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Longitudinal Evaluation of Serovar-specific Immunity to *Neisseria gonorrhoeae*

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The serovars of *Neisseria gonorrhoeae* that are predominant in a community change over time, a phenomenon that may be due to the development of immunity to repeat infection with the same serovar. This study evaluated the epidemiologic evidence for serovar-specific immunity to *N. gonorrhoeae*. During a 17-month period in 1992–1994, all clients of a sexually transmitted disease clinic in rural North Carolina underwent genital culture for *N. gonorrhoeae*. Gonococcal isolates were serotyped according to standard methods. Odds ratios for repeat infection with the same serovar versus any different serovar were calculated on the basis of the distribution of serovars in the community at the time of reinfection. Of 2,838 patients, 608 (21.4%; 427 males and 181 females) were found to be infected with *N. gonorrhoeae* at the initial visit. Ninety patients (14.8% of the 608) had a total of 112 repeat gonococcal infections. Repeat infection with the same serovar occurred slightly more often than would be expected based on the serovars prevalent in the community at the time of reinfection, though the result was marginally nonsignificant (odds ratio = 1.5, 95% confidence interval 1.0–2.4; $p = 0.05$). Choosing partners within a sexual network may increase the likelihood of repeat exposure to the same serovar of *N. gonorrhoeae*. Gonococcal infection did not induce evident immunity to reinfection with the same serovar. *Am J Epidemiol* 1999;149:353–8.

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Gonococcal disease is responsible for substantial morbidity, including pelvic inflammatory disease, infertility (1), and facilitation of human immunodeficiency virus (HIV) transmission (2, 3). In recent decades, significant progress has been made in understanding the structure and physiology of *Neisseria gonorrhoeae*. However, attempts to understand the immune response to the organism have met with limited success (4).

All gonococci express a porin protein, also known as Protein I, in the outer membrane (5). Variation in this protein has allowed the development of the serovar classification system, in which antibodies to porin are used to distinguish among strains of the organism (6, 7). The porin phenotype, or serovar, is a stable characteristic of a gonococcal strain (6). Classification by serovar has proven to be an invaluable tool for investigating the epidemiology of gonococcal disease. Serovar classification has been used to follow the spread of antimicrobial drug-resistant *N. gonorrhoeae*, to differentiate between treatment failure and reinfection, and to assess partner transmission patterns (8, 9).

Predominant serovars of *N. gonorrhoeae* vary with geographic location and change over time (9–11). The reasons for shifts over time in the predominant serovars in a community have not been fully elucidated; however, the existence of serovar-specific immunity has been hypothesized as one explanation for these patterns (12). Both clinical and laboratory studies support the concept that an immunologic response to porin may be important. Anti-porin antibodies can be measured in serum and cervical secretions following gonococcal infection in humans (13–15). In addition, antibodies raised against porin are opsonic and bactericidal and protect against damage to in vitro cell

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Abbreviations: HIV, human immunodeficiency virus; STD, sexually transmitted disease.

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culture (16–18). Epidemiologic evidence for serovar-specific immunity following gonococcal infection has been obtained in an African sex worker cohort (12). Such immunity might be an important force shaping the epidemiology of gonococcal disease and would be critical to the consideration of porin as a vaccine candidate. To assess evidence of the presence of serovar-specific immunity, we studied a cohort of patients from a sexually transmitted disease clinic in a community in rural North Carolina with a high incidence of repeat gonococcal infection (19).

MATERIALS AND METHODS

Study design

As part of the Sexually Transmitted Epidemic Prevention (STEP) project, we conducted a prospective cohort study at the sexually transmitted disease (STD) clinic of a rural county health department in North Carolina. This STD clinic handles approximately 60 percent of STD-related physician visits in the county. All county residents who visited the clinic for STD services during the 17-month study period (August 1992–January 1994) were enrolled. Subjects were examined by the clinic staff, and specimens were obtained from the male urethra and female endocervix for culture of *N. gonorrhoeae*. Rectal and pharyngeal cultures were performed as clinically indicated. All gonococcal isolates were transported to our laboratory for evaluation as described below. Subjects who returned for STD services during the study period were again examined and cultured. Repeat infection with *N. gonorrhoeae* was defined as a positive culture taken at least 2 weeks after treatment for a previous infection, or less than 2 weeks if an intervening negative culture had been documented.

Laboratory methods

Specimens were obtained using calcium alginate swabs for male urethras and Dacron swabs (du Pont de Nemours and Company, Inc., Wilmington, Delaware) for all other sites. Specimens were immediately streaked on Martin-Lewis agar plates and incubated at 37°C in a 5 percent carbon dioxide atmosphere for 48 hours. Colonies of *N. gonorrhoeae* were identified by colony morphology, oxidase testing, and Gram stain. Multiple colonies were frozen in 3 percent trypticase soy broth and 25 percent glycerol at –70°C. Serotyping was performed on 24-hour-old cultures grown on chocolate agar from the frozen stocks. Each isolate was identified as belonging to porin group PIA or PIB by a standard coagglutination method available commercially as the Phadebact Monoclonal GC Test (Boule Diagnostics AB, Huddinge, Sweden). IB serovars were identified using a previously described whole-cell enzyme-linked immunosorbent assay (20). IA serovars were identified by the *Neisseria* Reference Laboratory at the University of Washington Center for AIDS and STD (Seattle, Washington) using standard methods described by Knapp et al. (6). Monoclonal antibodies for both procedures were provided by the Division of AIDS, STD, and TB Laboratory Research, Centers for Disease Control and Prevention (Atlanta, Georgia).

Data analysis

The Pearson χ^2 test was used to compare the distributions of demographic data between patients who had one infection during the study period and those with repeat infection, or between those reinfected with the same serovar versus a different serovar, and to compare the distributions of serovars across time. Fisher's exact test was used when an expected cell value was less than five. The nonparametric Mann-Whitney *U* test was used to compare age and time to reinfection between groups. Odds ratios for the risk of repeat infection with the same serovar versus any different serovar were calculated using conditional logistic regression. To control for changes over time in the predominant serovars in the community, the comparison distribution for each repeat infection was the serovar distribution of all isolates obtained in the prior 4 weeks. An odds ratio less than 1 would indicate protection from reinfection with the same serovar. Reported probability values are nominal and were not adjusted for multiple comparisons.

RESULTS

Study population

A total of 2,838 county residents visited the STD clinic for STD services during the study period. Of these, 608 (21.4 percent) were found to be infected with *N. gonorrhoeae* at the initial visit. Ninety patients (14.8 percent of the 608) had a total of 112 repeat gonococcal infections, with a median time to reinfection of 112 days (range, 14–468 days). The majority of infections were urethral in men and cervical in women. Of 19 rectal cultures performed, two initial infections and two repeat infections were identified; of 112 pharyngeal cultures performed, two initial infections and three repeat infections were identified. One rectal and two pharyngeal infections were accompanied by genital infection with the same serovar. Table 1 shows the demographic characteristics of the study population, comparing persons who had one infection during the study period to those who had two or more infections.

TABLE 1. Characteristics of patients with gonococcal infection at a sexually transmitted disease clinic in rural North Carolina, by number of infections incurred during the study period, 1992–1994

	1 infection (n = 518)		≥2 infections (n = 90)		p value
	No.	%	No.	%	
Median age (years)	22.6 (13.8–74.1)*		22.5 (15.2–57.5)		
Race†					
African-American	493	95.2	90	100	0.04
White	19	3.7	0		
Other	6	1.2	0		
Male gender	352	68.0	75	83.3	0.003

* Numbers in parentheses, range.
† p value is for African Americans versus all other races.

Median age was similar for the two groups. A higher proportion of men than of women had repeat infections (17.6 percent vs. 8.3 percent; $p = 0.003$), and a higher proportion of African Americans than of whites had repeat infections (15.4 percent vs. 0 percent; $p = 0.04$).

Serovars

Overall, 26 different serovars were identified among the gonococcal isolates from this population. One dual infection, with serovars IB-1 and IB-2, was found; both were isolated from the cervix in a patient who did not have any subsequent infections. Throughout the study period, four serovars were predominant: IA-6, IB-2, IB-3, and IB-6 (figure 1). Together, these four serovars accounted for 554 (76.8 percent) of the 721 isolates. No other serovar accounted for more than 3.3 percent of isolates overall or more than 8.9 percent of isolates in any single quarter. The relative prevalences of the four major serovars changed significantly over time ($p < 0.01$).

Repeat infections

Fourteen different serovars were identified among repeat infection isolates. As with overall infections, serovars IA-6, IB-2, IB-3, and IB-6 were predominant, accounting for 94 (83.9 percent) of the 112 repeat isolates. Thirty-three repeat infections (29.5 percent) were caused by the same serovar as the subject's previous infection, while 79 (70.5 percent) were different. The odds ratio for repeat infection with the same serovar versus any different serovar was 1.5 (95 percent confidence interval 1.0–2.4), indicating a marginally nonsignificant trend (at the $\alpha = 0.05$ level) toward reinfection with the same serovar more often than would be expected based on the serovar distribution in the community at the time of reinfection (table 2). An odds ratio less than 1 would indicate protection from reinfection with the same serovar. The risk of repeat infection with the same serovar versus any different serovar was either greater than unity or no different from unity regardless of the serovar of first infection, the amount of time to reinfection, or the gender of the patient (table 2). Median age was similar for those reinfected with the same serovar and those reinfected with a different serovar (23.0 years vs. 22.0 years, respectively; $p = 0.18$). Time to reinfection was shorter for those reinfected with the same serovar than for those reinfected with a different serovar (median time to reinfection was 77 years vs. 129 days, respectively; $p = 0.01$).

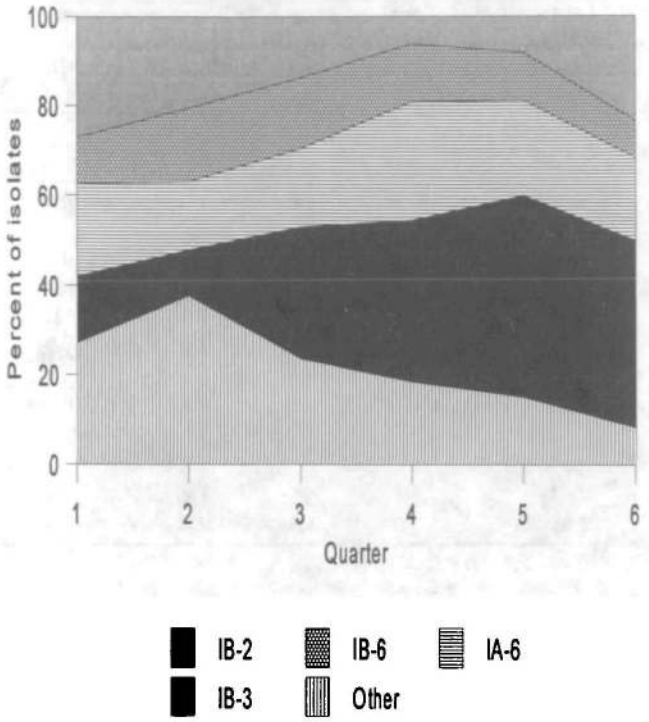

FIGURE 1. Changing prevalences of gonococcal serovars over a 17-month period (August 1992—January 1994) in a county in rural North Carolina ($p < 0.01$ for change in the relative prevalences over time).

DISCUSSION

In this study, we demonstrated the absence of protection against repeat infection with the same serovar of *N. gonorrhoeae* in a cohort of persons with repeated gonococcal infections in rural North Carolina. There was a marginally nonsignificant trend toward reinfection with the same serovar more often than would be expected given the serovars circulating in the community at the time of reinfection. Since immune

TABLE 2. Risk of repeat infection with the same serovar of *Neisseria gonorrhoeae* versus any different serovar among patients at a sexually transmitted disease clinic in rural North Carolina, 1992–1994

	No. of reinfections	OR†,‡	95% CI†
Overall	112	1.5	1.0–2.4
Serovar of first infection			
IA-6	21	1.4	0.5–3.6
IB-2	13	3.0	0.9–9.7
IB-3	27	4.6	1.9–11.1*
IB-6	18	0.7	0.2–2.5
Time to reinfection			
Early (≤12 weeks)	44	4.4	2.2–8.7**
Late (>12 weeks)	68	0.8	0.4–1.4
Gender			
Male	95	1.4	0.9–2.3
Female	17	2.3	0.8–6.4

* $p < 0.05$; **$p < 0.001$.
† OR, odds ratio; CI, confidence interval.
‡ An odds ratio greater than 1 indicates reinfection with the same serovar more often than would be expected based on the serovars prevalent in the community at the time of reinfection.

response might vary by the serovar of the organism and by the gender of the host, we examined the risk according to serovar of first infection and according to gender. However, in all groups, the risk of reinfection with the same serovar exceeded or was equal to the risk of reinfection with a different serovar. The numbers of subjects with rectal and pharyngeal infection were too small for assessment of the influence of infection site on this risk.

Biologic evidence that anti-porin antibodies decline 4–12 weeks after acute gonococcal infection (13) raises the possibility of transient serovar-specific immunity, which might be evident only with examination of early reinfections. We found, however, that patients reinfected within 12 weeks of initial infection were *more* likely to be reinfected with the same serovar than would be expected based on community serovar prevalences. This finding may result from the subjects' sexual partner choices, leading to greater than expected rates of reexposure to the initial serovar.

Our study contrasts with an earlier investigation which found evidence for serovar-specific immunity in a sex worker cohort (12). Several differences in the populations and study designs may account for the opposing findings. First, our cohort comprised mostly men, whereas the earlier cohort contained all women. There are clear biologic differences between genders in the pathophysiology of gonococcal infection, and there may be important differences in mucosal immunity (21). However, our results were no different when men and women were analyzed separately. Second, two thirds of the sex worker cohort were infected with HIV; we did not have information on the HIV status of our subjects, but other North Carolina STD clinics have HIV seroprevalences of 1.6–3.4 percent (22). A low rate of HIV infection, however, should increase the likelihood of detecting any existing immunity in our population. Third, as discussed above, sexual partner choice may have made our patients' likelihood of reexposure to the same serovar of *N. gonorrhoeae* greater than that for commercial sex workers.

Most importantly, we measured risk for reinfection with a particular serovar differently from the prior study. We demonstrated that the relative prevalences of serovars in our community changed substantially during the study period. This finding is consistent with previous literature (9–11), including the prior study of serovar-specific immunity (12). To account for the changing pattern of prevalent serovars, we used the serovar distribution in the 4 weeks prior to reinfection to establish the risk of infection with a particular serovar. The prior study used the serovar distribution for the entire study period (14 months) to establish this risk. Our method allowed us to account for the change over time in the predominant serovars in this community. Since the conclusion of our study, investigators in the United Kingdom have published findings similar to ours from a population of primarily homosexual men; however, risk was measured using the serovar distribution for the entire study period of 4 years (23).

Our study had three notable limitations. First, actual measurement of serovar exposure is not feasible, so the 4-week serovar distribution in the community was used as a proxy. The 4-week time period is probably appropriate for most patients; however, some patients may have been infected for more than 4 weeks before the clinic visit. Second, we excluded nonresidents of the county, because serovar patterns vary by geographic location (9–11); the inclusion of out-of-county strains in our analysis would have biased the measurement of circulating community strains. However, sociosexual networks are likely to cross county lines, so the pool of serovars to which a subject may have been exposed was not completely identified by our methods. Finally, our cohort was limited to patients seeking care at a health department STD clinic. Although this clinic diagnoses the majority of gonorrhea cases in the county, the relatively small number of women diagnosed there with repeat infections limited our ability to assess serovar-specific immunity in this group.

There has been considerable enthusiasm for a porin-based gonorrhea vaccine because of the relative conservation of porin sequences. This enthusiasm has been bolstered by the facts that 1) porin may play an important role in the pathogenesis of gonococcal dis-

ease (17, 18, 24), and immune interference with such a role could prevent infection; 2) systemic antibodies directed against the porin protein are generated as a result of mucosal infection (15); 3) porin antibodies can be bactericidal under some assay conditions (16–18); 4) work by Plummer et al. (12) suggested that gonococcal mucosal infection reduced susceptibility to reinfection with an organism with the same porin serovar; 5) an earlier, unsuccessful trial of a porin-based gonococcal vaccine appears to have failed, at least in part, because of inadvertent contamination of the vaccine preparation with gonococcal reduction-modifiable protein (25), which evokes generation of blocking antibodies that interfere with the function of bactericidal anti-porin antibodies (26); and 6) porin protein may serve as an adjuvant to the vaccine immune response (27).

Our results do not provide evidence for significant porin-specific immunity after naturally acquired infection. Because of the potential bias imposed by sexual partner choices, resulting in greater than expected rates of reexposure to the incident serovar, we cannot rule out a small protective effect of mucosal infection on the risk of reinfection by the same serovar. Rigorous testing of the possibility of porin-based immunity to gonococcal infection will require more precise knowledge of serovar exposure, either through careful collection of sexual network information or through controlled exposure in a human challenge model (28). We also cannot rule out the possibility that immunization by a porin protein formulated as a vaccine would result in a quantitatively and qualitatively different immune response, and hence better protection than is afforded by natural infection. Indeed, development of a gonococcal vaccine for either men or women requires a better understanding of the precise nature and durability of the mucosal immune response (21). Hopefully, porin vaccine candidates will soon be available for testing in the male human challenge model and thus will allow specific assessment of the potential for vaccine-induced, porin-based immunity to gonococcal infection.

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