on the surface of the gonococcus and is antigenically stable after passage *in vivo* in humans. Hicks et al. (7) recently showed that anti-H.8 antibodies were present in sera from several individuals before the onset of gonorrhoea. No difference with controls was seen. Unfortunately, their work did not deal with the 70K antigen described by West and Sparling (14) and us (9, 10), so caution must be used in comparing their results with ours.

The results obtained in humans infected with *N. gonorrhoeae* support the possible role of antibodies against the 70K common neisserial antigen in protection against gonorrhoea. Further investigations into the presence, isotype, and role of antibodies on genital mucosal surfaces will help us to better understand this aspect of natural defenses against gonococcal infection.

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