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Protein I: Structure, Function, and Genetics

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The study of the major outer membrane (OM) protein, protein I (PI), of *Neisseria gonorrhoeae* has proven to be as fascinating, and as frustrating, as every other aspect of this remarkable bacterium. However, intensive studies over the last few years have provided significant insight into the structure, function, and genetics of this abundant, surface-exposed porin protein. There are two forms of PI, which share up to 80% homology. The diversity of the exposed portion of the molecules among strains presents an ever-changing immunogenic and antigenic dilemma to the reinfected host. Yet, it is the variability of the PI surface epitopes that has provided a precise method of serotyping *N. gonorrhoeae*, making it possible to monitor the prevalance and spread of strains within the host population and correlate functional differences between strains with PI structural types.

Recent successes in cloning and sequencing of both PI structural subclasses have already contributed to our understanding of the mechanism(s) whereby *N. gonorrhoeae* generates PI structural diversity. Continued investigation is needed to clarify the relationship between PI type and disseminated disease, resistance to serum killing, auxotypic requirements, interaction with host cells, and resistance to antibiotics. Observations regarding the ability of PI to interact with eucaryotic membranes suggest a role for PI in the pathogenesis of *N. gonorrhoeae*, whereas immune responsiveness of patients to PI points to the possibility of a PI vaccine. Clearly, at the molecular level, PI represents all the mystery and intrigue that *N. gonorrhoeae* presents at the organism level. Certainly, much remains to be learned before we have a complete picture of the structure, function, and genetics of PI.

STRUCTURE

PI (73) is known to exist in two structurally related forms, designated subclass PIA and subclass PIB (61), which have different orientations in the OM (2, 4). A given strain or *N. gonorrhoeae* expresses a single, invariant PI of one of the other subclass, which accounts for up to 60% of the protein in the OM (36), whether grown aerobically or anaerobically (16). There is significant structural variation within the subclasses, resulting in proteins of different apparent molecular mass (52, 72), different isoelectric points (with some as low as 5.5, but most close to 8.0) (3), and unique immunological reactivities (6, 45, 61–63, 75). PIAs tend to be smaller than PIBs, ranging in apparent molecular mass from about 34 to 36.5 kilodaltons (kDa), whereas PIBs range from about 36 to 38 kDa (38, 52, 72, 73) (Fig. 1). Regardless of subclass, PIs appear to associate as trimeric porins which form hydrophilic channels through the OM (3, 19, 51, 78). Proteins of both subclasses show immunological variation (6, 45, 61–63, 75), which tends to be localized in surface-exposed portions of the molecules (13, 40, 42, 44, 46, 75). PIs also interact with other OM components, such as protein III (PIII) (55, 74) and lipooligosaccharide (LOS) (31), to form complex OM structures.

The amino acid sequences of a representative PIA (strain FA19) (14) and two PIBs (strains R10 [23] and MS11 [13]) are known. PIA and PIB have identical 19-amino-acid leader sequences which are cleaved to produce the functional protein (13, 14, 23). There is 65 to 80% homology between PIA and amino acid sequences of the two PIBs, confirming similarities previously observed by peptide mapping (2, 39, 40, 61, 72). Peptide mapping studies of PIAs and PIBs and sequence data for two PIBs (13) indicate that there is a higher degree of homology within the subclasses (2, 44, 61, 72). The predicted molecular masses from the deoxyribonucleic acid (DNA) sequences are about 34 kDa for the PIA and 35.5 to 36 kDa for the PIBs. These masses are remarkably close to the apparent molecular masses derived by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and are consistent with the masses of *Escherichia coli* porin proteins (14, 20, 23). Additionally, the PI sequences reveal an absence of long hydrophobic stretches and a lack of cysteine, and they have about 23% sequence homology with *E. coli* trimeric porins (23). Hydropathy patterns of the predicted PI sequences are also consistent with patterns of *E. coli* OmpC and OmpF porins (14), providing evidence that PI molecules act as porin proteins, forming hydrophilic channels across the OM (3, 19, 51, 78).

Sequence differences between the PIA and PIB subclasses result in different orientations of the PIs in the OM (Fig. 2). PIAs are oriented in the OM in such a manner that they have a relatively small portion (15 to 20 amino acids) of the protein exposed on the bacterial surface (2, 4, 42, 44, 68). PIBs traverse the membrane at least twice, having, perhaps, both termini embedded in the OM (2, 4, 68). Thus, PIAs are resistant to in situ proteolysis by trypsin and α-chymotrypsin (2, 4, 44, 68), with only a short portion of the molecule being susceptible to cleavage by proteinase K. Cleavage of purified PIAs from two strains of *N. gonorrhoeae* with leucine aminopeptidase, followed by immunoblot analysis, indicated that the N-terminus is the exposed portion of PIAs (42). A very recent study (13), in which shuttle mutagenesis was used to introduce a selectable marker near the PI gene, used transformation to locate surface epitopes of PIA and PIB in PIA-PIB hybrids. This study confirmed the N-terminal exposure of PIA, but suggested that there may be C-terminal exposure as well.

PIBs are very susceptible to in situ cleavage by exogenous proteases such as trypsin, α-chymotrypsin, and proteinase K and by neutrophil proteases such as elastase and cathepsin G (W. M. Shafer, personal communication), which cleave a central portion of the molecule, leaving two membrane-bound fragments (2, 4, 44). Cleavage of purified PIBs with CNBr (76) and endopeptidases (21), followed by immunoblot analysis, demonstrated that the central portion of PIB is the surface-exposed portion of the molecule. Analysis of PIA-PIB hybrids suggested that a portion of the N terminus may also be exposed in PIBs (13). The portions of the PIs that are exposed on the surface tend to be variable (13, 40, 42, 44, 46, 75) and possess epitopes which allow for immunological classification of strains based on the structural variation within the PIA and PIB subclasses (6, 46, 61–63, 75).

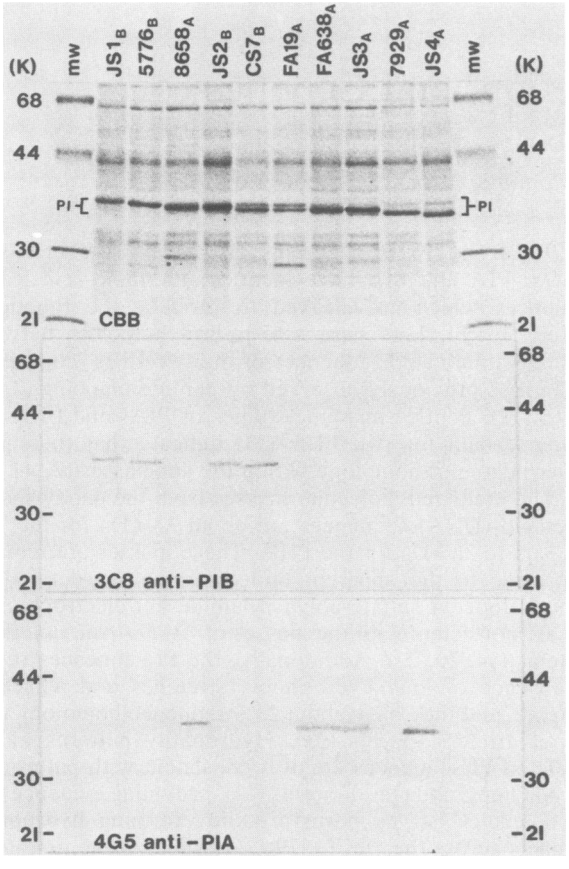

FIG. 1. Separation of whole-cell lysates of *N. gonorrhoeae* JS1, 5776, 8658, JS2, CS7, FA19, FA638, JS3, 7929, and JS4 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 12.5% gel. The top panel is the central portion of a Coomassie brilliant blue (CBB)-stained gel which shows the range of apparent molecular masses of PI. The apparent molecular masses, expressed in kilodaltons (K), of the PI of each strain, as determined by comparison with the Bio-Rad low-molecular-weight markers (mw) are as follows: JS1, 37.7 kDa; 5776, 36.9 kDa; 8658, 37.3 kDa; JS2, 36.3 kDa; CS7, 35.7 kDa; FA19, 35.7 kDa; FA638, 35.7 kDa; JS3 35.7 kDa; 7929, 35.2 kDa; and JS4, 34.6 kDa. The middle panel is an immunoblot using the PIB-specific 3C8 MAb (75), and the bottom panel is an immunoblot using the PIA-specific 4G5 MAb (75). Both blots were probed with anti-mouse IgG–horseradish peroxidase. The PI subclass of each strain, as determined by ^{125}I-labeled peptide mapping (42), is given as a subscript to each strain designation. Note that although the PIBs tend to be larger than the PIAs, there is considerable overlap in their apparent molecular masses. Also note that not all PIs bind these widely reactive anti-PI MAbs (e.g., strains JS3 and JS4), necessitating further analyses to determine the PI subclass of these nonreactive strains.

Initial efforts to serotype *N. gonorrhoeae* involved the use of antisera raised against OM vesicles (36). PI was a dominant antigen in these vesicles (36, 52), indicating that it might be useful in serotype analyses. Sandstrom and Danielsson (63), using absorbed polyclonal antisera in coagglutination assays, were able to separate *N. gonorrhoeae* into three serogroups designated WI, WII, and WIII. A second method of serogrouping, involving the use of anti-PI antibodies in an enzyme-linked immunosorbent assay, was developed by Buchanan and Hildebrandt (6). This system divided gonococci into nine serotypes. Further studies showed that both serogrouping systems correlated with the PI subclass: the WI strains and serogroup 1 to 3 have a PIA, and the WII and WIII strains and serogroups 4 to 9 have a PIB (61, 63). A series of PI-specific monoclonal antibodies (MAbs) have since been produced (Fig. 1) which react with different epitopes on or near the surface-exposed portion of the PI molecules (46, 75). On the basis of the reactivity patterns to these "banks" of PIA- or PIB-specific MAbs in coagglutination assays, systems of serotyping have been developed that classify strains into serovariants, or serovars. Thus, strains of *N. gonorrhoeae* can be described by their PI subclass and the reactivity of the PI with the serovar MAbs (e.g., PIA-1 indicates that this strain has a PIA that reacts with all six of the serovar MAbs) (46, 65, 75). The large number of serovar patterns demonstrates the wide structural variation in or around the surface-exposed regions of the PIs (45, 46, 75). Exposure analyses have confirmed the structural variability of the surface-exposed regions of the PIs (21, 40, 42, 44, 76).

Primary structural differences are not the only factors affecting PI structure in the OM. PI molecules interact to form trimeric porin structures (3, 51). PIII also interacts with PI in the OM in a ratio of three PIII molecules to one PI molecule, forming a complex that can be chemically cross-linked (55). Swanson et al. (74) confirmed an in situ association of PI and PIII by demonstrating that anti-PIII MAb coprecipitated PI. PIII may therefore participate in the formation, stabilization, and/or operation of the trimeric PI pore. A noncovalent association between LOS and PI has also been demonstrated (31). Although the nature of this association is not known, variation of LOS structure does correlate with differences in PI exposure in strains of *N. gonorrhoeae* having structurally identical PIAs or PIBs (R. C. Judd and W. M. Shafer, Mol. Microbiol., in press). Recent studies have demonstrated that PI is associated with peptidoglycan (PG) (S. A. Hill, Ph.D. thesis, University of Montana, Missoula, 1987), perhaps serving to anchor the OM to PG at critical sites. Neither protein IIs, pili, nor H.8 antigen appear to interact directly with PI in the OM (32, 60). A recent study by Robinson et al. (60) demonstrated that the availability of PI to bind PI-specific MAb and polyclonal antibody in intact cells varied extensively, even within the same culture. This raises the possibility that structurally identical PIs do not have the same orientation in all cells; that PI is not expressed in all cells; or that some other, as yet undescribed, interaction between PI and another OM component(s) is occurring differently in different cells (60).

FUNCTION

The known function of PI is to form hydrophilic pores across the OM (porin) (3, 19, 51). The porin function of PI is clearly necessary for the survival of the bacterium, allowing nutrients to penetrate into the cell and waste materials to exit. Other possible cellular functions of PI have been suggested, such as translocation into recipient membranes (3, 49) and association with PG (Hill, Ph.D. thesis). PI subclass has been associated with such traits as serum resistance (12, 29, 38, 57), resistance to antibiotics (1, 8, 33, 35, 59), auxotype (5, 24, 47, 54, 64), and expression of type 1 or type 2 immunoglobulin A1 (IgA1) protease (54). The role of PI in pathogenesis remains elusive, but the PI subclass has been associated with certain clinical states such as disseminated gonococcal infection and localized mucosal disease (5, 9, 50, 54, 56, 64).

The porin activity of PIs has been investigated by analyzing their ability to conduct electric currents when placed in lipid bilayers (3, 49, 51, 78). PIs inserted into artificial membrane bilayers conducted current in bursts, indicating

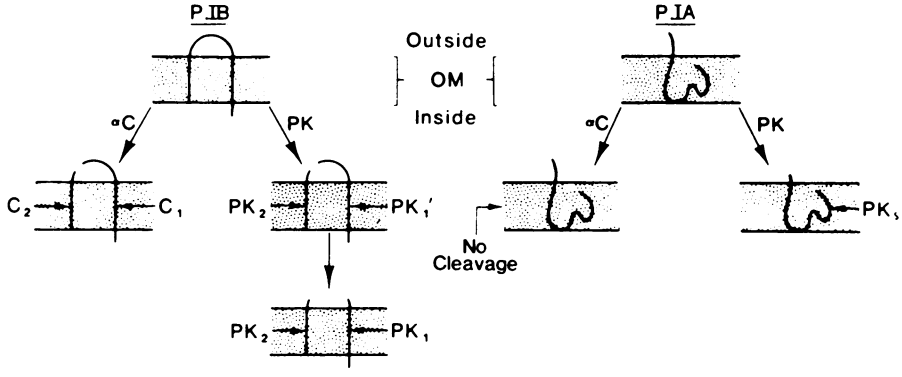

FIG. 2. Model of PIB and PIA in OMs of *N. gonorrhoeae* based on in situ cleavage by α-chymotrypsin (αC) or proteinase K (PK). The PIs were cleaved to yield the fragments shown in this model. C_1, C_2, PK_1', which is further cleaved to produce the PK_1 fragment, and PK_2 are produced when PIB-bearing strains are treated with α-chymotrypsin proteinase K. The PK_1 fragment is generated when PIA-bearing strains are treated with proteinase K. PIAs are resistant to in situ cleavage by α-chymotrypsin and trypsin. On the basis of these cleavage patterns, PIB has a central portion of the molecule exposed on the surface, with both termini embedded in the membrane, whereas PIAs have a short portion of the N terminus (42) exposed on the bacterial surface. Reproduced from *Infection and Immunity* (2) with permission of the publisher.

the pores existed in two states, open or closed (3, 49). Moreover, gonococcal PIs showed incremental voltage gating of current across a lipid bilayer in a manner characteristic of trimeric porins (20, 51). Thus, PIs appear to be porins with three PI molecules combining to form hydrophilic channels, through the OM (Fig. 3). The diameter of the pore has been estimated to be 2.5 nm (19), with an anion-selective channel ranging in Cl^-/K^+ selectivity from 6:1 (for PIBs) to 3:1 (for PIAs) (3, 78).

Further studies with lipid bilayers have indicated that gonococcal PIs spontaneously insert into these membranes in an inverted manner (3, 49). The rate of insertion of *N. gonorrhoeae* PIs was lower for PIBs (5 to 10 pores per h) than for PIAs (100 pores per h) when 10^6 organisms were placed in the test system. In comparison, *Neisseria sicca* did not transfer porin function to the bilayer, even when 10^9 organisms were used, whereas 10^6 organisms of *Neisseria meningitidis* transferred up to 1,500 pores per h (3, 49). Interestingly, gonococcal strains isolated from patients with disseminated disease had greater pore-forming activity than did strains isolated from mucosal sites (3), perhaps reflecting the observation that 80 to 90% of disseminated gonococcal infection isolates express the PIA subclass. It has been demonstrated that PI can be inserted into erythrocytes (3) and that transfer is facilitated when the recipient membrane is more fluid than the bacterial OM (3). The effect of this transfer on pathogenesis is unclear. It has been shown that neutrophils pretreated with purified PI had a decreased ability to exocytose granules and, in response to stimulation with *N*-formyl-Met-Leu-Phe, failed to increase their surface area and did not aggregate as well as control cells (25). PI has also been shown to bind calmodulin (3), a eucaryotic-cell-regulatory molecule. The effect of these activities on pathogenesis remains unclear, but is seems reasonable that PI mediates important events in host cells that contribute to bacterial survival and pathogenesis.

N. gonorrhoeae lacks a protein analogous to Braun's lipoprotein found in *E. coli* (26). Recently, PI has been shown to be one of several PG-associated proteins (30), suggesting that PI may help bind the OM to PG. This is supported by the observation that PI binds lectins that are specific for sugars found in gonococcal PG (Hill, Ph.D. thesis). Moreover, antibody raised against highly purified PIB (R.C. Judd, Abstr. Third Biannual UA/UC Conf. Pathogenic Bacteria, abstr. no. 5, p. 1) cross-reacted extensively with several other PG-associated proteins, while antibody made to a purified 60-kDa PG-associated protein reacts with PI, indicating that PI shares epitopes, probably of PG origin, with these molecules.

Protein I has been associated with several traits that are important to disease. The availability of serotyping reagents (46, 75) has made it possible to correlate PI subclass with many characteristics of the organism as well as the nature of the infection caused by a particular isolate. PIA has been associated with resistance to killing by normal human serum (29, 38, 57). As with most things gonococcal, the association is not absolute. A recent study comparing susceptibility to killing by normal human serum in transformant strains which have identical PIAs or PIBs in differing LOS and H.8 antigen backgrounds demonstrated that PI subclass does not account for serum resistance (R. K. Pettit, J. C. Szuba, and R. C. Judd, unpublished data), confirming that other bacterial components must play a role in resistance (11, 12, 69–71).

There does appear to be a relationship between PI subclass and antibiotic resistance. Several studies indicate that resistance to rifampin (8), thiamphenicol (8), ampicillin (59), and penicillin (59, 67) correlates strongly with the PIB subclass. Correlations between PI subclass and antibiotic resistance are somewhat dependent on geographical location (33, 35, 59), and both PIA- and PIB-bearing strains are capable of plasmid- and chromosome-mediated resistance to antibiotics. There is a consistent correlation between the arginine$^-$ hypoxanthine$^-$ uracil$^-$ (AHU^-) auxotype and PIA (5, 24, 47, 54, 64). Other auxotypes do not correlate as strongly with a particular PI subclass. Therefore, serotyping schemes have been developed which combine the PI serovar and auxotype to classify gonococcal isolates (24, 45).

Another association regarding PI subclass is the production of type 1 IgA1 protease, which is produced predominantly by AHU^-, PIA-bearing strains, and type 2 IgA1 protease, which is produced by many other auxotypes and serovars (54). The AHU^- auxotype and PIA subclass do correlate with the ability to cause disseminated infection (5, 9, 47, 50, 54, 56, 64); the majority (>85%) of blood isolates possess the PIA subclass of PI, and about 60% are AHU^- (47, 56). Mucosal isolates tend (>60%) to possess the PIB subclass of PI. It is important to note that both PIA- and

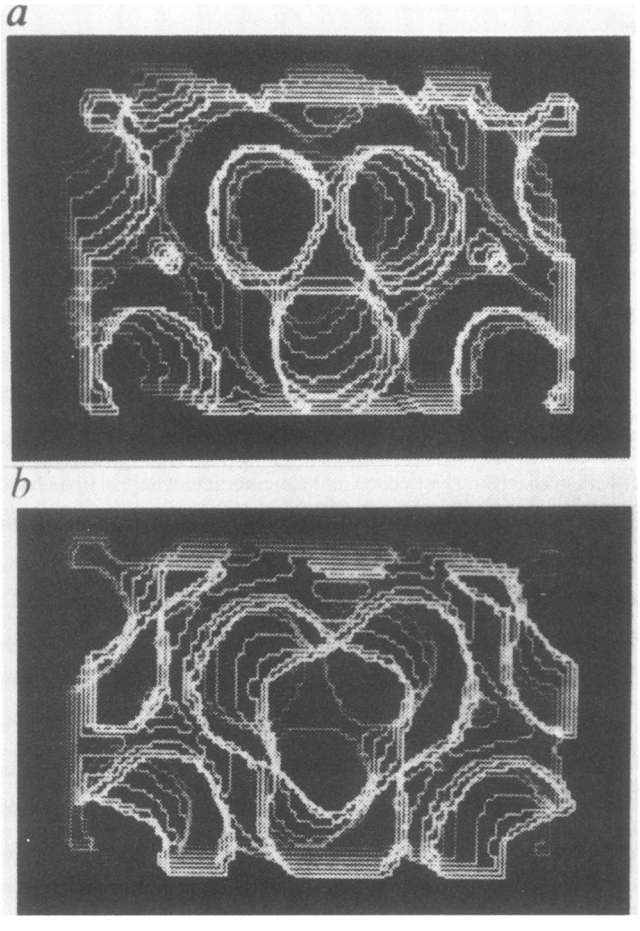

FIG. 3. Three-dimensional reconstruction of the small hexagonal form of a trimeric porin formed by *E. coli* matrix protein (OmpF) in reconstituted phospholipid membranes as viewed externally (a) and internally (b). Conductance measurements demonstrated a series of voltage-dependent openings and closings indicative of a trimeric pore as depicted in panel a. The three pores, which open and close independently, merge to form a single channel on the periplasmic side to the membrane (panel b). Conductance experiments on PI of *N. gonorrhoeae* inserted into lipid membranes demonstrated similar voltage-dependent gating data (51), indicating that PI forms trimeric pores analogous to those formed by the *E. coli* matrix protein as seen here. Reprinted from *Nature* (London) (20) with permission of the publisher.

PIB-bearing strains have been isolated from blood, joints, and mucosal sites (50, 56).

The host immune response to PI depends on previous immune status (28, 66) and site (48, 56) and duration (22, 28, 79) of infection. Several studies have demonstrated that normal human serum contains anti-PI antibody. In one study, normal human serum was shown to specifically inhibit anti-PI monoclonal antibody binding. This inhibiting antibody in normal human serum was opsonic (66). In a pre- and posturethral infection study, immunoblot analysis showed that 12 of 13 men possessed preformed anti-gonococcal antibody, some directed against PI, perhaps being cross-reactive antibody from previous exposure to *N. meningitidis* or other gram-negative organisms (28). Following infection, nine men showed a temporal increase in antibody levels against gonococcal antigens, including PI (28). A study comparing antibodies in serum and vaginal fluid from women with disseminated gonococcal infection, peritoneal inflammatory disease, and uncomplicated mucosal infections indicated that both IgG and IgA in vaginal fluid reacted more strongly with PI of the infecting strain of *N. gonorrhoeae* than did serum antibody (48). Radioimmunoprecipitation studies of serum from sexual partners suffering from localized mucosal infections demonstrated that both men and women produced anti-PI antibody in their sera in response to infection (79).

The role of anti-PI antibody in infection is unknown. However, the possibility that anti-PI antibody is important in limiting infection is supported by the observation that anti-PIII antibody blocked the bactericidal activity of anti-PI antibody in convalescent-phase serum (58), reflecting the close association of PI and PIII in the OM. When anti-PIII antibodies were removed from the serum, bactericidal activity was restored (58), indicating that anti-PI antibody in patient serum can kill *N. gonorrhoeae*.

Many anti-PIA and anti-PIB MAbs are able to activate the classical complement cascade, resulting in cell killing (27, 38, 77). The juxtaposition of the exposed PIA epitopes and other OM components seems to be critical in determining whether a particular MAb will be bactericidal, since MAbs that activate equivalent amounts of complement and that bind equivalently to cells showed marked differences in their ability to kill *N. gonorrhoeae* (38). A similar situation appears to occur with anti-PIB MAbs (77). The difference in killing is related to the manner in which the activated complement is deposited on the bacterial surface (37, 38). Anti-PIA and anti-PIB MAbs have also been shown to be opsonic and are able to inhibit the invasion of *N. gonorrhoeae* into epithelial cells (27).

The ability of anti-PI antibody to kill *N. gonorrhoeae*, combined with the relative abundance of PIs, their universal expression, the apparent structural similarity between PIs within each subclass, their antigenic stability within a strain, and the presence of anti-PI antibody in the mucus and serum, even following uncomplicated mucosal infection, has encouraged the use of PI as a potential vaccine. Purified PIB has been used, unsuccessfully, in vaccine trials (Sexually Transmitted Diseases 1986 National Institute of Allergy and Infectious Diseases Study Group Summary and Recommendations; K. K. Holmes, General Chairman; November 1987). In another study, recipients did show specific responses when injected with a PI preparation (F. Arminjon, M. Cadoz, S. A. Morse, J. P. Rock, and S. K. Sarafian, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, E92, p. 118). Attempts to improve the immunogenicity of PI have been made, especially by using liposomes as delivery vehicles (34). Such studies indicate that both PI-detergent complexes and PI-liposomes induce anti-PI antibodies but that PI-liposomes elicit the larger primary response (34).

The immunodominant portion of the PI molecule appears to be the most exposed region (13, 40, 42, 46, 75). Since this region is also the variable part of the PI molecule, several groups are attempting to identify conserved epitopes which are available to bind anti-PI antibody. One approach is to use MAbs to locate such regions (77). Another approach is to use cloned PI genes to synthesize peptides of PI (13, 14, 23), whereas others are using peptides generated by cleaving purified PIs and recovering surface-exposed fragments for immunogenicity and antigenicity studies (41, 43, 76).

GENETICS

The majority of information about the genetics of PI has come from transformation studies (12). As yet, no mutant

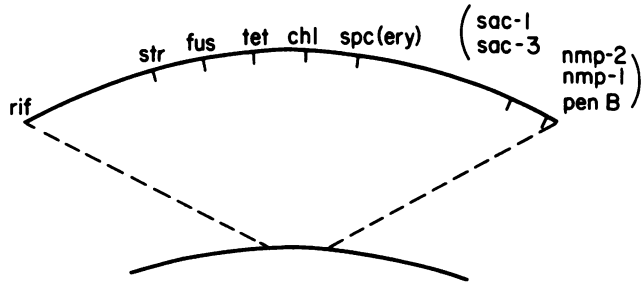

FIG. 4. Linked chromosomal genes for antibiotic resistance (rif, rifampin; str, streptomycin; fus, fusidic acid; tet, tetracycline; chl, chloramphenicol; spc, spectinomycin; ery, erythromycin; penB, nonspecific resistance to penicillin, tetracycline, and chloramphenicol), resistance to serum killing by normal human serum (sac-1 and sac-3), and PI structure (nmp-1 and nmp-2). All loci were mapped by three-factor transformation crosses. Parentheses indicate uncertainties regarding the positions of closely linked markers. Reproduced from *Annual Review of Microbiology* (12) with permission of the publisher.

strains lacking PI have been found, nor have strains been developed which have mutations in a PI gene (13, 14, 23). The availability of PI gene sequences for both PI subclasses (13, 14, 23) may prove invaluable in the generation of PI mutants, providing much-needed information about the structure and function of PI genes and the PI molecule. Transformation, by using both PIA- and PIB-specific MAbs (17), and by using a selectable marker linked to PI genes (13), has already provided several strains of *N. gonorrhoeae* expressing PIA-PIB hybrids, making functional and immunological studies possible.

Transformation studies have identified genetic loci, designated *nmp* (new membrane protein), that affect the apparent molecular mass of PI (10). Introduction of a particular *nmp* results in the expression of a unique PI (e.g., *nmp-1* results in the expression of a PIB-1, *nmp-3* results in a PIB-9, *nmp-4* results in a PIA-1, etc.) (17). The *nmp* loci are very closely linked to loci that influence serum resistance (*sac* [serum antibody complement]) (11) and to the *penB* locus (which is probably identical to the *nsr* locus [nonspecific resistance] described by Bygdeman et al. [7]), which produces low-level, nonspecific resistance to penicillin, chloramphenicol, and tetracycline. The *nmp* region is less closely linked to genes determining resistance to streptomycin (*str*) and spectinomycin (*spc*) and several other antibiotics (Fig. 4) (12). It now appears certain that *nmp* are structural genes for PI (13). Hybridization studies with PIB gene probes suggest that the structural PI gene exists in a single copy in the genome (23). This observation was confirmed by Carbonetti et al. (13), who demonstrated that the PIA and PIB genes are alleles of the same gene.

Transformation usually results in the expression of the donor PI which has the *nmp* locus linked to the *penB* locus (12), although occasionally the recipient PI is expressed (17). This verifies that the *penB* locus can be separated from *nmp*. In a study by Danielsson et al. (17), hybrid PIBs, which acquired unique serovars, were produced in about 6% of the transformants, in which the donor expressed PIB-1 and the recipient expressed a PIB-7, suggesting that recombination had occurred. A similar result was reported for PIA (15), when a transformant generated from a PIA donor and a PIB recipient expressed a PIA that had lost several of the parental PIA epitopes. Moreover, when PIB donor DNA was transformed into a PIA recipient, a single transformant expressed a hybrid PI which had epitopes of both the PIB and PIA molecules (17).

Such observations indicate that new PI molecules, expressing unique surface epitopes, can be generated by genetic exchange between different strains of *N. gonorrhoeae*. The occurrence of PIA-PIB hybrids is extremely rare in nature (46), but the diversity of PI serovars suggests that some form of natural recombination of PI genes does occur. A possible mechanism for in vivo genetic exchange comes from recent observations by Dorward and Judd (18) which demonstrated that naturally elaborated OM blebs of *N. gonorrhoeae* contain DNA. Further, it has been shown that blebs from strains of *N. gonorrhoeae* which possess the gene for penicillinase can transfer penicillin resistance to recipient, penicillin-susceptible strains with great efficiency in the presence of high levels of exogenous deoxyribonuclease (D. W. Dorward, C. F. Garon, and R. C. Judd, submitted for publication). Therefore, in vivo genetic exchange between coinfecting strains of gonococci might occur through bleb-mediated transfer of DNA. PI may form channels between the blebs (which have been shown to possess an abundance of PI molecules) and the recipient cells in a manner analogous to the inverted insertion of PI into eucaryotic cells (3, 49), allowing the DNA to pass from the bleb to the cell.

The close proximity of the *sac-1* and *sac-3* loci to the *nmp* loci may, in an unknown manner, contribute to the relationship of serum resistance and the PIA subclass. The mechanism by which the *sac-1* locus influences serum resistance is unknown, but the *sac-3* locus apparently effects LOS structure (71). Both *sac* loci influence serum resistance independently of the *nmp* loci, since the three loci are separable, and serum-resistant PIB-bearing organisms and serum-sensitive PIA-bearing organisms have been generated by transformation (12, 69). Recent reports of a 29-kDa molecule that correlates with serum resistance and disseminated disease (53) may help elucidate the mechanism(s) of serum resistance and its relationship to PI expression.

The availability of the amino acid sequences for one PIA (14) and two PIBs (13, 23) offers the opportunity to greatly increase our knowledge of the gonococcal PI. Although the PI genes have not been cloned and expressed in a stable cell line (the PIB sequences were elucidated from clones expressing overlapping, truncated PIs, as was the PIA sequence, although an intact PIA was expressed in a short-lived clone), it may now be possible to generate mutations in PI that will greatly improve our understanding of the structure, function, and genetics of PI and hence the role PI in the physiology, pathology, and immunobiology of *N. gonorrhoeae*.

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