

## NOTES

### Variation of *Neisseria gonorrhoeae* Protein II among Isolates from an Outbreak Caused by a Single Gonococcal Strain

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Gonococci isolated from localized urogenital infections usually possess one or more protein II (P.II) species in the outer membrane, but the structural and antigenic variation of these proteins among different gonococcal strains has made it difficult to determine if specific proteins of the P.II class are associated with particular sites or types of infection. A recent outbreak of gonorrhoea in Durham, N.C., was unusual in that over 200 isolates were derived from a single strain, which provided an opportunity to address these questions. The P.II profile of 54 isolates was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of outer membrane proteins. At least seven distinct P.II proteins were seen; no single protein or combination of proteins predominated in the different isolates, and there was no association of P.II profile with site of isolation. Gonococci recovered from the same patient at different times had different P.II profiles, confirming that P.II variation occurred in vivo.

The protein II (P.II) species of *Neisseria gonorrhoeae* are a family of heat-modifiable, outer membrane proteins that undergo a complex type of phase and antigenic variation. Some strains have been shown to produce at least six electrophoretically distinct P.II proteins (1, 13). A single organism is capable of expressing from zero to at least three P.II species in different combinations (8, 13, 16), and spontaneous changes in the state of P.II expression occur at a high frequency (11). Gonococci possessing certain P.II proteins often show increased colonial photo-opacity (8, 14), slightly increased resistance to some antibiotics (9), better attachment to buccal epithelial cells (9), altered serum resistance (6, 9), and decreased sensitivity to steroid hormones (12).

Gonococci expressing P.II are associated with infections localized to the urogenital mucosa. Organisms isolated from uncomplicated infection in men or at particular times in the menstrual cycle in women often express one or more P.II proteins (5, 17). Gonococci isolated from a disseminated gonococcal infection generally lack P.II (3, 5). However, because of the variation in electrophoretic mobility and antigenicity of P.II proteins of different strains (15), it has been difficult to draw conclusions about the extent of P.II variation in vivo or about the possible association of a specific P.II with the site of isolation or type of infection. Zak et al. (17) recently characterized P.II profiles of strains from seven groups of sexual partners; gonococci from the same group (up to three partners) were derived from the same strain but had differences in P.II expression. That study confirmed that P.II variation does occur in vivo, but because of the limited number of isolates available from each strain, it was impossible to draw conclusions about the number of different P.II proteins that were expressed by

each strain or about the infection site specificity of P.II expression.

An outbreak of penicillin-resistant gonorrhoea that occurred in 1983 in Durham, N.C. (4), gave us an opportunity to address some of these questions about in vivo expression of P.II. Nearly 200 of the isolates collected during this outbreak were derived from a single strain. We examined the P.II profiles of some of these isolates by sodium dodecyl sulfate-polyacrylamide gel electrophoresis to assess the extent of P.II variation in this collection.

All samples surveyed met the following criteria for belonging to the same strain: identical antibiotic MICs, the same protein I monoclonal antibody serovar (WII/WIII, serovariant IB-1) and apparent molecular weight by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and identical nutritional requirements (proline auxotrophs). The clinical and epidemiological features of the outbreak and the methods used in strain typing will be described separately (H. Faruki, R. N. Kohmescher, W. P. McKinney, and P. F. Sparling, submitted for publication). To minimize shifts in P.II profile occurring during passage in the laboratory, samples were passaged only once after initial isolation and frozen at  $-70^{\circ}\text{C}$ . Gonococci were grown on Difco GCB agar with the supplements of Kellogg et al. (7) for 18 to 20 h at  $37^{\circ}\text{C}$  in 4%  $\text{CO}_2$ . Colony phenotypes were determined by examining colonies with a dissecting microscope, using the criteria of Swanson (13).

An additional qualification for inclusion in this survey was that at least 90% of the colonies from a sample be of the same photo-opacity when examined with a dissecting microscope. Since colonial opacity is often associated with P.II expression, a sample containing a mixture of opacity variants could contain organisms expressing different P.II species. Heterogeneous P.II populations would be difficult to interpret, as it could be possible that some of the organisms changed P.II expression after initial isolation. Approximately 20% of the

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FIG. 1. P.II profiles of eight penicillin-resistant isolates (lanes A through H) recovered from male patients. Materials in lanes A through H were solubilized at 37°C for 60 min, and lanes A' through H' show the same preparations solubilized at 100°C for 5 min. The P.II proteins in lanes F and G showed the same migration after 37°C solubilization but migrated with different apparent molecular weights after 100°C solubilization. Therefore, they are distinct P.II species. Molecular weight standards (lane S): phospholipase B, 94,000; bovine serum albumin, 68,000; ovalbumin, 43,000; carbonic anhydrase, 30,000; soybean trypsin inhibitor, 20,100; and lysozyme, 14,300. P.I, Protein I.

TABLE 1. P.II expression in isolates from randomly chosen male and female patients from the Durham, N.C., outbreak of gonorrhoea

Source	No. of isolates examined	No. of different P.II proteins	No. of different combinations of P.II proteins	No. of P.II proteins expressed per isolate
Male urethra	13	6	12	1-3
Female cervix	13	7	13	0-3

clinical isolates displayed a mixed colonial opacity and were not studied further.

Outer membranes were isolated by rapid passage of gonococci through a 22-gauge needle in 200 mM lithium acetate buffer, pH 6.0 (8). Cellular debris was centrifuged and discarded. Outer membrane blebs were pelleted by centrifugation at  $100,000 \times g$  for 2 h at 4°C. Pellets were suspended in water and stored at -70°C. Samples containing approximately 25 µg of protein were solubilized at 37°C for 60 min or at 100°C for 5 min and were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 4 to 30% gradient gels with Laemmli buffers (10) as previously described (1). Gels were stained with Coomassie brilliant blue.

We characterized urethral isolates from randomly chosen male patients, cervical isolates from randomly chosen fe-

TABLE 2. Pairs of strains isolated from two infected sites of one patient or from the same site at two different times

Source	No. of pairs examined	No. of pairs with identical P.II proteins	No. of pairs with one P.II the same	No. of pairs with different P.II proteins
Same patient, different sites <sup>a</sup>	7	3	2	2
Same patient, different time <sup>b</sup>	7	0	1	6

<sup>a</sup> Cervical and urethral isolates obtained at the same time.

<sup>b</sup> Time interval between samples ranged from 5 days to 2 months.

male patients isolates from different anatomical sites of the same patient (cervical and urethral isolates from females), and isolates from the same patient over a period of time (treatment failures or repeat infections). At least seven electrophoretically distinct P.II species were seen in this collection of 54 isolates. With the exception of one isolate from a female patient that produced no P.II protein, all of the gonococcal isolates examined produced from one to three P.II species. Figure 1 shows representative profiles of isolates from male patients. Results of other comparisons are summarized in Tables 1 and 2. Isolates from a single patient at different times were heterogeneous in P.II composition. A more homogeneous pattern was observed with isolates from two infected sites of a single patient. Of the seven pairs examined, three had identical P.II patterns; in two other pairs, one of the two P.IIs was identical in apparent molecular weight. There was no detectable association of particular P.II species with the sex of the host or the site of isolation.

These results confirm and extend those of Zak et al. (17), who demonstrated that P.II variation of gonococci does occur in vivo. By analyzing a larger number of isolates derived from a single gonococcal strain, we were able to test the hypothesis that differences in P.II profile of a strain may be associated with differences in its ability to establish or maintain infection in particular microenvironments within the host. If different P.II proteins conferred different tissue tropisms upon gonococci, then organisms isolated from a particular anatomical site might be expected to be similar in P.II expression. We detected no association of particular P.II species with infection in different sites but did confirm that almost all of the isolates from uncomplicated gonococcal infection by this strain produced one or more P.II proteins.

Factors that regulate P.II expression or that influence the selection of different P.II-bearing variants in the host are unknown. Antibody specific for P.II is present in sera from patients with uncomplicated disease (17), and anti-P.II antibody can be bactericidal for gonococci expressing that protein (1). Therefore, it has been suggested that the immune response to P.II might influence the survival of particular P.II-bearing gonococci. Other host factors that might affect the selection of different P.II variants include proteases (2) and steroid hormones (12). Further studies are required to understand mechanisms for the generation of P.II diversity

and the selective pressures that operate in the infected host to maintain that diversity.

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