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Characterization of *Neisseria gonorrhoeae* Protein II Phase Variation By Use of Monoclonal Antibodies

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The protein II (P.II) outer membrane proteins of *Neisseria gonorrhoeae*, which have been implicated in gonococcal pathogenesis, have been previously shown to undergo a type of phase variation in which expression of any of several different forms of the proteins may be switched on or off. We identified six electrophoretically distinct forms of P.II proteins (designated P.IIa through P.IIf) within strain FA1090, and we isolated colonial variants of FA1090 that expressed only one of the six different P.II protein forms. Two monoclonal antibodies that bound specifically and differentially to P.II proteins were produced. One antibody bound to proteins P.IIb and P.IId and was bactericidal for all colonial variants expressing P.IIb. The second antibody bound to P.IIa and was bactericidal for colonial variants expressing P.IIa. P.II protein profiles of survivors of antibody killing indicated that multiple P.II protein species may be expressed on a single bacterium and that P.II protein switching in the gonococcus is nonrandom.

The outer membrane (OM) protein IIs (P.II proteins) of *Neisseria gonorrhoeae* are a class of related cell surface components that undergo a type of phase variation in which expression of any of several different forms of the proteins may be switched on or off by the gonococcus. Within a strain, gonococcal variants may express no, one, or several different P.II protein(s) (15, 22, 28). P.II proteins within a strain may differ in both molecular weight and antigenicity (16, 25, 26). Antigenic differences are predominantly in surface exposed regions (26). P.II proteins also show certain similarities, including sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) heat modifiability (15, 22), similar peptide maps (7, 23), and common antigenic determinants which are not surface exposed (26).

Phase variation of P.II proteins may be related to gonococcal virulence. A number of reports indicate that gonococcal variants that differ in their expression of P.II proteins also differ in their intracellular adhesion (8, 16, 27), survival in normal human serum (11, 16), cytotoxicity (27), and protease (1) and steroid (19) sensitivities. P.II protein phase variation may thus enable gonococci to survive in a variety of different microenvironments within the host. Significantly, clinical isolates from different loci in the host and from different stages of the menstrual cycle have different P.II protein profiles (3, 10).

The regulation of gonococcal P.II protein phase variation is poorly understood (17). The study of P.II protein switching has been hindered by a lack of easily assayable and differentiable phenotypes associated with the different P.II proteins. Colonial photo-opacity partially correlates with the P.II protein profile of a colony (22, 28). Dark colonies tend to possess P.II proteins. Gonococci without P.II proteins tend to form light colonies. It is important, however, to note that the converse of each of the last two statements is not always true (18).

We have developed monoclonal antibodies to probe gonococcal colonies directly for the expression of certain forms of P.II proteins. Nachamkin et al. have previously reported monoclonal antibody 1090-10.1 (hereafter designated McAb 10.1), which binds differentially to colonial opacity variants of gonococcal strain FA1090 (18). Sugasawara et al. have reported that McAb 10.1 binds to a P.II protein of FA1090 (21). Here we describe the various P.II protein forms of FA1090, report a second monoclonal antibody, and identify the specific P.II proteins to which each of these antibodies bind. We also describe how these antibodies have been used to analyze P.II protein expression.

MATERIALS AND METHODS

Strains. *N. gonorrhoeae* FA1090 and FA19 have been described (18, 20). FA1090 is a serum-resistant, prototrophic strain isolated from a patient with probable disseminated gonococcal infection. Gonococci were grown on Difco GCB agar with the supplements of Kellogg et al. (13). Opacity variants were identified on GCB agar by the criteria of Swanson (22). Nonpiliated colonial variants were used in all experiments.

Production of hybridomas. Cloned hybridomas secreting specific monoclonal antibodies were produced and screened as described by Nachamkin et al. (18). Spleen cells from BALB/c mice immunized with gonococcal OM were fused with Sp2/0 myeloma cells. Membrane samples used for immunization were prepared by Sarkosyl extraction (6) or lithium acetate extraction (described below). Antibodies secreted by the hybridomas were screened for binding to gonococci by solid-phase radioimmunoassay (18) or enzyme-linked immunosorbent assay (ELISA) (5). Monoclonal antibody heavy-chain isotypes were analyzed by immunoelectrophoresis (Universal film agarose) by using subclass-specific antisera against mouse immunoglobulins (Litton Bionetics). Monoclonal antibody-containing ascites fluid was produced as previously described (18). All immunological assays described in this report used monoclonal antibodies in ascites.

Colony blot radioimmunoassay. Gonococcal colonies were assayed for binding of monoclonal antibodies by a modification of the colony blot radioimmunoassay of Henning et al. (9). Colonies were blotted with autoclaved disks of Whatman 42 filter paper. Filters were dried at 37°C and then incubated for 1 h at 37°C in a solution of monoclonal antibody ascites

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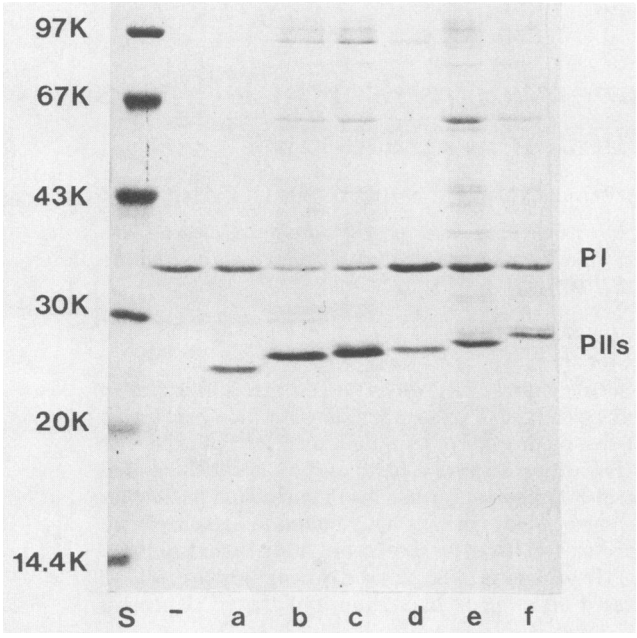

FIG. 1. P.II proteins of *N. gonorrhoeae* FA1090. Lithium acetate-extracted OMs were solubilized at 37°C for 60 min and electrophoresed on a 4 to 30% polyacrylamide gradient gel. Lane S, Electrophoretic molecular weight standards; lane −, P.II⁻ variant; lanes a through f, variants P.IIa through P.IIf, respectively.

diluted into 10.0 mM sodium phosphate (pH 7.2)–0.85% sodium chloride–0.5% bovine serum albumin (PBS-BSA). Filters were washed with agitation in three changes of PBS-BSA for a total of 15 min at room temperature. Washed filters were incubated at 37°C in PBS-BSA containing 0.1 μCi of radioiodinated staphylococcal protein A (Amersham Corp.) per ml and then were washed again as before. Dried filters were autoradiographed with Kodak XR-5 and a Cronex enhancing screen.

OM preparation. OMs were prepared by mechanical shearing of OM blebs from gonococcal cells in 200 mM lithium acetate buffer (pH 6.0)–10.0 mM EDTA. Shearing force was provided either by vigorous agitation of the cell suspension in the presence of glass beads (12) or by repeated rapid passage of the cell suspension through a 21-gauge needle (15). Cells were removed by centrifugation at 12,000 × *g* for 10 min. Membrane material in the supernatant was pelleted by centrifugation at 100,000 × *g* for 2 h and then was suspended in water.

SDS-PAGE. SDS-PAGE was performed with the discontinuous buffer system of Laemmli (14). Linear gradients of 4 to 30% were utilized. Electrophoresis was at 200 V (constant voltage) at 4°C for 24 h. OM gels were stained with Coomassie brilliant blue. All gel reagents were from either Bio-Rad Laboratories or Bethesda Research Laboratories.

Western transfer. Binding of monoclonal antibodies to specific antigens was analyzed by Western transfer by the method of Burnette (2), except that Nonidet P-40 was omitted from the washes. Polyacrylamide gel electrophoretic patterns of OMs were transferred to Schleicher & Schuell BA85 nitrocellulose at 8 V/cm overnight in a Bio-Rad Transblot device. Filters were either stained with Coomassie blue or probed with monoclonal antibody ascites, washed, incubated in 5.0 nCi of radioiodinated staphylococcal protein A (Amersham) per cm^2 of filter, and washed again. Autoradiography was done as described above. The Coomassie blue-stained filters were used to align the autoradiograph signals with the corresponding SDS-PAGE bands.

Bactericidal assay. Complement-dependent bactericidal activity of the monoclonal activity ascites for specific strain FA1090 variants was determined by a modification of the serum bactericidal assay of Eisenstein et al. (4). The final serum concentration was 25%. The final ascites dilution was 1:80. Incubation was for 45 min at 37°C.

Analysis of survivors from bactericidal assay. Colonies that grew after the bactericidal assay were individually expanded, and OMs were prepared from each by lithium acetate extraction. The P.II protein profile was then determined by SDS-PAGE as described above.

ELISA. ELISA was performed essentially as described by Engvall and Perlman (5). Strain FA1090 P.II protein variants analyzed by ELISA were from colonies grown overnight and suspended in phosphate-buffered saline (135 mM NaCl, 2.5 mM KCl, 0.9 mM KH_2PO_4, 6.4 mM Na_2HPO_4) at an optical density at 750 nm of 0.1. Cells were fixed to the plates with 0.25% glutaraldehyde, probed with monoclonal antibody, incubated with goat anti-mouse immunoglobulin G alkaline phosphatase conjugate (Sigma Chemical Co.), and then incubated with Sigma phosphatase substrate. Plates were read on a MR 580 Microelisa Auto Reader (Dynatech Instruments) at a wavelength of 405 nm.

RESULTS

Monoclonal antibodies specific for P.II proteins. Sugasawara et al. have shown that McAb 10.1 binds to a P.II protein of strain FA1090 (21). We were interested in determining the specific P.II protein to which this monoclonal antibody binds. To characterize the P.II proteins of strain FA1090, we screened lithium acetate-extracted OM preparations of colonial variants for P.II proteins by SDS-PAGE. Variants were selected either randomly from a parental stock or as survivors of the bactericidal activity of McAb

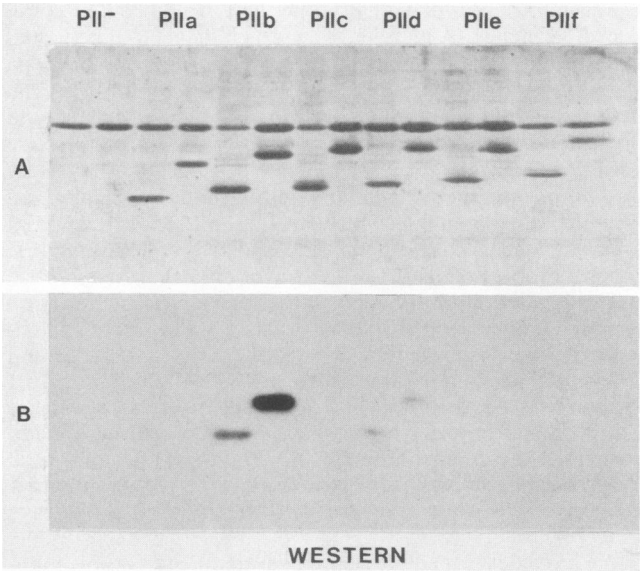

FIG. 2. Binding of McAb 10.1 to strain FA1090 P.IIb and P.IId demonstrated by Western transfer. (A) SDS-PAGE of FA1090 P.II variants. OMs in each pair of lanes were solubilized at 37 (left) and 100°C (right). (B) Autoradiograph of McAb 10.1-probed Western transfer of the gel in (A).

10.1. Among the variants, we found P.II protein bands of six distinct electrophoretic mobilities. We isolated six FA1090 variants, each of which possessed only a single P.II protein band, and a seventh variant which possessed no P.II protein band (Fig. 1). The six P.II proteins were designated P.IIa through P.IIf in order of increasing apparent molecular weight. All of the P.II proteins were heat modifiable (Fig. 2).

P.II proteins were present in both phenotypically opaque and transparent colonial variants, although variants lacking a P.II protein were always transparent. The number of major heat-modifiable bands present in colonial variant OM preparations ranged from zero to three. In addition to the major bands, most colonial variants possessed minor heat-modifiable bands, always of a higher apparent molecular weight than the major bands (data not shown). Protein I (P.I protein), identified in Fig. 1, is another of the major surface proteins of the gonococcus (12). The ratio of P.II to P.I protein, as indicated by electrophoretic band density, differed among the variants.

We used lithium acetate-extracted OM from a variant of strain FA1090 expressing P.IIa to immunize mice for production of a second monoclonal antibody, McAb H138.2. We assayed the binding of both McAb 10.1 and McAb H138.2 to specific P.II protein-bearing variants of FA1090 by colony blot and by ELISA (Table 1). By colony blot, McAb 10.1 bound only to variants possessing either P.IIb or P.IId. McAb H138.2 bound only to P.IIa-expressing variants. The monoclonal antibodies bound to all colonial variants that expressed the appropriate P.II target antigen bands, regardless of how many other P.II protein bands the colonial variant possessed. Analysis by ELISA confirmed the results of the colony blot assay.

The gonococcal antigens to which McAb 10.1 and McAb H138.2 bound were determined by SDS-PAGE and Western transfer. McAb 10.1 bound to SDS-PAGE bands corresponding to the forms of P.IIb and P.IId solubilized at 37 and 100°C; it did not bind to the other P.II protein bands (Fig. 2). There was greater binding of the antibody to P.IIb bands than to P.IId bands. McAb H138.2 bound only to the forms of P.IIa solubilized at 37 and 100°C (Fig. 3). The stained filters indicated equal transfer to and retention on the filters by all P.II protein bands.

McAb 10.1 has previously been typed as an immunoglobulin G2a (18). McAb H138.2 is of the immunoglobulin G3 subclass.

Demonstration of phase variation by colony blot. The P.II protein-specific monoclonal antibodies have enabled us to probe entire populations of gonococcal colonies for the expression of P.II antigens. Using the colony blot radioimmunoassay, we directly demonstrated the existence of a minority population that differs in its expression of P.II proteins. A cloned colonial variant of strain FA1090 that expressed P.IIa was plated, and the colonies were tested for binding of the P.IIb- or P.IId-specific McAb 10.1. Most of the colonies did not bind the antibody (Fig. 4). However, within the population of colonies, there was a subpopulation which bound McAb 10.1, reflecting a change to expression of P.IIb or P.IId.

TABLE 1. Binding of McAb 10.1 and McAb H138.2 to P.II variants of strain FA1090

Protein	McAb 10.1		McAb H138.2	
	Colony blot	ELISA[a]	Colony blot	ELISA
P.II⁻	−	0.018	−	0.002
P.IIa	−	0.032	+	0.115
P.IIb	+	0.672	−	0.003
P.IIc	−	0.000	−	0.001
P.IId	+	0.416	−	0.001
P.IIe	−	0.009	−	0.003
P.IIf	−	0.000	−	0.000

[a] Values show absorbance at 405 nm. Results are average of two experiments.

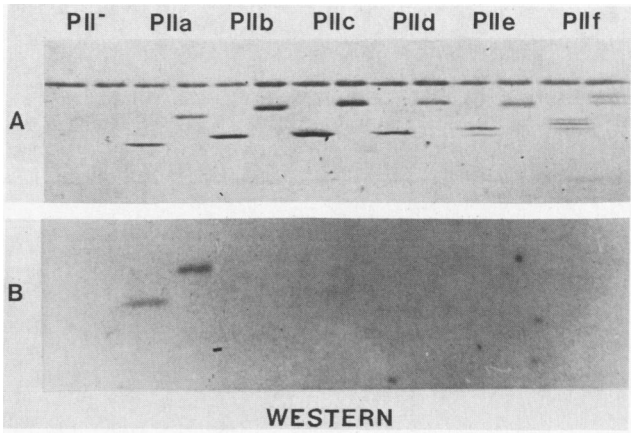

FIG. 3. Binding of McAb H138.2 to FA1090 P.IIa demonstrated by Western transfer. (A) SDS-PAGE of FA1090 P.II variants. OMs in each pair of lanes were solubilized at 37 (left) and 100°C (right). (B) Autoradiograph of McAb H138.2-probed Western transfer of the gel in (A).

Analysis of P.II protein expression by monoclonal antibody bactericidal activity. McAb 10.1 exhibited complement-dependent bactericidal activity against P.IIb-expressing variants of strain FA1090 (Table 2). All other FA1090 variants, including those expressing P.IId, were not susceptible to killing by McAb 10.1. Greater than 99% killing was seen in all bactericidal assays when McAb 10.1 and a colony variant expressing P.IIb were used. Colonial variants expressing P.IIb plus another P.II protein were killed as efficiently as those containing P.IIb alone (Table 2). McAb H138.2 was bactericidal only for variants expressing P.IIa (data not shown).

Next, we determined the distribution of P.II protein species among the minority constituents of a population. The minority constituents were defined as those organisms within a susceptible population which survived the bactericidal activity of a monoclonal antibody. This was routinely less than 1% of a population (Table 2). Variants that expressed P.IIb, or P.IIb in combination with a second P.II protein, were subjected to McAb 10.1 bactericidal activity (Table 3). When the initial variant expressed P.IIb, most of the survivors lacked any P.II protein. When a variant expressing P.IIb in combination with a second P.II protein was used, most of the survivors expressed only the second P.II protein. A small percentage of survivors exhibited other P.II proteins. Those survivors which displayed P.IIb were sensitive to the P.IIb monoclonal antibody after reexposure. In similar experiments with the P.IIa-specific McAb H138.2 and a P.IIa-expressing variant, 23 of 25 survivors expressed no P.II protein; 2 of the survivors expressed other P.II protein species.

DISCUSSION

Gonococcal cell surface components such as the P.II OM proteins have been implicated in pathogenic processes (8,

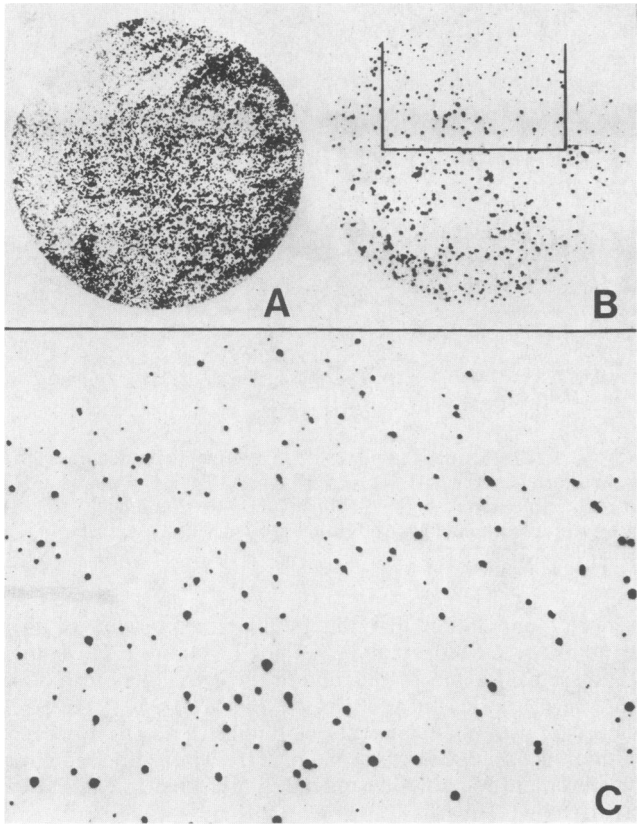

FIG. 4. Colony blot of colonies derived from a P.IIa-expressing variant with McAb 10.1 as probe. (A) Coomassie blue-stained filter indicating colony density. (B) Autoradiograph of colony blot filter probed with McAb 10.1 (C) Enlargement of bracketed area in (B).

16, 27). P.II proteins have been reported to undergo a type of phase variation (17, 25). To date, this variation has been studied primarily through the partially correlated phenotype of colonial opacity (17, 25). We describe here the variant P.II protein forms of gonococcal strain FA1090 and the affinity reagents and techniques that have enabled us to examine P.II protein expression directly.

Six distinct P.II protein forms were found within strain FA1090. This is consistent with the number of different P.II proteins found in other gonococcal strains by Swanson (25) and Heckels (7). McAb 10.1 bound to the species designated P.IIb and P.IId and not to any of the other four P.II proteins. McAb H138.2 bound only to P.IIa. These specificities were suggested by the colony blot and ELISA analyses and were confirmed by the Western transfer experiments. McAb 10.1 may have a greater avidity for P.IIb than for P.IId, as indicated by ELISA and the Western transfer experiments. The difference in signal intensity between P.IIb and P.IId in the Western transfer experiments was probably not due to differences in the efficiency of transfer or retention of the two proteins. Both antibodies bound to P.II antigens that had been processed in very different fashions in each of the assays: dried in the colony blot, glutaraldehyde fixed in the ELISA, and SDS-denatured and electrophoresed in the Western transfer.

The binding of the monoclonal antibodies in the colony blot radioimmunoassay provided a simple method for assaying whole populations of gonococcal colonies for the expression of P.II proteins. Previous analyses of whole populations have depended on the colonial opacity phenotype (17, 25). We examined a population of colonies derived from a cloned variant that expressed P.IIa and thus did not bind P.IIb-specific McAb 10.1, and we demonstrated that within the population there existed minority constituents that did bind the monoclonal antibody.

The monoclonal antibodies McAb 10.1 and McAb H138.4 were bactericidal for P.IIb-expressing variants and P.IIa-expressing variants, respectively. This suggests that these antibodies bind to the native forms of their respective target antigens. The absence of bactericidal activity of McAb 10.1 for FA1090 variants bearing P.IId, despite the presence of such activity for P.IIb-bearing variants, may reflect differences in the avidity of McAb 10.1 for the two P.II proteins, or it may reflect differences in the surface exposure of the two P.II proteins. P.II proteins have been shown to have surface-exposed portions (7, 24), but it is not known whether there are differences in the extent of exposure of different P.II proteins of a strain.

We have exploited the bactericidal activity of McAb 10.1 to analyze P.II protein expression in two ways. First, by demonstrating that colonial variants expressing P.IIb plus a second P.II protein were just as susceptible to monoclonal antibody bactericidal activity as those expressing P.IIb alone, we have demonstrated that one organism is capable of expressing two P.II proteins. If the colonial variants with two P.II proteins represented a mixture of organisms, each expressing a single P.II proteins, then the efficiency of killing by the monoclonal antibody would have been much lower.

Second, we have shown that P.II protein switching in the gonococcus is nonrandom. Surviving minority constituents selected by exposure of a population to the bactericidal effect of a P.II monoclonal antibody were primarily derivatives that switched off a single P.II protein. In this experiment, a switch from a P.IIb to a P.IIb plus any other P.II protein would not be detected since the variant would still be sensitive to P.IIb-specific antibody. In P.IIb-expressing populations, the majority of survivors of killing with a P.IIb-specific antibody expressed no P.II protein. In populations that expressed P.IIb plus a second P.II protein, the survivors of the bactericidal assay generally expressed the second P.II protein only. Thus, among the switching events detected by this method, there was not a random distribution of the possible P.II protein profiles of the minority constituents. The relatively small number of survivors different from the switched-off P.IIb derivatives could reflect variants that underwent two switch events, one that switched off P.IIb and a second that switched on a new P.II protein. Work by Swanson supports this notion of P.II protein switching by single independent steps (25). Alternatively, P.II protein phase variation might involve both a single-step on-off

TABLE 2. Bactericidal activity of FA1090 P.IIb-specific monoclonal antibody

FA1090 P.II profile	Relative survival (%)[a]
P.IIb	<1
P.IIb,a[b]	<1
P.IIb,e	<1
Other non-P.IIb variants	70–120
No P.II	115

[a] Viability calculated as [(CFU after exposure to antibody and complement)/(CFU in controls without antibody and complement)] × 100. Killing occurred only with complement plus antibody; omission of either or heating of the serum at 56°C for 20 min abolished the bactericidal activity.

[b] One variant expressing two P.II proteins.

TABLE 3. P.II profiles of minority populations within P.IIb-expressing colonial variants[a]

Profile before P.IIb antibody exposure (no. of bactericidal expts)	Profile of survivors of bactericidal expts[b]
P.IIb (6)	No P.II, 105; P.IIa, 2; P.IIb, 5; P.IIc, 0; P.IId, 4; P.IIe, 3; P.IIf, 2
P.IIb,e (2)	P.IIe, 41; P.IIe,d, 1
P.IIb,a (2)	P.IIa, 46; P.IIa,d, 1; no P.II, 1

[a] Minority populations were defined as those organisms within a susceptible population which survived the bactericidal activity of a monoclonal antibody.

[b] Values show number of survivors in each category.

switch and a second, though less common, direct change from one P.II protein to another.

Much work has been done on the pathobiological significance of gonococcal P.II OM proteins. Little is known about the regulation of expression of these components. The immunological reagents and techniques described here have facilitated our initial investigations of P.II protein expression. Monoclonal antibodies should be useful tools for characterizing surface variation in a variety of microorganisms.

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LITERATURE CITED

1. **Blake, M. S., E. C. Gotschlich, and J. Swanson.** 1981. Effects of proteolytic enzymes on the outer membrane proteins of *Neisseria gonorrhoeae*. Infect. Immun. **33**:212–222.
2. **Burnette, W. N.** 1981. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulphate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. **112**:195–203.
3. **Draper, D. L., J. F. James, G. F. Brooks, and R. L. Sweet.** 1980. Comparison of virulence markers of peritoneal and fallopian tube isolates with endocervical *Neisseria gonorrhoeae* isolates from women with acute salpingitis. Infect. Immun. **27**:882–888.
4. **Eisenstein, B. I., T. J. Lee, and P. F. Sparling.** 1977. Penicillin sensitivity and serum resistance are independent attributes of strains of *Neisseria gonorrhoeae* causing disseminated gonococcal infection. Infect. Immun. **15**:834–841.
5. **Engvall, E., and P. Perlman.** 1972. Enzyme-linked immunosorbent assay ELISA. III. Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen coated tubes. J. Immunol. **109**:129–135.
6. **Guymon, L. F., D. L. Walstad, and P. F. Sparling.** 1978. Cell envelope alterations in antibiotic-sensitive and -resistant strains of *Neisseria gonorrhoeae*. J. Bacteriol. **136**:391–401.
7. **Heckels, J. E.** 1981. Structural comparison of *Neisseria gonorrhoeae* outer membrane proteins. J. Bacteriol. **145**:736–742.
8. **Heckels, J. E.** 1982. Role of surface proteins in the adhesion of *Neisseria gonorrhoeae*, p. 301–304. *In* D. Schlessinger (ed.), Microbiology—1982. American Society for Microbiology, Washington, D.C.
9. **Henning, U., H. Schwarz, and R. Chen.** 1979. Radioimmunological screening method for specific membrane proteins. Anal. Biochem. **97**:153–157.
10. **James, J. F., and J. Swanson.** 1978. Color/opacity colonial variants of *Neisseria gonorrhoeae* and their relationship to the menstrual cycle, p. 338–343. *In* G. F. Brooks, E. C. Gotschlich, K. K. Holmes, W. D. Sawyer, and F. E. Young (ed.), Immunobiology of *Neisseria gonorrhoeae*. American Society for Microbiology, Washington, D.C.
11. **James, J. F., E. Zurlinden, C. J. Lammel, and G. F. Brooks.** 1982. Relationship of protein I and colonial opacity to serum killing of *Neisseria gonorrhoeae*. J. Infect. Dis. **145**:37–44.
12. **Johnston, K. H., K. K. Holmes, and E. C. Gotschlich.** 1976. Serological classification of *Neisseria gonorrhoeae*. I. Isolation of the outer membrane complex responsible for serotype specificity. J. Exp. Med. **143**:751–758.
13. **Kellogg, D. S., Jr., W. L. Peacock, Jr., W. E. Deacon, L. Brown, and C. I. Pirkle.** 1963. *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. J. Bacteriol. **85**:1274–1279.
14. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
15. **Lambden, P. R., and J. E. Heckels.** 1979. Outer membrane protein composition and colonial morphology of *Neisseria gonorrhoeae* strain P9. FEMS Microbiol. Lett. **5**:263–265.
16. **Lambden, P. R., J. E. Heckels, L. T. James, and P. J. Watt.** 1979. Variations in surface protein composition associated with virulence properties in opacity types of *Neisseria gonorrhoeae*. J. Gen. Microbiol. **114**:305–312.
17. **Mayer, L. W.** 1982. Rates of in vitro changes in gonococcal colony opacity phenotypes. Infect. Immun. **37**:481–485.
18. **Nachamkin, I., J. G. Cannon, and R. S. Mittler.** 1981. Monoclonal antibodies against *Neisseria gonorrhoeae*: production of antibodies directed against a strain-specific cell surface antigen. Infect. Immun. **32**:641–648.
19. **Salit, I. E.** 1982. The differential susceptibility of gonococcal opacity variants to sex hormones. Can. J. Biochem. **60**:301–306.
20. **Sarubbi, F. A., Jr., E. Blackman, and P. F. Sparling.** 1974. Genetic mapping of linked antibiotic resistance loci in *Neisseria gonorrhoeae*. J. Bacteriol. **120**:1284–1292.
21. **Sugasawara, R. J., J. G. Cannon, W. J. Black, I. Nachamkin, R. L. Sweet, and G. F. Brooks.** 1983. Inhibition of *Neisseria gonorrhoeae* attachment to HeLa cells with monoclonal antibody directed against a protein II. Infect. Immun. **42**:980–985.
22. **Swanson, J.** 1978. Studies on gonococcus infection. XIV. Cell wall protein differences among color/opacity colony variants of *Neisseria gonorrhoeae*. Infect. Immun. **21**:292–302.
23. **Swanson, J.** 1980. ^{125}I-labeled peptide mapping of some heat-modifiable proteins of the gonococcal outer membrane. Infect. Immun. **28**:54–64.
24. **Swanson, J.** 1981. Surface-exposed protein antigens of the gonococcal outer membrane. Infect. Immun. **34**:804–816.
25. **Swanson, J.** 1982. Colony opacity and protein II compositions of gonococci. Infect. Immun. **37**:359–368.
26. **Swanson, J., and O. Barrera.** 1983. Immunological characteristics of gonococcal outer membrane protein II assessed by immunoprecipitation, immunoblotting, and coagglutination. J. Exp. Med. **157**:1405–1420.
27. **Virji, M., and J. S. Everson.** 1981. Comparative virulence of opacity variants of *Neisseria gonorrhoeae* strain P9. Infect. Immun. **31**:965–970.
28. **Walstad, D. L., L. F. Guymon, and P. F. Sparling.** 1977. Altered outer membrane protein in different colonial types of *Neisseria gonorrhoeae*. J. Bacteriol. **129**:1623–1627.