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Genital Inoculation of Male Baboons with *Neisseria gonorrhoeae*

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Eight male baboons inoculated intraurethrally with *Neisseria gonorrhoeae* failed to shed gonococci or develop serum antibody. Urethral inoculation, preceded by epididymal inoculation, elicited an anamnestic antibody response.

Recently, gonococcal urethritis in great apes was produced by intraurethral inoculation of male chimpanzees (*Pan troglodytes*) (9). The infection was sexually transmitted by male chimpanzees to female cagemates, causing asymptomatic cervical infection (3). This chimpanzee model, though, has several disadvantages. Chimpanzees are expensive to procure and maintain, requiring elaborate caging facilities and extensive spatial requirements not readily available in most nonhuman-primate colonies. They present a considerable public health hazard and may be an endangered species. A more suitable simian model is needed and, if found, would facilitate studies of the pathogenesis, virulence, and immunogenicity of gonococcal infection as well as provide a system for vaccine evaluation. Hill (7) reviewed the literature on animal inoculations with *N. gonorrhoeae* up to the early 1940s. Although few in number, all attempts to infect the genital tract of both sexes of nonhuman primates (species largely unidentified) were unsuccessful. Twenty-five years later, Dodin (6) reported urethritis in a prosimian (*Lemur macaco*), produced by inoculating the epididymis with exudate from a male patient with gonorrhea. Although the urethral discharge contained polymorphonuclear leukocytes with intracellular diplococci, infection was apparently not confirmed by reisolation of *N. gonorrhoeae* from the discharge. In an attempt to find a suitable simian that could be more universally employed as a model, gonococcal inoculation of the baboon was undertaken.

Eight male baboons (*Papio cynocephalus*), weighing 9 to 27 kg, were utilized. The baboons, inoculated in pairs, received from one to four inoculations. A total of eight inoculations was performed. The inocula consisted of: (i) fresh urethral discharge from a male patient with gonorrhea; (ii) fresh, pooled urethral discharge from five male patients with gonorrhea; (iii and iv) first-passage type 1 gonococcal colonies from the urethra of a male patient (performed twice with different patients); (v) first-passage pooled type 1 gonococcal colonies from the urethra of three male patients; (vi) a laboratory strain of *N. gonorrhoeae*, which had been repeatedly passed as type 1 colonies after isolation from the cervix of a female patient; (vii and viii) a type 1 strain (Mel) of *N. gonorrhoeae* used in the successful production of urethritis in chimpanzees (2) and in subcutaneous chamber infections of rabbits, guinea pigs, hamsters, and mice (1) (performed twice), kindly provided by Robert J. Arko, Center for Disease Control, Atlanta, Ga.

The gonococcal organisms were grown on Thayer-Martin agar (without hemoglobin), the agar medium of Kellogg et al. (8), or GC agar base with IsoVitaleX (Baltimore Biological Laboratories, Baltimore, Md.). The organisms were incubated for 24 h in a candle extinction jar at 36.5°C. Organisms were removed from the agar medium and suspended in Trypticase soy broth, although occasionally phosphate-buffered saline or *Neisseria* defined fluid medium (5) was employed. The titer of the inocula, as determined by dilution plate counts, ranged from 5×10^5 to 2×10^{11} colony-forming units per ml. Inocula prepared from urethral discharge of patients yielded lower titers than laboratory-cultivated strains. The urethra was inoculated 14 times, the conjunctiva (of one eye) was inoculated four times, and the pharynx, rectum, and epididymis were inoculated twice each. Gonococcal organisms, suspended in 0.5 ml of medium, were inoculated through a size 3.5 FR urethral catheter inserted 2 to 4 cm into the urethra. The glans was compressed with the fingertips for 5 to 10 min, and the urethra was intermittently massaged to expose the entire penile urethra to the inoculum. Prior to inoculation, on four occasions, the urethra was traumatized by vigorous swabbing. Similar vol-

umes were instilled into the pharynx and rectum, whereas a lesser volume was placed on the conjunctiva. The epididymis was inoculated by percutaneous injection of 0.5 ml of medium with suspended gonococci (3×10^8 colony-forming units of first-passage type 1 colonies from the urethra of a male patient).

Swabs of the inoculated sites were obtained at 3- to 4-day intervals up to 14 days after inoculation. Specimens were streaked on chocolate agar and Thayer-Martin plates and incubated for 48 h in 2.5% CO_2 at 37°C. The criterion used for a presumptive diagnosis of urethral colonization by *N. gonorrhoeae* was growth on Thayer-Martin medium of oxidase-positive gram-negative diplococci. All cultures were negative for *N. gonorrhoeae,* including some cultures performed 24 h after inoculation. No naturally occurring *Neisseria* were cultured from the urethra of male baboons, although the urethra was always heavily colonized by gram-positive cocci and occasionally by gram-negative rods.

A discharge was not observed from any of the inoculated sites. Urine sediment was examined, at frequent intervals, after three of the eight inoculations, for the presence of leukocytes, but significant numbers (≥15 in at least two of the five high-power fields) were not found. Although urethral cultures were consistently negative after epididymal inoculation, the epididymis and spermatic cord were swollen for two weeks, and one baboon also developed inguinal lymphadenopathy. Since the injections were performed percutaneously, it could not be ascertained whether the inoculations were actually intraepididymal.

Paired sera, obtained prior to inoculation and at 3 to 4 weeks postinoculation, were assayed for gonococcal antibodies by the radioactive pili antigen binding assay (4) with rabbit antiserum to baboon immunoglobulin G. The percentage of antigen bound remained the same in all sera, except that from the two baboons after epididymal inoculation of gonococci. The two baboons exhibited a 150 to 225% increase in antigen binding at 1 week after inoculation which persisted for at least 5 weeks (Fig. 1). They also had two urethral inoculations prior to epididymal inoculation which failed to produce an antibody response, whereas an anamnestic response was demonstrated to a urethral inoculation performed 6 weeks after epididymal inoculation. Once the baboons were sensitized to gonococci by parenteral inoculation, we detected a serological response to urethral inoculation. This suggests that pili antigen is processed by the immune system after urethral inoculation, although the expression of resistance to urethral colonization by gonococci remains to be elucidated. Failure to achieve gonococcal urethritis indicates that the baboon is unsuitable as a model.

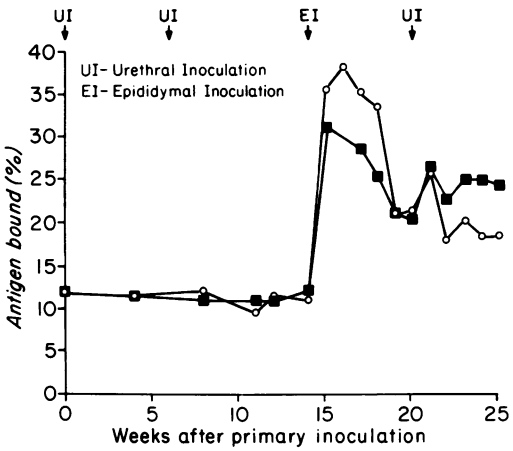

FIG. 1. *Course of serum antibody in two baboons after urethral and epididymal inoculations of N. gonorrhoeae.*

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