Antibody to Rmp (Outer Membrane Protein 3) Increases Susceptibility to Gonococcal Infection

Francis A. Plummer,*[§] Hilary Chubb,* J. Neil Simonsen,* Milcha Bosire,[§] Leslie Slaney,* Ian Maclean,*
Jackoniah O. Ndinya-Achola,[§] Peter Waiyaki,[‡] Robert C. Brunham*

*Departments of Medical Microbiology, Medicine, and Community Health Sciences, University of Manitoba, Winnipeg, Manitoba,
Canada R3E 0W3; [‡]Centre for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya;
and [§]Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya

Abstract

The severe adverse effects of gonococcal infection on human fertility suggests that *Neisseria gonorrhoeae* would exert powerful selection for the development of a protective immune response in humans. *N. gonorrhoeae* is an obligate human pathogen and must persist in humans to survive. Since it is an ecologically successful organism, it must have evolved strategies to evade any human immune response it elicits. In a longitudinal study among 243 women working as prostitutes and experiencing frequent gonococcal infection, younger women, women with HIV infection, and women with antibody to the gonococcal outer membrane protein 3 (Rmp) were at increased risk of infection (adjusted odds ratio 3.4, CI95% 1.1–10.4, $P < 0.05$). Rmp is highly conserved in *N. gonorrhoeae* and the blocking of mucosal defences may be one of its functions. As similar proteins occur in many gram negative mucosal pathogens, the enhancing effect of such proteins may be a general strategy whereby bacteria evade human immune responses. (*J. Clin. Invest.* 1993. 91:339–343.) Key words: *Neisseria gonorrhoeae* • Rmp (outer membrane protein 3) • blocking antibody • mucosal infection • HIV

Introduction

In considering the ecology of human gonococcal interactions, we know that: (*a*) *Neisseria gonorrhoeae* is a frequent human pathogen (1, 2); (*b*) it has no reservoir apart from the human genital tract; (*c*) gonococcal infection has a deleterious effect on human reproductive health; and (*d*) *N. gonorrhoeae* is a ubiquitous pathogen, and an individual may experience multiple infections. From these precepts, two conclusions about the ecologic interactions of humans and gonococci follow logically. First, over evolutionary time, gonococcal infection would powerfully select for effective human defence mechanisms against the organism. Second, if *N. gonorrhoeae* is to survive, it must do so in spite of any human defence mechanisms, and thus these defence mechanisms have probably in turn selected *N. gonorrhoeae* for strategies that evade them.

The latter appears to have happened in that *N. gonorrhoeae* is an ecologically successful human pathogen.

Much more is known about the strategies used by *N. gonorrhoeae* to evade human defences than about the defences themselves. Antigenic diversity of the porin protein, outer membrane protein 1 (Por)[1] (3–6), and phase and antigenic variation of the adhesins, outer membrane protein 2 (Opa), and pilus (7–12) are probably examples of such strategies. In contrast, protein 3 (Rmp), an outer membrane protein physically associated with Por, is conserved (13, 14). Rmp elicits antibody that blocks killing of *N. gonorrhoeae* by normal or immune human sera, causing the aberrant deposition of complement on the outer membrane (15–17). Here we describe increased risk of gonococcal mucosal infection in the presence of antibody to Rmp.

Methods

Study design and patient population. 243 women working as prostitutes in the community of Pumwani, Nairobi, Kenya, were enrolled in the study. Women were examined and cultured for *N. gonorrhoeae* monthly between March 1985 and July 1986. Observation was stopped at that point because of rapid changes in the use of condoms by the women and their clients; before July 1986, condom use was essentially nil.

Enrollment and follow up of women have been previously described (18–20). Briefly, demographic, sexual, reproductive, and medical histories were obtained in a standard format interview and complete physical examinations, including vaginal speculum and bimanual examinations, were performed at enrollment. Specimens for culture of *N. gonorrhoeae* and for serology for HIV and gonococcal immunoblot were obtained. Women were scheduled for follow up at 2-wk intervals. At each visit, interval symptoms were reviewed and a genital examination was performed. Endocervical swabs for gonococcal culture were obtained. All women returned to the clinic 3–4 d later for results, at which time treatment for gonorrhea was administered, if necessary. 4 d after treatment, a test of cure culture was performed.

Laboratory methods. Specimens for *N. gonorrhoeae* culture were immediately inoculated on modified Thayer-Martin media, transported to the laboratory within 3 h, and incubated at 37° for 48 h. *N. gonorrhoeae* was identified by colony morphology, gram stain, and oxidase reactivity and later confirmed by determination of nutritional requirements (20). Cultures were stocked frozen in skim milk with 10% glycerol for later serotyping. Por serovar determinations were performed using the monoclonal antibody typing system of Knapp and Sandstrom (6).

Five Por serovars, 1B1, 1B3, 1B5, 1A4, and 1A6, comprised 81% of the serovars in the population (20). The potential role of outer membrane protein antibody in mediating resistance to gonococcal infection

1. *Abbreviations used in this paper:* 95% CI, 95% confidence intervals; Opa, outer membrane protein 2; OR, odds ratio; Por, outer membrane protein 1; Rmp, outer membrane protein 3.
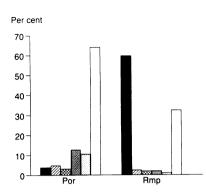
Figure 1. The frequency of detectable antibodies to the gonococcal outer membrane proteins Por and Rmp of 5 independent serovars in 243 women working as prostitutes. ■, No antibodies; □, antibodies to one serovar; ■, antibodies to two serovars; ▨, antibodies to three serovars; □, antibodies to four serovars; and □, antibodies to five serovars.

was assessed by determining the frequency of serum antibody at enrollment to the outer membrane proteins, Por and Rmp, of these five Por serovars circulating in the population by immunoblot (Fig. 1). Standardized whole cell preparations of 18-h cultures of each serovar were solubilized by boiling in SDS and 2-mercaptoethanol, separated by SDS polyacrylamide electrophoresis in 12.5% gels (21), and transferred to Immobilon P, polyvinylidene fluoride membranes (Millipore Corp., Bedford, MA) by electroblotting (22). Sera were preabsorbed with 100 μg each of whole cell preparations of *Neisseria sicca, Neisseria meningitidis, Neisseria lactamica, Haemophilus influenzae, Haemophilus ducreyi,* and *Haemophilus parainfluenzae* at 4°C overnight followed by centrifugation to remove the organisms. Membranes were preblocked with a 5% skim milk in Tris-buffered saline and incubated with absorbed sera overnight at 4°C. Immunostaining followed standard protocols, using goat anti–human IgG conjugated to horseradish peroxidase (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) and diaminobenzidine with cobalt chloride (Sigma Chemical Co., St. Louis, MO) (23). Biotinylated and prestained molecular weight standards (Bio-Rad Laboratories, Richmond, CA) were included on the blots. The first available serum from each of the 243 study women was tested in this assay. Only observations recorded after the date of serum collection were analyzed. Blots were read visually by an observer blinded to the results of clinical and epidemiologic studies. Molecular weight standards and monoclonal antibodies were used to determine the antibody specificity of antigen antibody reactions. Antibodies to the two outer membrane proteins were then correlated to the subsequent occurrence of infection.

Data analysis. Demographic and clinical parameters and the frequency of outer membrane antibodies were correlated with the frequency of gonococcal infection. Odds ratios with 95% confidence intervals, chi square tests, and Student's *t* test were used to compare risks, proportions, and means, respectively. Logistic regression was used to perform multivariate analysis.

Results

There were 1,944 study visits (excluding test of cure visits) and 870 gonococcal infections. 23 women experienced no gonococcal infections. These women are compared to those who experienced one or more infections in Table I. Several factors were related to the occurrence of gonococcal infections: young age, duration of prostitution, the presence of cervical ectopy, and the use of oral contraceptives increased the risk of gonococcal infection. The use of condoms was too infrequent to analyze (19).

The frequency of antibody, at baseline, to the two outer membrane proteins of the five-serovar panel is shown in Fig. 1. The presence of Por antibodies to the five-serovar panel at enrollment was not related to the subsequent risk of gonococcal infection. Por antibody was associated with the number of study visits.

The relationship of demographic, physiologic, and behavioural factors to Rmp antibody are shown in Table II. Antibody to Rmp was associated with younger age and with the number of sex partners per day but not with Por antibody.

The presence of antibody to Rmp at baseline increased the risk of one or more gonococcal infections by more than threefold (odds ratio [OR] = 3.54, 95% confidence intervals [95% CI] 1.2–10.8, $P < 0.001$) (Fig. 2). Antibody to Rmp also increased the rate of gonococcal infection. As shown in Fig. 3 *A,* the mean annual rates of gonococcal infection were 4.6±4.7, and 6.0±5.2 in women without and with one or more Rmp antibodies, respectively ($P < 0.03$, *t* test). The mean rate of gonococcal infection per 100 visits was 19±25, and 13±20 in the presence and absence of Rmp antibody, respectively ($P < 0.05$, *t* test) (Fig. 3 *B*). Although Rmp is thought to be highly conserved, of those women with detectable Rmp antibodies, a small proportion (7.4%) did not have Rmp antibodies to all

Table I. Comparison of Demographic, Sexual Behavior, and Other Parameters in Women with No Gonococcal to Women with One or More Gonococcal Infections

	No gonococcal infections n = 23	One or more infections n = 220	P
Age (mean yr±SD)	35.2±8.8	29.5±5.8	< 0.0001
Duration of prostitution (mean mo±SD)	70.7±64.2	39.1±42.2	< 0.055
Length of follow up (mean mo±SD)	6.3±5.7	10.6±4.7	< 0.0001
Number of study visits (mean±SD)	4.1±2.5	7.0±4.8	< 0.01
Sex partners per day (mean±SD)	3.6±2.3	3.9±2.2	= 0.61
Using oral contraceptives*	4 (17)	86 (39)	< 0.06, OR = 3.0, CI95% 1.0–9.3
Using other contraceptives	0	3 (1)	= 0.67
Using condoms	1 (4)	12 (6)	= 0.79, OR = 1.3, CI95% 0.2–7.5
Using prophylactic antibiotics	1 (4)	39 (18)	= 0.18, OR = 4.7, CI95% 0.7–45.0
Cervical ectopy present	0 (0)	49 (22)	< 0.08, OR > 6.6, CI95% 0.91–50.2
HIV-1 seropositive	14 (61)	154 (70)	= 0.51, OR = 1.5, CI95% 0.6–3.9

* Figures in parentheses are percentages.

Table II. Relationship of Antibody (Ab) to One or More Rmp's from the Panel to Demographic and Epidemiologic Parameters

	Rmp Ab negative n = 145	Rmp Ab positive n = 98	P
Age (mean yr±SD)	30.8±7.0	28.9±4.9	< 0.016
Duration of prostitution (mean mo±SD)	43.6±49.9	39.9±40.8	= 0.82
Length of follow up (mean d±SD)	10.3±4.9	10.1±5.1	= 0.72
No. of study visits (mean±SD)	7.5±6.5	8.7±6.8	= 0.19
Sex partners per d (mean±SD)	3.6±2.0	4.0±2.5	= 0.24
Using oral contraceptives*	57 (39)	31 (34)	= 0.45, OR = 1.4, CI95% 0.8–2.5
Using prophylactic antibiotics	27 (19)	23 (23)	= 0.45, OR = 0.7, CI95% 0.3–1.3
Cervical ectopy present	24 (17)	16 (16)	= 1.0, OR = 1.0, CI95% 0.5–2.1
HIV-1 seropositive	96 (66)	72 (73)	= 0.29, OR = 0.3, CI95% 0.4–1.3

* Figures in parentheses are percentages.

five serovars. The subset of women with no Rmp antibody and no gonococcal infections did not account for the entire effect seen in those whom were antibody positive vs. antibody negative. Excluding this subset from the analysis, the association between Rmp antibody and increased risk of gonococcal infection continued to show a trend towards significance (mean rate of gonococcal infection per 100 visits 19.4±2.5 vs. 14.8±2.0, $P = 0.078$, t test). The mean number of Rmp antibodies to the panel was 0.87±1.94 in women with no gonococcal infections vs. 1.9±2.34 in women with one or more gonococcal infections ($P < 0.05$, t test). Rmp antibody appeared to enhance the risk of infection both in the absence and the presence of Por antibody; there was no difference in the enhancing effect when stratified for the presence of Por antibody.

The interrelationships of demographic, physiologic, and behavioural factors and antibody to Rmp to gonococcal infection were analyzed by logistic regression. As shown in Table III, age > 29 reduced the risk of gonococcal infection (adjusted OR = 0.3, 95%CI 0.1–.83, $P < 0.03$). Oral contraceptive use (adjusted OR 3.0, 95%CI 0.95–9.2, $P = 0.062$) and antibody to Rmp increased the risk of gonococcal infection (adjusted OR = 3.4, 95%CI 1.1–10.4, $P < 0.05$). Since the number of times an individual is sampled influences the likelihood of detecting gonococcal infection, we included the number of cultures in the model. This did not change parameter estimates (results not shown). Similarly the number of sexual partners did not influence the results.

Discussion

It is apparent that a complexity of interacting host and organism factors, some facilitating infection, some acting to prevent
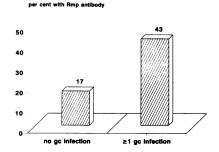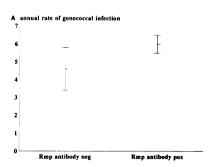
infection, determine if gonococcal infection follows exposure. Study of the system consisting of gonococcal and human prostitute populations may produce unique insights into the ecology and biology of gonorrhoea, not feasible by study of its components in isolation. Some of the advantages of studying this particular ecologic system are that it is a relatively homogeneous population, both ethnically and in terms of sexual behaviour, residing in one geographically distinct location, with very high prevalence and incidence rates of common sexually transmitted infections. Moreover, because all women sell sex, the contribution of differences in sexual behaviour in determining the occurrence of sexually transmitted disease is minimized. While prostitutes may not be representative of all women, they are one important component of the gonococcal core group in many societies and are probably key in the transmission dynamics of *N. gonorrhoeae*. Thus, they are central to the ecologic relationships between human populations and gonococcal populations. Further because of the intensity of their exposure to gonococci, the interaction of ecologic forces may be more obvious and easier to detect. This study demon-
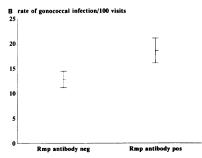

A annual rate of gonococcal infection

per cent with Rmp antibody

Figure 2. The frequency of antibody to Rmp in women with none and ≥ 1 gonococcal infections. OR = 3.5, CI95% 1.2–10.8, $P < 0.001$.

B rate of gonococcal infection/100 visits

Figure 3. (*A*) Annual rate of gonococcal infection in the presence and absence of Rmp antibody. $P < 0.03$, t test. (*B*) Per 100 visit rate of gonococcal infection in the presence and absence of Rmp antibody. $P < 0.05$, t test.

Table III. *Logistic Regression Analysis of Epidemiologic and Immunologic Risk Factors for One or More Gonococcal Infections*

	Odds ratio	Adjusted 95% confidence Intervals	P
Age > 29	0.3	0.1–0.83	< 0.03
Oral contraceptive use	3.0	0.95–9.2	= 0.062
Antibody to Rmp	3.4	1.1–10.4	< 0.05

The model did not change when the number of gonococcal cultures, condom use, and sexual partners per day were entered as independent variables.

strates the complexity and importance of the interaction between the gonococcal outer membrane, the host immune system, and physiologic factors in determining susceptibility to *N. gonorrhoeae* infection. Host physiologic and immune factors act in competing directions in determining if infection results from exposure.

The effect of age and duration of prostitution on the risk of gonococcal infection in this study may reflect acquired immunity to *N. gonorrhoeae* or physiologic alterations related to age or frequent sexual exposure. Although not independently associated with gonococcal infection after multivariate analysis, cervical ectopy increased the risk of one or more gonococcal infections by exposing a greater area of columnar epithelium to the organism or as a marker for a susceptible physiologic state. The effect of oral contraceptive use on susceptibility to gonococcal infection may be related to the association of oral contraceptives and ectopy (24, 25), as it was in this study (14% of women with ectopy in non–oral contraceptive users compared to 31% in users [OR = 2.8, 95%CI 1.4–5.7, P < 0.005]). Alternatively or in addition, an effect of hormonal contraceptives on the systemic or local immune system (26, 27) could account for the altered susceptibility.

The subset of women who did not acquire gonococcal infection are interesting. From this study, it cannot be determined if this resistance to gonococcal infection is inherent, the result of the absence of factors increasing susceptibility or to immunity. Further study of populations exposed to gonorrhoea but who are uninfected would be of interest to exploring the possibility that there is some acquired protective immunity to *N. gonorrhoeae*.

The absence of a detectable effect of antibody to Por and the risk of gonococcal infection is in contrast to our previous findings on reduced risk of homologous serovar infection after an initial infection. This is possibly related to the sensitivity of the immunoblot in detecting Por specific antibody, the epitope specificity of the antibody detected in this system, or the potential interaction between Rmp antibody and bactericidal Por antibody. More detailed study of epitope specificity of Por and Rmp antibodies and quantitative analysis would help to address these possibilities.

The most important finding of this study is the correlation of increased risk of gonococcal infection in frequently exposed women with the presence of antibody to Rmp. The blocking effect of Rmp antibody was first described in association with the blocking of killing of *N. gonorrhoeae* by normal and immune human sera and was thought to be potentially important

in disseminated gonococcal infection. This blocking phenomenon has no previously known role in mucosal infection. Perhaps the killing effect of normal human serum is an important mucosal defense against *N. gonorrhoeae*. Alternatively, as antibody to Rmp also blocks killing by Por antibody, in this study, Rmp antibody may be blocking specific protective immunity (28). From an ecologic perspective, the enhancing or blocking effect of antibody to Rmp has a logical appeal. *N. gonorrhoeae* invests considerable resources into diversity of the antigens it presents to the human host. Por, Opa and pilus are all very variable. In contrast, Rmp has apparent antigenic stability within the species and the genus *Neisseria* (14, 28). A blocking of host immune mechanisms would provide an ecologic rationale for this conservation.

The demonstration that Rmp antibody enhances susceptibility to mucosal infections with *N. gonorrhoeae* is of considerable interest and importance, but also may have a more general relevance in understanding the immunobiology of gram negative bacterial infections. Rmp-like proteins occur in many pathogenic bacteria, particularly those causing mucosal infections. Rmp is closely related to the outer membrane protein 3 protein of *Escherichia coli* (29) and the class 4 protein of *N. meningitidis* (14, 28). Some of these proteins have been shown to play a role similar to gonococcal Rmp, in blocking bacterial killing by normal human serum. The conservation of this protein across species and genera suggests that it may be a general mechanism whereby bacteria evade human defenses. In broader terms, the link between enhancing or blocking antibodies and an evolutionary conserved outer membrane protein may have significance for understanding the pathogenesis of mucosal bacterial infection.

Acknowledgments

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