INFECTION AND IMMUNITY, July 1986, p. 229–233
0019-9567/86/070229-05$02.00/0

Vol. 53, No. 1

A Common Neisserial Antigen Evidenced by Immunization of Mice with Live *Neisseria meningitidis*

PAUL M. V. MARTIN,[1]* ALFREDO LAVITOLA,[1]† LEILA AOUN,[1] ROSEMARY ANCELLE,[2] ANNE C. CREMIEUX,[2] AND JEAN Y. RIOU[1]

Unité d'Ecologie Bactérienne, Institut Pasteur, 75015 Paris, France[1]; and Institut National de la Santé et de la Recherche Médicale, U13, IMET, Hopital Claude Bernard, 75019 Paris, France[2]

Received 20 December 1985/Accepted 18 March 1986

Mice immunized with live meningococci developed antibodies directed against various antigens of *Neisseria gonorrhoeae* and *Neisseria meningitidis*, as demonstrated by immunoblotting. An antigen of 70 kilodaltons appeared to be common and stable in all gonococcal strains tested and highly immunogenic in mice infected with *N. meningitidis*.

Numerous studies on *Neisseria gonorrhoeae* have shown a high variability of surface-exposed structures, from strain to strain and among different variants of the same strain (5, 16, 24). Such diversity, observed on in vitro-grown organisms, has been demonstrated in vivo in animal models (17, 21) and in human partners (30). Changes of surface antigens could obviously play a role in both adaptation of the organisms to host environment as receptors and avoidance of host defences. This largely explains the failure of gonococcal vaccines. Stability is of crucial necessity for an antigen to become a reliable vaccine. On an other hand, the fact that humans can develop several gonococcal attacks probably reflects (i) the high variability of protective antigens as pili (4) or outer membrane proteins—proteins I and II (4, 6)—and (ii) the low immunogenicity of constant and stable antigens present in all gonococcal strains, as demonstrated for conserved protein portions in pili (23). Also, early treatment and eradication in males and their female partners, as well as the strict location of the disease to the genital tract in most cases, can account for the lack of a sufficient immune response directed against the constant outer membrane antigens, providing these antigens would play a role in protection against a second gonococcal infection.

N. gonorrhoeae and *Neisseria meningitidis* present a high degree of DNA-DNA homology, as evidenced by hybridization experiments (21). Since the numerous antigenic relationships described by Geizer (11) on whole bacterial extracts, several antigenically related surface-exposed structures were studied. Sensitivity to a set of R-type pyocins from *Pseudomonas aeruginosa* demonstrated similar structures in lipopolysaccharides (LPS) (3). By using monoclonal antibodies, epitope identities were shown in LPS antigens (1), outer membrane proteins of different molecular weights (9), and pili (28) from both species. Immunoglobulin A1 (IgA1) proteases share structural communities, as demonstrated by nucleotide sequence homology between the IgA1 protease genes of both gonococci and meningococci (14). More recently, quantitative comparisons of 48 selected polypeptidic spots, derived from *N. gonorrhoeae* with high-resolution two-dimensional polycarylamide gel electrophoresis, showed from 36 to 57% resemblance between the gonococcus and representative strains of meningococci (13). Because immunization of mice by *N. meningitidis* elicited bactericidal antibodies against *N. gonorrhoeae* (10), we used this animal model as a tool to determine the most commonly cross-reacting antigenic determinants of both species.

Cultures of *N. meningitidis* N1912 (serogroup A), N1913 (serogroup B), F2 (B, serotype 2), F9 (B, serotype 9), N4573 (serogroup C), N4578 (serogroup Y), N1982, N1505, N1309 (non groupable strains), and N5425 (a noncapsulated mutant of group B) were grown overnight on Gonomeningo (GC) agar base (Institut Pasteur Production) at 37°C in an candle instinction jar. Serotype 2 (T2), 4 (T4), 6 (T6), 7 (T7), 8 (T8), and 9 (T9) reference strains of *N. gonorrhoeae* (7); *N. gonorrhoeae* N4007 (Proline auxotype) and N4024 (zero auxotype), *N. mucosa* N405, *N. perflava* N407, *N. sicca* N408, *N. flava* N410 and *N. lactamica* N4627, N1043, N841, and N167 were cultured in the same conditions. One strain each of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* and *Yersinia enterocolitica* N381 and WA were cultured on Mueller-Hinton agar (Institut Pasteur). Pathogen-free Swiss mice (Institut Pasteur) were immunized at 7-day intervals by subcutaneous injections of about 10^8 live meningococci (either N1912 group A or F2 group B) suspended in a chemically defined medium (27). Paired mice were bled by retroorbital puncture before each immunization procedure on days 1, 21, 28, and 41.

Paired sera were pooled to yield a sufficient quantity, assayed for bactericidal antibodies without extrinsic source of complement by a micromethod (19), and frozen at −70°C. Duplicate serial dilutions of mice serum were made in defined medium, and T2 gonococci were added (2×10^4 to 4×10^4 organisms per ml). The mixture was incubated for 40 min at 37°C and plated on agar. Bactericidal antibody titers were taken as the highest serum dilution which killed more than 50% of the inoculum. Experiments with heated (1 h, 56°C) mice serum were performed in parallel, as a control of the killing reaction. Antigenic preparations were performed as follows. Bacteria were suspended (1×10^9 to 2×10^9 per ml) in saline and sonicated for six times for 30 s in a Sonifier cell disruptor (B-30; Branson Sonic Power Co.; output power, 3), until a clear suspension was obtained. The sonicated suspensions were centrifuged (10 min, 10,000 × *g*), and the pellet was discarded. The supernatant was assayed for protein content by the method of Lowry et al. (18) and kept at −20°C until used. Antigens (20 μg of protein) were applied to a sodium dodecyl sulfate–12.5% polyacryl-

* Corresponding author.
† Permanent address: Cattedra di Microbiologia, Departement di Biologia e pathologia cellulare e moleculare, 2d Facolta de medicine e chirurgia, 80131 Napoli, Italy.

TABLE 1. Bactericidal antibody titers of sera from mice immunized with *N. meningitidis* against *N. gonorrhoeae* strain T2

Mouse serum no.[a]	Reciprocal of bactericidal antibody titer postimmunization[b]				
	Day 1	Day 7	Day 21	Day 28	Day 41
Expt. 1[c]					
1	<4 (1×10^8)	<4 (2×10^8)	<4 (1×10^9)	>32 (1.6×10^8)	64
2	4 (1×10^8)	<4 (2×10^8)	<4 (1×10^9)	>32 (1.6×10^8)	64
3	4 (1×10^8)	<4 (2×10^8)	<4 (1×10^9)	32–64 (1.6×10^8)	>128
4	8 (1×10^8)	<4 (2×10^8)	<4 (1×10^9)	64 (1.6×10^8)	>128
5	<4 (1×10^8)	<4 (2×10^8)	<4 (1×10^9)	32–64 (1.6×10^8)	64
Expt. 2[d]					
6	<8 (1×10^9)	NT (4×10^8)	64 (2×10^8)	32 (4×10^8)	128
7	<8 (1×10^9)	NT (4×10^8)	128 (2×10^8)	64 (4×10^8)	128
8	<8 (1×10^9)	NT (4×10^8)	64 (2×10^8)	NT (4×10^8)	128
9[e]	<8 (1×10^9)	NT (4×10^8)	<8 (2×10^8)	<8 (4×10^8)	<8
10	<8 (1.4×10^9)	NT (4×10^8)	8 (3×10^8)	64 (4×10^8)	32
11	<8 (1.4×10^9)	NT (4×10^8)	16 (3×10^8)	>256 (4×10^8)	>256
12	NT (1.4×10^9)	NT (4×10^8)	NT (3×10^8)	NT (4×10^8)	NT

[a] Blood samples from two mice were pooled after each bleeding.
[b] Inoculum size is given in parentheses. Number of live *N. meningitidis* organisms subcutaneously injected. Mice were immunized at day 14 with 1×10^8 for experiment 1 and with 3.2×10^8 organisms for experiment 2. NT, Not tested.
[c] Paired mice (one to five) were immunized with serogroup A *N. meningitidis*.
[d] Paired mice were immunized with group A (6 to 8), serogroup B (10 to 12) *N. meningitidis*. Mice in serum group 9 were nonimmunized controls.
[e] Nonimmunized controls (paired mice).

amide gel, and electrophoresis was performed by the discontinuous buffer system of Laemmli (15). Gels were stained for protein visualization with Coomassie brilliant blue and for LPS with the silver stain method of Tsai and Frasch (26). Migration of proteins of known molecular weight (Pharmacia Fine Chemicals, Uppsala, Sweden) indicated the apparent molecular weight of the different antigens. Electrotransfer to nitrocellulose (BA 85, 0.45 μm; Schleicher and Schüll, Dassel, Federal Republic of Germany) was done as described by Burnette (8) at 200 mA for 2 h in a Bio-Rad Transblot cell. After overnight exposure to a 1/200 dilution of immune serum in phosphate-buffered saline–0.5% (wt/vol) bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.), nitrocellulose foils were washed, incubated with radioiodinated protein A (0.05 μCi/ml), extensively dried, and autoradiographed with Kodak XR-5 films for 24 h to 3 days at −80°C.

Table 1 shows the rise in bactericidal antibody titer against serotype 2 *N. gonorrhoeae* T2. The bactericidal antibody titer rose on day 28 in the first experiment and at least 1 week earlier in the second experiment. Controls did not show a rise in bactericidal antibody titers. For technical reasons, sera no. 12 were not tested. To determine which cross-reacting antibodies are directed, each pair of sera from nos. 1 to 12, obtained on days 21, 28, and 31, was tested at a 1/200 dilution against crude antigenic preparations from group A (N1912) or B (F2) meningococci and serotype 2 gonococci (T2) by immunoblotting. Typical of all tested sera, cross-reacting antibodies appeared to be preferentially directed against one antigen with an apparent molecular weight of 70,000 (70K antigen) (Fig. 1, arrow). Antibodies reacting against the homologous immunizing strain (Fig. 1, lane 11) were mainly directed against LPS (bottom), major outer membrane proteins of 28K to 40K, and an antigen of 20K. When tested against gonococcal (lane 10) or group A meningococcal (lane 12) antigenic preparations, sera strongly reacted with one, two, or three antigens located in the 17K-to-35K zone and loosely with several other antigens (Fig. 1 and 2). But with all tested sera, the 70K antigen was regularly evidenced by immunoblotting on group A and B meningococci and the T2 strain of gonococcus. When 12.5% polyacrylamide gels were stained by Coomassie brilliant blue, 4 faint bands appeared in that region, whereas numerous (about 70) other peptidic bands were visualized.

In Fig. 1, the slightly stained band just beyond the 70K antigen (apparent molecular weight, 66,000) was frequently but not always recovered. Polyacrylamide gels were also silver stained for LPS by the method of Tsai and Frasch, and LPS was present in all three antigenic preparations at an apparent molecular weight of less than 14,000. Sera from mice immunized with group B *N. meningitidis* organisms showed cross-reacting antibodies directed against LPS of group A meningococci (Fig. 1), but not of *N. gonorrhoeae* T2. Anti-LPS antibodies were detected by Western blotting at day 21, in sera nos. 6, 7, 10, and 12 (Table 1) but were

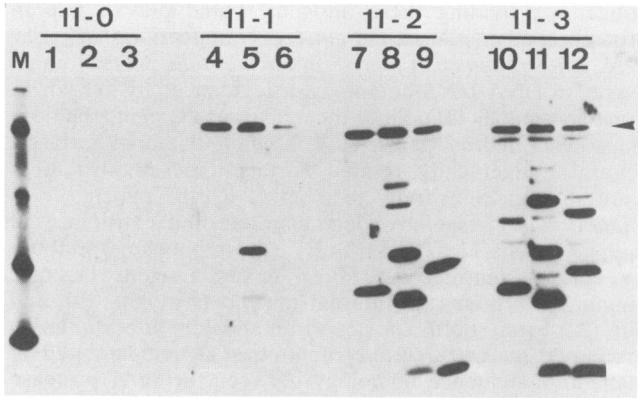
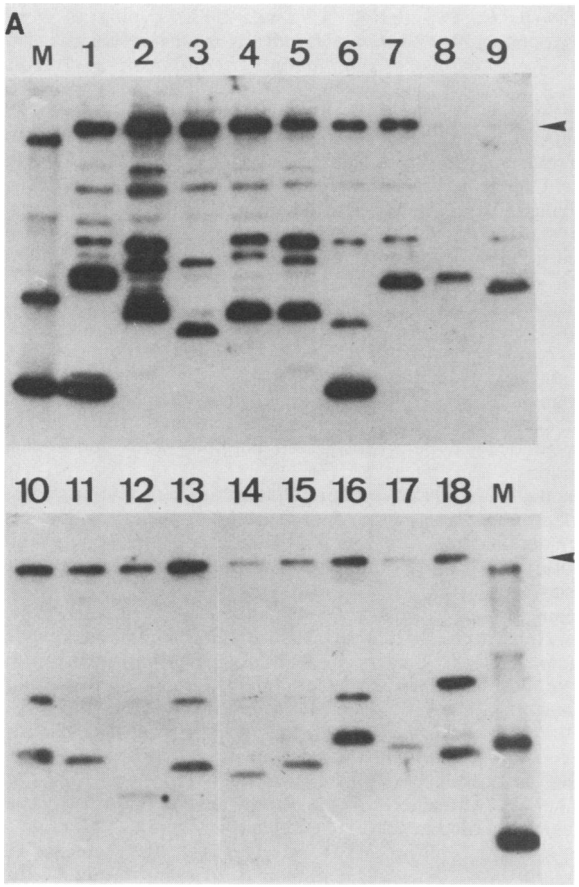


FIG. 1. Autoradiogram of immunoelectrophoretic transfer of gonococcal and meningococcal antigenic preparations. Sonicated bacteria were solubilized at 100°C, subjected to sodium dodecyl sulfate–12.5% polyacrylamide gel electrophoresis and Western transfer. Lanes: 1, 4, 7, and 10, *N. gonorrhoeae* T2; 2, 5, 8, and 11, *N. meningitidis* F2 (group B); 3, 6, 9, and 12, *N. meningitidis* N1912 (group A); M, molecular mass markers of 115, 67, 37, 22.5, and 14.4 kilodaltons. All lanes were developed with a 1/200 dilution of mice serum (no. 11; see Table 1) obtained on day 1 (11-0), day 21 (11-1), day 28 (11-2), and day 41 (11-3). This autoradiogram was developed for 65 h and is typical of six experiments.



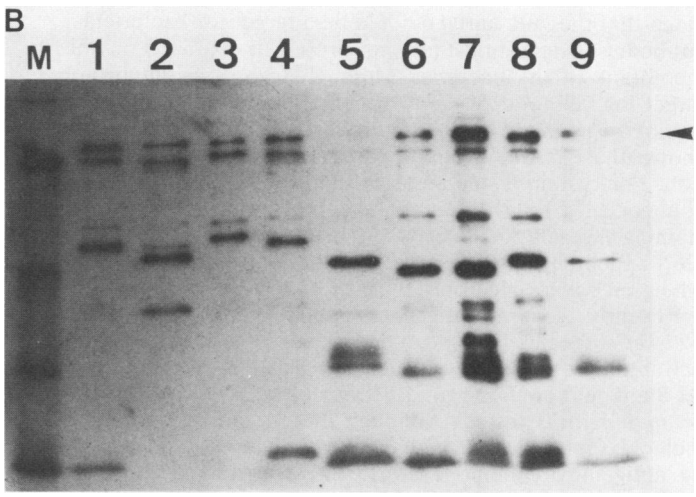


FIG. 2. Autoradiograms of immunoelectrophoretic transfer of *Neisseria* spp. antigenic preparations. Sonicated bacteria were solubilized at 100°C and subjected to 12.5% sodium dodecyl sulfate-12.5% polyacrylamide gel electrophoresis and Western transfer. (A) Lanes: 1, N1912; 2, F2; 3, F9; 4, N1943; 5, N4573; 6, N4578; 7, N1982; 8, N1505; 9, N1309; 10, N5425; 11, T2; 12, T4; 13, T6; 14, T7; 15, T8; 16, T9; 17, N4007; 18, N4024. (B) Lanes: 1, N405; 2, N407; 3, N408; 4, N410; 5, N4627; 6, N1043; 7, N841; 8, N167; 9, N4625; M, molecular mass markers (see legend to Fig. 1). All lanes were developed with a 1/200 dilution of mice serum (no. 11; see Table 1) obtained on day 41. Autoradiograms were developed for 65 h.

directed against meningococcal, and not gonococcal, antigenic extracts (data not shown). Antigenic preparations from 10 strains of *N. meningitidis*, 8 strains of *N. gonorrhoeae* of different geographical and epidemiological origins, and 1 strain each of *N. flava*, *N. mucosa*, *N. sicca*, and *N. perflava*, and 4 strains of *N. lactamica*, representative of nonpathogenic *Neisseria* species, were applied to a 12.5% polyacrylamide gel and transferred to nitrocellulose filters. Nitrocellulose was allowed to react with a 1/200 dilution of hyperimmune mice serum (Fig. 1, lanes 10 through 12), and cross-reacting antibodies were revealed by radioiodinated protein A. All strains of *N. gonorrhoeae* possessed the 70K antigen (Fig. 2A). Other cross-reacting antigens were variable in molecular weight from strain to strain. Of the nine strains of *N. meningitidis*, the six groupable strains exhibited the 70K antigen (Fig. 2A, lanes 2 to 7). Of the three nongroupable strains (lanes 8 to 10), N1505 did not possess the 70K antigen, whereas N1309 and N1982 did. Figure 2B shows that all but one (*N. lactamica*) nonpathogenic species possessed the 70K antigen. None of the antigenic preparations obtained from non-*Neisseria* species contained cross-reacting antigens in the 70K region.

Immunization of mice with live serogroup A or B meningococci elicited cross-reacting antibodies directed against several gonococcal antigens. Of 22 immunized mice whose paired sera were tested by immunoblotting, all exhibited antibodies for a 70K antigen. Only this antigen was regularly evidenced by cross-reacting antibodies. Other antigens revealed by this method were proteins of different molecular weights, comprised between 17K and 35K. Antigens of 17K to 35K from *N. gonorrhoeae* strains (Fig. 2A) are of variable molecular weight from strain to strain, and do not always correspond to antigens of *N. meningitidis* (immunizing strain) of the same molecular weights (Fig. 1). This either indicates that proteins from both species, but of different apparent molecular weight, share common epitopes, as described for the H:8 antigen (9), or reflects a quantitative difference in the production of the same protein by *N. gonorrhoeae* and *N. meningitidis*. More interestingly, when mouse sera were tested against antigens obtained from a representative series of *Neisseria* strains, all but one strain of nonagglutinable *N. meningitidis* (and one strain of *N. lactamica*) possessed the 70K antigen. Proteins in the same region were stained by Coomassie brilliant blue and not by LPS silver stain, indicating a probable peptidic nature of the 70K antigen. Whether the fact that one nonagglutinable meningococcus lacked this protein is interesting in regard to pathogenicity should be further investigated on a larger number of strains.

Table 1 shows that sera tested at day 21 (second experiment) were always bactericidal for *N. gonorrhoeae* T2. In sera nos. 7, 8, and 11, cross-reacting antibodies evidenced by the immunoblotting procedure were directed only against the 70K common antigen in gonococci.

Further investigations on these three polyclonal sera were done to insure their specificity. First, transfer from gel to nitrocellulose was performed in the presence of dipolar ionic detergent (Calbiochem-Behring, La Jolla, Calif.), by the method of Mandrell et al. (19). Second, IgG anti-mouse IgM (Nordic I.L., Tilburg, The Netherlands) were reacted with nitrocellulose sheets after incubation with mouse immune sera and before the addition of radiolabeled protein A. In both experiments, no supplementary band was visualized; the 70K antigen was evidenced, i.e., early immune antisera contained IgM directed only against it. This possibly indi-

cated that the 70K antigen could be a target for bactericidal antibodies, suggesting that this protein is exposed on the membrane of the bacteria. Although LPS is an important target for killing of *N. gonorrhoeae* by antibodies to either *N. gonorrhoeae* or *N. meningitidis*, Tramont et al. (25) have shown that surface proteins participate in the killing reaction. For certain tested sera, antibodies cross-reacting with gonococcal LPS (bottom of the blots, as controlled by staining the gels for LPS by the method of Tsai and Frash [26]) appeared only after the third or fourth immunization, when mice were bled on day 28 or 41.

Recently, several antigens common to most pathogenic *Neisseria* species have been described. By use of monoclonal antibodies, Cannon et al. (9) described a 20K protein (H-8 antigen) common to all strains of *N. gonorrhoeae* and *N. meningitidis* tested. Although the H-8 antigen varies in molecular mass between 20 and 30 kilodaltons, it appears to be antigenically homogeneous. There was consistently a positive band in the region between 20 and 30 kilodaltons, except for four nonpathogenic *Neisseria* species (Fig. 2B, lanes 1 to 4). It is likely that the antigen detected in that region is the H-8 antigen. Other cross-reacting antigens were described as LPS (1), pili (28), and other proteins. Numbers of pathogenic *Neisseria* species produce and excrete an IgA1 protease which cleaves human IgA1 heavy chains at the hinge region. First studies by Blake and Swanson (2) on purification of gonococcal IgA1 protease described active proteins with apparent molecular weights of 65,000 and 70,000. More recent studies by Halter et al. (12) showed that highly purified extracellular IgA1 protease has a molecular weight of 105,000. Improved methods of enzyme purification and detection, as well as use of monoclonal antibodies (M. S. Blake and E. C. Gotschlich, 1984, Meeting on Pathogenic *Neisseria*, Asilomar, Calif., abstr. no. 34), demonstrated IgA1 protease activity in proteins of 110K and 65K. The small-molecular-weight fragment probably contains the enzyme activity site. Whether the common 70K protein we describe in this paper is related to an IgA1 protease fragment should be investigated. However, it should be noted that although we did not recover the 70K antigen in sonicates from one strain of nonagglutinable meningococcus, most nonpathogenic *Neisseria* species tested exhibited the 70K antigen. West and Sparling recently described (29) an iron-repressible protein of 70K that was present in crude membrane extracts from two strains of *N. gonorrhoeae* and was expressed when the bacteria were grown in the presence of either Desferal or a low iron saturation of transferrin. However, when 12 recent clinical isolates were tested for the presence of this protein, only 50% of them exhibited it (29).

An antigen common to all pathogenic *Neisseria* strains has an obvious interest for immunoprophylaxis. More investigations on its nature, function, surface exposure, and in vivo expression and stability are necessary to ascertain its possible interest. Are the results obtained in mice with the 70K antigen significant in the human situation? This is the purpose of future work.

LITERATURE CITED

1. **Apicella, M. A., T. M. Bennet, C. A. Hermerath, and D. E. Roberts.** 1981. Monoclonal antibodies analysis of lipopolysaccharide from *Neisseria gonorrhoeae* and *Neisseria meningitidis*. Infect. Immun. **34**:751–756.
2. **Blake, M. S., and J. Swanson.** 1978. Studies on gonococcal immunoglobulin A protease, p. 285–289. *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.
3. **Blackwell, C. C., and J. A. Law.** 1981. Typing of nonserogroupable *Neisseria meningitidis* by means of sensitivity to R-type pyocines of *Pseudomonas aeruginosa*. J. Infect. **3**:370–378.
4. **Buchanan, T. M., D. A. Eschenback, J. S. Knapp, and K. K. Holmes.** 1980. Gonococcal salpingitis is less likely to recur with *Neisseria gonorrhoeae* of the same principal outer membrane protein antigenic type. Am. J. Obstet. Gynecol. **138**:978–980.
5. **Buchanan, T. M., and J. F. Hildebrandt.** 1981. Antigenic specific serotyping of *Neisseria gonorrhoeae*: characterization based upon principal outer membrane protein. Infect. Immun. **32**:985–994.
6. **Buchanan, T. M., W. A. Pearce, G. K. Schoolnick, and R. J. Arko.** 1977. Protection against infection with *Neisseria gonorrhoeae* by immunization with outer membrane protein complex and purified pili. J. Infect. Dis. **136**:S132.
7. **Buchanan, T. M., J. Swanson, K. K. Holmes, S. L. Kraus, and E. C. Gotschlich.** 1973. Quantitative determination of antibody with gonococcal pili. Changes in antibody with gonococcal infection. J. Cli. Invest. **52**:2896.
8. **Burnette, W. N.** 1981. "Western blotting" electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. **12**:195–203.
9. **Cannon, J. G., W. J. Black, I. Nachamkin, and P. Stewart.** 1984. Monoclonal antibody that recognize an outer membrane antigen common to the pathogenic *Neisseria* species but not to most nonpathogenic *Neisseria* species. Infect. Immun. **43**:994–999.
10. **Cremieux, A. C., A. Puissant, R. Ancelle, and P. Martin.** 1984. Bactericidal antibodies against *Neisseria gonorrhoeae* elicited by *Neisseria meningitidis*. Lancet **ii**:930.
11. **Geizer, I.** 1977. Typing of the gonococcus, p. 49–65. *In* F. A. Skinner, P. D. Walker, and H. Smith, (ed.), Gonorrhoea, epidemiology and pathogenesis. Academic Press, Inc., London.
12. **Halter, R., J. Polher, and T. Meyer.** 1984. IgA protease of *Neisseria gonorrhoeae*: isolation and characterisation of the gene and its extracellular product. EMBO J. **3**:1595–1601.
13. **Jackson, P., M. J. Thornley, and R. J. Thompson.** 1984. A study by high-resolution two-dimensional polyacrylamide gel electrophoresis of relationships between *Neisseria gonorrhoeae* and other bacteria. J. Gen. Microbiol. **130**:3189–3201.
14. **Koomey, J. M., and S. Falkow.** 1984. Nucleotide sequence homology between the immunoglobulin A1 protease genes of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Haemophilus influenzae*. Infect. Immun. **43**:101–107.
15. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
16. **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.
17. **Lambden, P. R., J. E. Heckels, L. T. James, and P. J. Watt.** 1979. Variation in surface protein composition associated with virulence properties in opacity types of *Neisseria gonorrhoeae*. J. Gen. Microbiol. **114**:305–312.
18. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
19. **Mandrell, R. E., and W. D. Zollinger.** 1984. Use of a Zwitterionic detergent for the restoration of antibody-binding capacity of electroblotted meningococcal outer membrane protein. J. Immunol. Methods **67**:1–11.
20. **Martin, P. M. V., P. V. Patel, N. J. Parsons, and H. Smith.** 1983. Induction of serum resistance in recent isolates of *Neisseria gonorrhoeae* by a low molecular weight fraction of guinea pig serum. J. Infect. Dis. **148**:334.
21. **Penn, C. W., N. J. Parsons, D. R. Veale, and H. Smith.** 1978. Correlation with different immunotypes of gonococcal antigens associated with growth *in vivo*. J. Gen. Microbiol. **105**:153–157.
22. **Riou, J. Y., M. Guibourdenche, and M. Y. Popoff.** 1983. A new taxon in the genus *Neisseria*. Ann. Microbiol. Inst. Pasteur (Paris) **105**:153–157.

23. **Rothbard, J. B., R. Fernandez, and G. K. Schoolnick.** 1984. Strain-specific and common epitopes of gonococcal pili. J. Exp. Med. **160**:208–221.
24. **Swanson, J.** 1978. Studies on gonococcal infection. XIV. Cell wall protein difference among color/opacity variants of *Neisseria gonorrhoeae*. Infect. Immun. **21**:292–302.
25. **Tramont, J. C., J. C. Sadoff, and M. S. Artenstein.** 1974. Cross-reactivity of *Neisseria gonorrhoeae* and *Neisseria meningitidis* and the nature of the antigens involved in the bactericidal reaction. J. Infect. Dis. **130**:240–247.
26. **Tsai, C. M., and C. E. Frasch.** 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. **19**:115–119.
27. **Veale, D. R., C. W. Penn, and H. Smith.** 1981. Factors affecting the induction of phenotypically determined serum resistance of *Neisseria gonorrhoeae* grown in media containing serum or its diffusible components. J. Gen. Microbiol. **122**:235–245.
28. **Virji, M., and J. E. Heckels.** 1983. Antigenic cross-reactivity of *Neisseria* pili: investigations with type- and species-specific monoclonal antibodies. J. Gen. Microbiol. **129**:2761–2768.
29. **West, S. E. H., and P. F. Sparling.** 1985. Response of *Neisseria gonorrhoeae* to iron limitation: alterations in expression of membrane proteins without apparent siderophore production. Infect. Immun. **47**:388–394.
30. **Zak, K., J. L. Diaz, D. Jackson, and J. E. Heckels.** 1984. Antigenic variation during infection with *Neisseria gonorrhoeae*: detection of antibodies to surface proteins in sera of patients with gonorrhoea. J. Infect. Dis. **149**:166–173.