INFECTION AND IMMUNITY, May 1983, p. 816–819
0019-9567/83/050816-04$02.00/0
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Vol. 40, No. 2

Confirmation of Association of Protein I Serotype of *Neisseria gonorrhoeae* with Ability to Cause Disseminated Infection

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Received 6 December 1982/Accepted 27 January 1983

Previous work indicates that strains of *Neisseria gonorrhoeae* isolated in Seattle, Wash., and Atlanta, Ga., show an association between serotypes 1 and 2 of protein I of the outer membrane and the ability to cause disseminated infection (T. M. Buchanan and J. F. Hildebrandt, Infect. Immun. 32:985–994, 1981). By using the same serotyping system, we confirmed the association between those serotypes and both disseminated infection and serum resistance in strains from North Carolina. Some strains of the same serotype had protein I species with different apparent molecular weights.

Neisseria gonorrhoeae most commonly causes a mucosal infection limited to the urogenital tract. In a small percentage of cases, however, the organism originates from a urogenital, rectal, or oropharyngeal focus to establish an invasive, systemic infection, referred to as disseminated gonococcal infection (DGI). Strains from patients with DGI tend to share a constellation of traits that are found less frequently among strains from patients with uncomplicated genital infection (UGI). These include penicillin sensitivity (22), resistance to the bactericidal action of normal human serum (16), and unusual nutritional requirements (auxotrophy) (4, 11). There is little evidence regarding the mechanism by which some or all of these characteristics of gonococci might contribute to their virulence.

Particular surface antigens may also be associated with the ability to cause invasive infection. Protein I (PI) of the outer membrane is one of the major surface proteins of the gonococcus and has been shown to vary both chemically and antigenically among different strains (9, 10, 14, 20, 21). The serotyping system of Buchanan and Hildebrandt (2) uses an enzyme-linked immunosorbent assay, with an antigen consisting primarily of PI, in which nine different antigenic types of PI are recognized. Two of the serotypes predominate in invasive gonococci isolated from patients in Seattle, Wash., and Atlanta, Ga. The finding that a limited number of serotypes may be responsible for most invasive disease suggests the possibility that PI has a role in the pathogenesis of DGI. The importance of antibody to PI in protection against invasive disease is suggested by the finding that gonococcal salpingitis is unlikely to recur with a strain of the same PI serotype as the strain causing the first episode of infection (1). If PI does have a role in gonococcal pathogenesis and if antibody to PI is indeed protective, then the association of a limited number of PI serotypes with DGI would seem to increase the likelihood of developing a PI-based vaccine for invasive disease. As Buchanan and Hildebrandt have pointed out (2), a confirmation of the association between particular PI serotypes and invasive disease will require the serotyping of gonococcal strains from geographical areas other than those already studied to eliminate the possibility that the observed associations were local phenomena.

To that end, we studied a total of 40 strains isolated from patients in Durham, Charlotte, and Chapel Hill, N.C., from 1974 to 1981. We accepted as definite DGI strains only those that were isolated from blood or joint fluid; 20 strains from our collection satisfied those criteria. These were compared with 20 strains from patients with UGI, selected randomly from strains isolated from patients in Durham during the years in which the majority of the DGI strains were isolated (1974 to 1976). Some of the DGI strains were among those studied by Eisenstein et al. (5), although PI serotypes were not determined in that study.

The strains were characterized by PI serotype (2) and sensitivity or resistance to the bactericidal action of normal human serum (13) (Table 1). The majority (31 to 40) of these strains reacted with more than one PI serotype, although not all serotypes were represented. Buchanan and Hildebrandt (2) have shown that serotypes 1 and 2 are closely related and that these serotypes are found frequently among DGI strains from Atlan-

TABLE 1. PI serotype and sensitivity or resistance to bactericidal action of normal human serum in 40 *N. gonorrhoeae* strains

Strain and characteristics[a]	No. of strains of serotype[b]:									
	1,2	2	1,2,3	5	5,6	5,6,7	8	9	8,9	Nontypable
DGI strains (n = 20)	10	2	4	0	2	1	1	0	0	0
Serum resistant	10	2	4		2	1	1			
UGI strains (n = 20)	4	2	1	3	3	0	1	3	1	2
Serum resistant	4	2	1	1	2		0	0	0	0
Serum sensitive	0	0	0	2	1		1	3	1	2

[a] A strain was defined as serum resistant if there was ≥70% survival after exposure to a 1:4 dilution of pooled normal human serum at 37°C for 30 min; strains classified as serum sensitive were resistant (<70% survival) to a 1:16 dilution of serum.

[b] A serotype of 1,2 indicates that a strain types as both serotype 1 and serotype 2. The enzyme-linked immunosorbent assay test for serotyping was performed as described by Buchanan and Hildebrandt (2). Polystyrene tubes were coated with PI antigen, incubated with rabbit immune sera, washed, incubated with goat anti-rabbit antibody linked to horseradish peroxidase, washed, and incubated with substrate and chromogen. Antibody to PI was quantified by optical density at 490 nm. The serotype of gonococcal strains was determined by preincubating organisms with antiserum; a strain was accepted as a given serotype if the antiserum-organism mixture produced >30% reduction, as compared to controls, in the enzyme-linked immunosorbent assay.

ta and Seattle. In Table 1, strains of serotype 1 and/or 2 were significantly more common among isolates from patients with DGI (16 of 20) than among isolates from patients with UGI (7 of 20) ($P < 0.01$; chi-square test of homogeneity). It seems unlikely that possession of serotype 1 or 2 antigens is the only factor involved in dissemination, since 35% of UGI strains also possessed these antigens. One possibility is that those UGI strains were capable of establishing an invasive infection, but no invasion occurred because of early treatment or active host defense factors. Serum resistance occurred more frequently among DGI strains (20 of 20) than UGI strains (10 of 20).

The data also show associations between the PI serotype of the 40 strains and serum sensitivity or resistance ($P < 0.01$). All 23 strains of serotypes 1 and/or 2, including local isolates, were serum resistant; 7 of the 17 strains of other serotypes were serum resistant. Of six strains of serotypes 8 and/or 9, five were serum sensitive.

The basis for the association between PI serotypes 1 and 2 and serum resistance is not clear. Similar associations have been reported previously, leading to speculation that PI might be directly involved in serum resistance or sensitivity (7, 8). However, genetic experiments have demonstrated that PI differences are not necessarily responsible for serum resistance and that distinct genetic loci affect PI and serum resistance (3, 17). Further work will be needed to determine the mechanism underlying this association.

The observation that 16 of the 20 DGI strains were of the same or closely related serotypes led us to ask whether or not these strains each possessed the same PI in the outer membrane. Outer membrane proteins of the 20 DGI strains were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, with two different gel systems (Fig. 1). There were differences in PI migration of some of these strains, both among and within serotype groups. The four DGI strains that were of serotypes other than 1 and/or 2 each had a PI with an apparent molecular weight of 38,200 (determined by the gel system of Shapiro et al. [18]). Of the 16 strains of serotypes 1 and/or 2, 13 were essentially indistinguishable in terms of the electrophoretic mobility of PI in both gel systems (36,700 daltons). The migration of PI from these strains was the same as that from strain FA19, which is a serotype 1,2 strain (urogenital isolate) that has been used in a variety of studies (3, 6, 15, 19). There were three strains in the serotype 1 and/or 2 group that showed an apparent molecular weight of PI that was different from that of the majority of the strains: strain FA1094 (serotype 1,2,3), 37,700; strain FA1088 (serotype 1,2), 36,900; and strain FA1092 (serotype 1,2), 36,900. Thus, 1 of the 4 serotype 1,2,3 strains and 2 of the 12 serotype 1,2 or 2 strains had a PI of different electrophoretic mobility than the others in each group. In addition to differences in apparent molecular weights of PI in these strains, there were differences in other outer membrane proteins, which might affect properties of these strains other than PI serotype. Figure 1 shows the migration of proteins of representative strains in the two gel systems. The differences in electrophoretic mobility of the proteins may not reflect actual differences in molecular weights, since some differences in

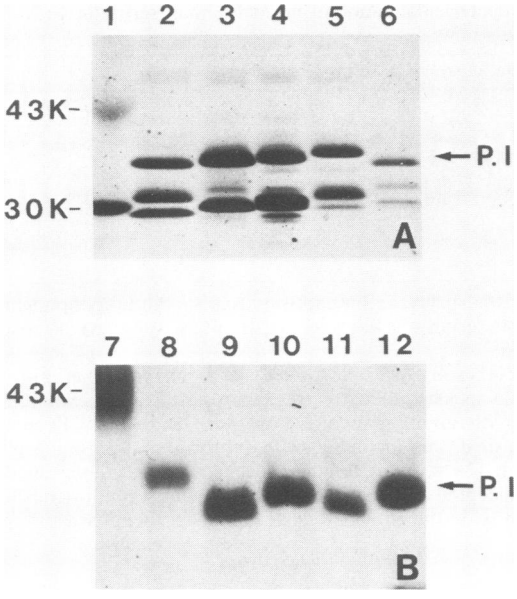

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of outer membrane proteins of representative DGI strains. Outer membranes were purified by Sarkosyl (Geigy Chemicals) extraction (6). (A) Protein profiles in a gradient gel system (4 to 30% acrylamide) with the buffers of Laemmli (12). (B) Profiles of proteins in the system of Shapiro et al. (18) on a 10% acrylamide gel. In both systems, the ratio of acrylamide to bisacrylamide was 30:0.8. Lanes 1 and 7, molecular weight standards; lanes 2 and 8, strain FA1013 (serotype 5,6,7); lanes 3 and 9, strain FA19 (serotype 1,2); lanes 4 and 10, strain FA1094 (serotype 1,2,3); lanes 5 and 12, strain FA1088 (serotype 1,2); lanes 6 and 11, strain FA1095 (serotype 1,2,3). Note that the order of the final two strains is different in the two panels. Only the relevant portion of each gel is shown.

electrophoretic mobility of particular proteins were revealed by one system and not by the other. However, even if the different proteins did not differ in true molecular weight, the differences in their mobility reflected some structural differences. The structures mediating serotypes 1 and 2 are presumably conserved, and the differences will probably be found in other parts of the molecule (14). Further analysis of these proteins will be necessary to determine the structure of the serotype antigenic domains and to clarify the genetic mechanisms responsible for the existence of a given serotype peptide sequence on higher or lower apparent molecular weight forms of PI.

These results provide information from a new geographical region that confirms the association of PI serotypes 1 and 2 with DGI and serum resistance. These associations are significant, even though we surveyed a limited number of strains. Studies of this type are important for assessing how generalized a particular association might be. There is no available evidence of possible functional differences in PI species of different serotypes. In the future, it will be important to try to understand the underlying mechanisms for the observed associations between PI serotype and invasiveness. Meanwhile, it may be possible to use this association for practical purposes, such as a PI-based vaccine to prevent invasive gonococcal disease.

We thank Bonita Gandhi, Diana Barritt, and Charlotte Richards for technical assistance, Kant Bangdiwala for assistance with statistical analysis, and Lynn Brooks for preparation of the manuscript.

This research was supported by Public Health Service grants AI15036 and AI92618 from the National Institute of Allergy and Infectious Diseases.

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