Resistance to Gonorrhea Possibly Mediated by Bacterial Interference

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Two men with no prior history of urethritis failed to develop gonorrhea after sexual exposures to women with genital gonococcal infection. Usual methods of prophylaxis, such as antibiotics or condoms, were not employed. The aerobic bacterial flora of these men’s urethras consisted of several bacteria, some of which inhibited the in vitro growth of *Neisseria gonorrhoeae*. The hypothesis is suggested that bacterial interference may have played a role in protecting these men from gonorrhea.

It is well established that not all men experimentally or sexually exposed to *Neisseria gonorrhoeae* develop gonorrhea (1, 3, 5). The following two case reports contain a possible explanation for this variability in the transmission of gonorrhea.

MATERIALS AND METHODS

Case reports. A. L., a 35-year-old white male, was referred to our venereal disease clinic after epidemiological investigations of Y. T., a 25-year-old white female found to have cervical gonorrhea in routine screening at a family planning clinic. Two other sexual contacts of Y. T. were diagnosed as having gonorrhea. A. L. and Y. T. had been having sexual relations three to four times a week for 4 months, and this continued for the 2-week interval between the time Y. T. was cultured and the time she came to the venereal disease clinic. A cervical culture in our clinic the day of treatment confirmed the diagnosis of gonorrhea. A. L. denied dysuria or urethral discharge, and physical examination showed no urethral exudate before or after penile stripping and prostatic massage. He also denied any prior venereal diseases (non-specific urethritis, herpes, syphilis, chancroid, granuloma inguinale, lymphogranuloma venereum, pediculosis pubis, and condyloma acuminata) or the use of condoms or systemic antibiotics. Identical histories were elicited by the examining physician (N. E.) and a venereal disease epidemiologist.

H. T., a 20-year-old negro male, came to our clinic concerned about *N. gonorrhoeae* infection after his regular sexual partner, S. L., was diagnosed as having gonorrhea on the basis of a positive cervical culture. S. L. had two other male contacts both of whom developed gonorrhea. One of these men denied sexual contact with anyone else during the prior 6 months. H. T. and S. L. had been having sexual relations five to six times a week for 6 weeks continuing until the day of S. L.’s treatment. The length of time S. L. was infected was indeterminable, but 10 days had intervened between the time S. L. was cultured and the time she was brought into the clinic, notified of the infection, and treated. H. T. denied the use of condoms, antibiotics, or other prophylactic procedures. He also denied any prior venereal disease. A venereal disease epidemiologist obtained an identical history from H. T. Physical examination of H. T. revealed no evidence of urethritis even after penile stripping and prostatic massage.

Y. T. and S. L. were treated with aqueous procaine penicillin G (4,800,000 units) and probenecid (1 g). A. L. and H. T. were not treated and resumed sexual relations with Y. T. and S. L. within a week of the latter’s treatment. Y. T. and S. L. were recalled to the clinic 7 days after therapy for posttreatment evaluation.

Routine cultures for *N. gonorrhoeae* from Y. T.’s and S. L.’s cervixes and from A. L.’s and H. T.’s urethras were plated on selective media, incubated at 36 C in a candle extinction jar, and examined for growth after 24 and 48 h. Gonococci were identified on the basis of colony morphology, positive oxidase reaction, Gram stain, and the fermentation of dextrose but not maltose, lactose, or sucrose. The isolate from A. L. was also studied with a *N. gonorrhoeae* fluorescent antibody conjugate.

The first 20 ml of A. L.’s and H. T.’s voided urine was filtered through a 0.45-μm cellulose acetate filter; the filter paper was placed on GC Medium Base supplemented with Isovitalex (GCB medium) and incubated at 36 C for 48 h. This technique can detect to 5 colony-forming units of *N. gonorrhoeae* in 10 ml of urine, and the medium not only supports the growth of *N. gonorrhoeae* but also that of many aerobic bacteria found in the urethra.

Bacteria isolated on the GCB medium were tested for bacterial interference against virulent colony type I *N. gonorrhoeae* (4). A modification of Counts’
technique for bacteriocin assay was used (2). This consisted of swabbing a single linear streak of the bacteria to be studied across a plate of GCB medium. The plate was incubated at 36°C in a candle extinction jar for 48 h. The linear streak of confluent bacterial growth was then removed with sterile swabs, with care being taken not to disturb the agar surface. The cleaned plates were exposed to chloroform vapors for 24 h and then airdried in a laminar flow hood for 1 h.

The plates were then cross-streaked with CDC (Center for Disease Control) strain 2686 colony type I N. gonorrhoeae, replaced in the candle extinction jar, reincubated for 24 h, and then checked for the pattern of gonococcal growth.

Absence of gonococcal inhibition was evidenced by growth of gonococci over the entire plate. Inhibition produced a pattern of gonococcal growth at the periphery of the plate and absence of growth in the area of the linear streak. To exclude the possibility that the gonococcal inhibition was due to depletion from the medium of a substrate needed for gonococcal growth, the two bacteria producing inhibition were further tested in an identical manner, but a thin layer of GCB medium was layered over the original medium before it was crossed streaked with N. gonorrhoeae. Inhibition of N. gonorrhoeae was measured as before.

The cultures producing gonococcal inhibition were submitted to the Special Bacteriology Unit, Bacteriology Branch, CDC, for identification. Additional N. gonorrhoeae and Staphylococcus epidermidis strains were obtained from the genital area of patients attending Georgia’s DeKalb County venereal disease clinic.

RESULTS

Routine selective media cultures revealed N. gonorrhoeae from the cervices of Y. T. and S. L., and posttreatment cultures were negative for N. gonorrhoeae. Similar cultures from the urethras of A. L. and H. T. were negative for N. gonorrhoeae. The gonococcus from S. L. was lost during in vitro passage, but the isolate from Y. T. (Y. T. gonococcus [GC]) was available for additional studies.

GCB cultures of urine from A. L. were also negative for N. gonorrhoeae but did reveal two isolates which were tested for gonococcal inhibition. One of these showed no inhibition, but the other inhibited gonococci (strain 2686 and Y. T. GC) in each of the two assay techniques. GCB cultures from H. T. were also negative for N. gonorrhoeae but yielded growth of a single species which inhibited N. gonorrhoeae (strain 2686 and Y. T. GC) in the assays. Both gonococcal inhibitors were identified as S. epidermidis, and the zones of inhibition extended 10 to 15 mm beyond the margin where the first bacteria had been grown (Fig. 1).

The sequence of bacterial growth on GCB medium was reversed. N. gonorrhoeae (strain 2686 and Y. T. GC) was initially grown in a linear streak on the medium, and after chloroform treatment the plates were cross-streaked with the S. epidermidis from A. L. and H. T. There was no inhibition of the staphylococcus in the area of prior N. gonorrhoeae cultivation.

Eight randomly selected strains of S. epidermidis and 74 N. gonorrhoeae clinical isolates were tested in the inhibition system. All 74 strains of N. gonorrhoeae were inhibited by the staphylococcus from A. L. and H. T. Four of the eight strains of S. epidermidis inhibited CDC strain 2686, Y. T. GC, and the 74 other strains of N. gonorrhoeae; the other four S. epidermidis did not inhibit any of the 76 N. gonorrhoeae strains.

The anterior urethras of 75 men without venereal disease were cultured on GBC medium, and all bacterial isolates were tested for gonococcal inhibition. Three hundred and eleven isolates were obtained, and 18% of these inhibited the gonococcus. S. epidermidis accounted for 70% of the inhibitors, i.e., 39 of the 75 men harbored gonococcus-inhibiting S. epidermidis in their urethra.

DISCUSSION

A statistical study by Holmes et al. (3) indicated that a male has a 22% chance of contacting gonorrhea from a single sexual exposure with an infected female. Pariser et al. (5), in a clinical study, reported that of 115 males exposed to N. gonorrhoeae-infected females, 17 had a symptomatic infection. Of the remaining
98, 26 had positive urine or prostatic secretion cultures, or both, for *N. gonorrhoeae*. This is an infection rate of 37.4%.

Working with human volunteers, Brown et al. (1) directly inoculated male urethras with either a swab containing cervical secretions or material from a 24- to 48-h-old *N. gonorrhoeae* culture originally taken from infected females. Cervical secretions infected five out of nine volunteers, and the cultured gonococci infected 10 of 12 men. These studies indicate that not every male exposed to *N. gonorrhoeae* sexually or experimentally will become infected. The mechanism(s) for this resistance to infection is not understood. Natural immunity resulting from a prior gonococci infection may be one reason. Another possible explanation is resistance conferred by the normal urethral bacterial flora which inhibit the growth of the gonococcus.

The two male patients had repeated sexual exposures with women known to have gonorrhea, but they were resistant to infection. Their total number of exposures is difficult to determine since the onset of the infection in the females is impossible to document; however, sexual relations occurred for 10 days (H. T.) to 2 weeks (A. L.) between the time of the initial gonococcal cultures and the time the women were notified of the problem and referred to the clinic for treatment. H. T. was therefore resistant to gonococcal infection after at least seven exposures, and A. L. resisted at least eight exposures. If one assumes a 78% chance of escaping gonorrhea after a single exposure to an infected female (3), one would expect escape after seven exposures to occur 17.6% of the time and escape after eight exposures to occur 13.7% of the time (Binomial Test).

Acquired immunity from a previous infection appears to be an unlikely explanation for the resistance of A. L. and H. T. to gonorrhea, because a thorough history failed to elicit prior signs or symptoms suggestive of a gonococcal infection. Other explanations, such as the use of condoms or systemic antibiotics, were similarly excluded. *S. epidermidis* isolated from each contact's urethra was capable of inhibiting *N. gonorrhoeae* in vitro. If it does so in vivo, these men may have been protected from infection with *N. gonorrhoeae* by bacteria in their urethral flora. A prospective study will be needed to determine the overall clinical significance of this bacterial interference.

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