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Structure and Function of Pili of Pathogenic *Neisseria* Species

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PILI AND THEIR ASSOCIATION WITH VIRULENCE

Pili are hairlike filamentous appendages which extend several micrometers from the bacterial surface and have long had an important role in the pathogenesis of gonococcal infections. Pioneering studies by Kellogg et al. demonstrated that a loss of virulence was observed when gonococci were subjected to repeated laboratory subculture and that this change was associated with a change in colony morphology of the bacteria when grown on solid media (21). Four characteristically different colonial forms, designated types T1, T2, T3, and T4, could be observed; primary isolates produced predominantly small, domed, highlighted colonies (T1 and T2), whereas the laboratory subculture resulted in an increasing proportion of large, flat colonial forms (T3 and T4). Each type could, however, be stably maintained by careful colony selection during subculture, and T1-T2 colonial forms retained their virulence for human volunteers (20). Subsequent electron-microscopic studies revealed that the T1-T2 colonial forms produced pili, whereas the T3-T4 colonial forms did not (54). This association between piliation and virulence prompted a considerable body of work on the structure, function, immunochemistry, and genetics of gonococcal pili and, subsequently, related studies of meningococci.

PILUS STRUCTURE

Subunit Structure

Pili can be obtained from gonococci by utilizing their ability to form crystalline aggregates under appropriate ionic conditions. Repeated cycles of disaggregation followed by crystallization can produce pilus preparations of high purity (2). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of such preparations from both gonococci and meningococci revealed the presence of a predominant polypeptide, pilin, with a molecular weight which varied between strains in the range 17,000 to 22,000 (2, 5, 38). This led to the concept that pili were entirely composed of a repeating array of identical pilin subunits. Improved resolution on sodium dodecyl sulfate-polyacrylamide gel electrophoresis has revealed that pilus preparations may contain two closely moving bands representing heterogeneity of the pilin protein (34; J. E. Heckels and P. R. Lambden, unpublished observations). Even greater heterogeneity can be observed when pilin preparations are subjected to isoelectric focusing (22). It is unclear whether such heterogeneity is due to minor modifications such as deamination of glutamine and asparagine residues or truly represents the presence of distinct pilin molecules. Recently, it has also been suggested that pilus preparations may contain minor amounts of accessory proteins which could play an important functional role (31) similar to the tip-located pilus proteins of uropathogenic *Escherichia coli* (26).

The primary sequence of pilin molecules from gonococcal strains R10 and MS11 were first determined by Schoolnik et al. (42), who found by using protein sequencing that pilin molecules contain approximately 160 amino acid residues with methionine at positions 7 and 92 and a single disulfide loop occurring between residues 140 and 151. Cleavage at the methionine residues produced three peptides, CNBr-1 (comprising the first 7 residues from the N terminus), CNBr-2 (residues 8 to 92), and CNBr-3 (residues 93 to 159). By comparing the sequences of the fragments from each strain, the authors suggested that the CNBr-2 region was conserved between strains, whereas the carboxy-terminal CNBr-3 showed significant differences, which were responsible for antigenic differences between strains (39). This model has subsequently been refined by sequence analysis of cloned pilin genes from both inter- and intrastrain pilus variants (see below).

Meningococcal Pilins

Pili isolated from meningococci by the disaggregation methods outlined above have been found to have a similar morphology and composition to those of gonococcal pili with subunit M_r in the range 17,000 to 21,000 (33, 49). Amino acid sequencing revealed significant N-terminal homology with gonococcal pili through to residue 50 (17, 33), and antibodies raised against the CNBr-2 peptide of gonococci cross-react with the meningococcal pili (49), suggesting considerable similarities between the two species.

In a subsequent study, two monoclonal antibodies (SM1 and SM2) which recognize conserved epitopes in all gonococcal pili so far examined (69) were used to detect piliation in meningococcal isolates. A large proportion failed to react with both antibodies but were nevertheless shown by electron microscopy to be piliated (9). These strains also failed to react on Western immunoblots with polyclonal antisera raised against gonococcal pili, but did react with the same sera on immune precipitation under nondenaturing conditions, to reveal pilins with M_r in the range 13,000 to 16,000. Recent studies with synthetic peptides have located the epitopes recognized by antibodies SM1 and SM2 to separate epitopes located in CNBr-2 and CNBr-3, respectively. Thus, meningococci express one of two quite distinct classes of pili; class 1 pili closely resemble gonococcal pili, whereas class 2 pili are composed of smaller pilins which lack epitopes in both the CNBr-2 and CNBr-3 regions but do contain conformational determinants which are shared with class 1 and gonococcal pili. The genes encoding expression of the class 1 pili have been cloned and sequenced, confirming the similarity with gonococcal pili (35a), but the detailed structure of the class 2 pili is not yet known. Clearly, the distinct structural differences between the two types of meningococcal pili may have important implications for their role in pathogenesis.

Quaternary Structure

The way in which pilin monomers fold and assemble to form the quaternary structure of the actual pilus is not yet

known. One model based on a combination of methods of secondary structure prediction and protein homology has suggested that pilin contains four antiparallel α-helices similar to tobacco mosaic virus coat protein (7). Preliminary X-ray-crystallographic analysis of pilus crystals appears to confirm the model and should ultimately provide information on how the pilin subunits assemble into the mature pilus structure (34). Such information should provide valuable information in identifying those functionally important domains of the pilus which interact with the host.

ROLE OF PILI IN PATHOGENESIS

Adhesion to Mucosal Surfaces

The ability of gonococci to attach to the mucosal surfaces of the genital tract and to multiply there despite the flow of mucus and other body fluids is the essential first stage in the pathogenesis of gonorrhea. Subsequent events include uptake by nonciliated columnar epithelial cells and intracellular multiplication, followed by invasion of subepithelial connective tissues (74). Following the observation that virulent colonial forms of gonococci differed from avirulent forms in their possession of pili, many studies have demonstrated that pili facilitate adhesion to a wide range of different cell types. Thus, Pil^+ gonococci show an advantage over Pil^- variants in their attachment to tissue culture cells (50), vaginal epithelial cells (27), fallopian tube epithelium (74), and buccal epithelial cells (36).

These studies led to the view that the role of gonococcal pili in virulence was associated with their ability to promote adherence to the mucosal surfaces of the genital tract, a view which was strengthened by the observation that isolated pili readily attached to epithelial cells (4). Results of experiments in which gonococci were chemically modified to alter their surface charge suggest that pili participate in the first stage of a two-stage attachment process (16). Initially, pili are able to overcome the electrostatic barrier which exists between the negatively charged surfaces of the gonococcus and the host cell. This increases the probability of a closer approach, leading to a stable adhesion which involves other components of the gonococcal surface, including outer membrane protein PII (23), and may occur even when the bacterial cell is nonpiliated. The precise mechanism by which pili are able to overcome the electrostatic barrier is unclear; one possibility is that because of their small surface area, pili are less sensitive to the electrostatic repulsive forces than the surface of the bacterium is.

Fresh isolates of meningococci are also invariably piliated, but the role of pili in pathogenesis has been less extensively studied than that of gonococcal pili, and consideration of their interaction with host cells is complicated by the additional presence of a hydrophilic capsule on the surface of the bacteria. Nevertheless, pili do mediate adhesion of meningococci to nasopharyngeal cells and may therefore be important factors in establishing the carrier state (48). Isolates cultured from the blood and cerebrospinal fluid of patients with meningococcal disease are also piliated (9, 48, 49), and pili have been directly demonstrated in the cerebrospinal fluid of a child with meningococcal meningitis (47). Whether pili play any role in the ability of meningococci to transgress the blood-brain barrier or interact with meningeal tissues remains unclear.

Nature of the Pilus-Host Cell Interaction

Host cell receptors. Despite the established role of pili in adhesion to epithelial cells, the molecular basis of this interaction remains unclear, although considerable specificity is seen in in vitro experiments with respect to both anatomical location and the species from which the tissue is isolated. Thus, piliated gonococci adhere to isolated fallopian tubes from humans and higher primates but not to equivalent tissues from other animals (18). Purified gonococcal pili attach at a much higher density to cervical-vaginal and buccal epithelial cells than to erythrocytes, leukocytes, or fibroblasts (35). Similarly, piliated meningococci attach in much greater numbers to human nasopharyngeal and buccal epithelial cells than they do to anterior nasal cells (48).

Although the specificity of attachment with regard to both species and cell type tends to suggest recognition by pili of specific ligands on the epithelial cell surface, only limited evidence is available about the nature of such an epithelial cell receptor. Buchanan et al., using purified pili, reported that adhesion was inhibited by the addition of a number of different purified gangliosides or by pretreatment of buccal epithelial cells with exoglycosidases (5). They suggested that the human cell receptor was likely to resemble a ganglioside in structure. A similar conclusion was reached in studies with oligosaccharide-deficient clones of Chinese hamster ovary cells (14). In contrast, Trust et al. (66) reported that gangliosides had little effect on the adhesion of Pil^+ gonococci to buccal epithelial cells. However, pretreatment of the buccal cells either with sodium periodate or with a mixture of neuraminidase and glycosidase did reduce the attachment of Pil^+ gonococci to the level seen with a Pil^- variant. Thus, the consensus of opinion implicates carbohydrate moieties present on the surface of epithelial cells as potential gonococcal pilus receptors, but their precise identity remains far from clear.

Pilus receptor-binding domain. The nature of the region(s) on the pilin molecule involved in recognition of host cells is also far from clear. Gubish et al. reported that the CNBr-2 fragment, obtained from pilin treated with cyanogen bromide, bound to Chinese hamster ovary cells in similar amount to intact pili, whereas the carboxy-terminal CNBr-3 did not attach (14). Adhesion was reduced by periodate or galactosidase treatment, suggesting that sugars present on the pilin were required for optimal attachment (14). Schoolnik et al., using erythrocytes, also reported that CNBr-2 (residues 8 to 84) had adhesive properties (43). In subsequent studies they reported that a tryptic fragment encompassing residues 31 to 111 bound to human endocervical cells but not to buccal epithelial cells or HeLa cells and postulated that this region encompassed the receptor-binding domain (42).

Since the studies described above and in the previous section were carried out with different strains and different cell types, it is perhaps not surprising that the nature of the pilus-host cell interaction has not been unambiguously defined. Additional complexity in interpretation occurs because the majority of the studies were carried out before the extent of potential structural and antigenic variation of pili was appreciated. Such variations may have an important influence on the interaction of pili with a variety of different cell types (see below).

Other Possible Pilus Functions

Interaction with PMN. The interaction of Neisseria species with polymorphonuclear leukocytes (PMN) has important consequences for the potential outcome of an infection. Several early studies showed that Pil^+ gonococci were more resistant to phagocytosis than were the equivalent Pil^- variants (8, 10, 12, 58), suggesting an important additional

role of pili in virulence. Swanson et al., however, suggested that pili had only a minor role in gonococcus-PMN interactions and that an additional outer membrane component termed leukocyte association factor dominated the interaction (56, 57). Subsequently, leukocyte association factor was recognized to have the biochemical properties characteristic of outer membrane protein PII (53), and it was demonstrated that Pil^- PII^- variants showed considerably less interaction with PMN than did their Pil^- PII^+ equivalents (24, 37).

Since the original studies were carried out before knowledge of PII variation was available, the association between piliation and resistance to phagocytosis was in some doubt, since changes in PII expression may have accompanied those in pilus expression. We therefore used a panel of variants of strain P9 with defined differences in pilus and/or PII expression in a chemiluminescence assay to determine initial interactions and in a phagocytic killing assay to determine the ultimate fate of the gonococci (72). In this study, leukocyte interaction was synonymous with possession of PII. When pairs of variants expressing the same PII but either Pil^+ or Pil^- were compared, the chemiluminescence response was determined in each case by the particular molecular species of PII present, with pili having a negligible effect. Moreover, pili did not inhibit either uptake or intracellular killing, confirming the predominant role of PII in PMN interactions and suggesting that pili have little effect in resistance to phagocytosis by PMN.

Transformation. Gonococci are naturally competent for transformation throughout their growth cycle, with piliated cells being transformed at much higher frequencies than nonpiliated cells (45), although the mechanism by which pili enhance transformation is not known. Recently it was suggested that since gonococci readily undergo autolysis, they are therefore constantly exposed to their own deoxyribonucleic acid, leading to transformation events which may play a critical role in antigenic variation in pilus expression (44).

VARIATION IN PILUS EXPRESSION

Phase Variation

The original observations which led to the identification of pili on gonococci are the result of expression being subject to phase variation (2), so that although pili are lost on repeated subculture, Pil^- cells may revert to the Pil^+ phase. Genetic analysis of phase variation has revealed complex mechanisms of piliation control, with two classes of Pil^- variants (52). Members of one group are unable to revert to pilus production, whereas members of the other group revert at high frequency. The possible role of the Pil^- phase in infection is unclear, but it appears likely that the reduced attachment associated with loss of piliation may allow the gonococci to leave the site of initial colonization and gain access to other locations. Similar considerations also apply to the spread of meningococci from the nasopharynx into the blood and cerebrospinal fluid. It has also been suggested that a nonadherent interim state could enhance gonococcal transmission, with reversion to the Pil^+ phase allowing subsequent adhesion to the mucosal surfaces of the infected individual (52).

Antigenic Variation

Considerations of the role of pili in pathogenesis are dominated by their extreme structural and antigenic diversity. Early studies revealed that gonococcal pili are immunogenic for laboratory animals (3) and that patients with gonorrhea develop anti-pilus antibodies (6). Despite their apparent considerable structural homology, based on amino acid analysis and peptide mapping, pili from different strains were found to be antigenically distinct. Antisera to purified pili raised in rabbits showed only limited cross-reactivity with pili from other strains, the amount of shared antigenicity between pili from heterologous strains usually being less than 10% (2).

Even greater antigenic diversity is generated by the fact that pili produced by a single strain undergo antigenic variation. Lambden et al. (25) purified pili from colonial opacity variants of strain P9 and found that pili from the transparent type had pilin with a subunit molecular weight of 19,500 (α-pili), whereas those from an opaque type had a molecular weight of 20,500 (β-pili). Another study with a number of strains showed that pilin M_r usually, although not always, varied between pairs of opacity variants (41). These observations suggested that alterations in subunit M_r might be linked to opacity and hence PII expression. However, further studies with strain P9 revealed two further pilus types (γ and δ) and showed that their expression was not linked to PII expression (22). This conclusion was confirmed by a study of other strains, which showed that a single strain could produce at least a dozen different pilus types (51). Antisera raised against variant pili produced by a single strain display only limited antigenic cross-reactivity (68). Recent studies of the genetic mechanisms of pilus variation show that the potential repertoire of pili expressed by a single strain may be even greater than indicated above (30).

Antigenic Shift during Gonococcal Infection

The possible occurrence of antigenic variation during natural infection was less easily established because of the ethical need for prompt antibiotic therapy, but was confirmed by comparing isolates taken from different sites in sexual partners. Gonococci were cultured from the urethras of male patients and from the urethras and cervices of their female partners, and surface antigen preparations were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (11). Within each group of patients, the auxotype and the serotype of the outer membrane protein PI were identical, confirming their clonal derivation, but considerable variations were seen in expression of both pili and outer membrane protein PII. For example, one strain expressed pili of M_r 17,500 in the urethra of a male, 18,500 plus 19,000 in the urethra of a female partner, 17,000 in her cervix, and 18,300 in the cervix of a second female contact (75). Indeed, the M_r of pili differed between the isolates from the cervices and urethras of all female patients examined, suggesting that antigenic shift occurs commonly during the course of gonococcal infection. This view was confirmed by observations of isolates obtained during an outbreak caused by a penicillin-resistant gonococcus strain (46). In subsequent studies, human volunteers were subjected to urethral challenge and the pilins of the gonococci isolated during the resulting infection were analyzed (55). All reisolates were found to express pili which were structurally and antigenically distinct from those expressed by the input gonococci. The occurrence of extensive variation in each of the reported studies suggests that antigenic shift in pilus expression occurs commonly during the course of the natural infection and must play an important role in pathogenesis of gonococcal disease.

Antigenic Shift during Meningococcal Infection

Laboratory investigation of possible meningococcal antigenic variation is hampered by the fact that the changes in colony morphology which led to the discovery of antigenic variation in gonococci are not readily observed with meningococci. Nevertheless, variations in pilus M_r were detected following nonselective laboratory subculture of one strain (33). The occurrence of antigenic shift during meningococcal infection has been investigated by comparison of paired isolates obtained from the blood, cerebrospinal fluid, and nasopharynges of patients (59). Isolates from any individual produced identical deoxyribonucleic acid fingerprints and showed stability in expression of the class 2/3 and H.8 antigens, confirming their origin as a single strain. Variation in pilus expression was detected not only in strains expressing pili which contained the conserved gonococcal epitope recognized by monoclonal antibody SM1, but also in a strain which expressed non-SM1-reacting pili. Subsequent studies have revealed extensive variation in the M_r of SM1-reactive pilins produced by strains isolated from symptomatic and asymptomatic infected individuals during an epidemic of serogroup A meningitis, confirming the likely widespread occurrence of antigenic shift (1). Since meningococci produce one of two distinct classes of pili, both of which can undergo antigenic shift during infection, their antigenic repertoire appears to be equal to or even greater than that of gonococci.

Role of Antigenic Shift in Pathogenesis

Genetic studies (30) show that pathogenic *Neisseria* species have evolved with a significant proportion of their genome devoted to complex genetic mechanisms designed to ensure a continual change in the antigenic nature of the pili which they express. This, combined with the widespread occurrence of antigenic shift during natural infection, suggests that it must confer significant survival advantages on the bacteria, presumably allowing adaptation to a changing external environment. Clearly, one potential benefit would be expected to occur in interactions with the host immune system. Many studies have shown the potential protective effect of antibodies directed against gonococcal pili (see below). Thus, the ability to switch to expression of antigenically distinct pili would allow the gonococci to evade the effect of antibodies directed against the original colonizing variants. The effect would also be enhanced by a concomitant switch in the nature of the PII expressed (75). Although the role of anti-pilus antibodies in meningococcal disease is unclear, similar considerations may also apply and could, for example, explain the persistence of nasopharyngeal carriage despite the presence of the host immune response (13).

An additional potential effect of antigenic variation may influence the ability of both gonococci and meningococci to colonize host cells. Purified α- and β-pili from gonococcal strain P9 show striking differences in their ability to adhere to buccal epithelial cells, with α-pili showing a much greater affinity (25). This advantage was lost when the buccal cells were treated with glycosidases, and it was suggested that the α-pili recognize an oligosaccharide present on the surface of buccal cells but that β-pili do not (66). In contrast, when the piliated variants were compared for their ability to attach to and invade Chang conjunctival cells growing in tissue culture, the β-piliated variant showed much greater adhesion and hence virulence than did the α-piliated variant (68). The altered specificity for different cell types in vitro suggests that in vivo variation might endow the gonococci with the ability to colonize a variety of cell types found at different anatomical locations. Similar factors may well operate during meningococcal infection. Differences between the abilities of different strains to adhere to bucal epithelial cells and erythrocytes have been associated with differences in the mechanism of attachment of the pili which they express (65). The ability to interact specifically with different cell types may well be important in the pathogenesis of meningococcal disease, since the varied symptoms of infection result from a complex and poorly understood series of interactions between the bacteria and a variety of host cells.

STRUCTURAL AND IMMUNOCHEMICAL BASIS OF ANTIGENIC VARIATION

Considerable information has now accumulated from sequencing pilus structural genes from variants of a number of different strains expressing a variety of distinct pili. Variants have been isolated by colony selection (15, 32) and following natural infection (15) and experimental infection of both animals (32) and human volunteers (55). A clear model of the structural basis of pilus variation has emerged (Fig. 1). Pilins can be considered to contain three major regions, a region encompassing approximately the first 53 amino acids, which is highly conserved between pilins, a semivariable region (residues ca. 54 to 114), and a hypervariable region at the carboxy terminus. Structural variations in the semivariable region arise from amino acid substitutions, but in the hypervariable region insertions and deletions of up to four amino acids occur. Within the hypervariable region, two conserved sequences occur centered around the two cysteine residues (residues ca. 121 and 154) which form the disulfide bridge and loop; this may result from conservation of sequences at the deoxyribonucleic acid level that are involved in the genetic mechanisms of pilus variation (30). Sequence analysis of the cloned gene for expression of the SM1-reactive class of pilin from one meningococcal strain also conforms to this model (35a).

Thus, despite distinct antigenic specificity, variant pilins show a considerable degree of structural homology. Comparison of published variant pilin sequences from gonococcal strains MS11 (29) and P9 (32) and the SM1-reactive pilin from meningococcal strain C311 (35a) shows that they have approximately 80% of their amino acid residues in common. Variation in approximately 20% of the pilin must generate the extensive antigenic diversity seen, with conserved regions apparently being immunorecessive. In one series of studies, synthetic peptides were synthesized corresponding to a series of regions of one MS11 pilin. Immunization with intact pili was found to produce antibodies directed predominantly against peptides equivalent to residues 121 to 134 and 135 to 151, corresponding to the hypervariable region within the disulfide loop (39). Low levels of antibodies were directed against a weakly immunogenic determinant between residues 48 and 60. The authors suggested that pili had evolved so that the most immunogenic domain, the disulfide loop, was located on the surface of the pilin molecule and that amino acid substitutions could occur in this region, altering antigenic specificity without disrupting regions critical for pilus function.

The immunochemistry of intrastrain antigenic variation has been investigated by using monoclonal antibodies raised against variant pili of strain P9 (69, 73). The immunodominance of type-specific epitopes was confirmed in that from over 200 antibodies screened, only 1 monoclonal antibody

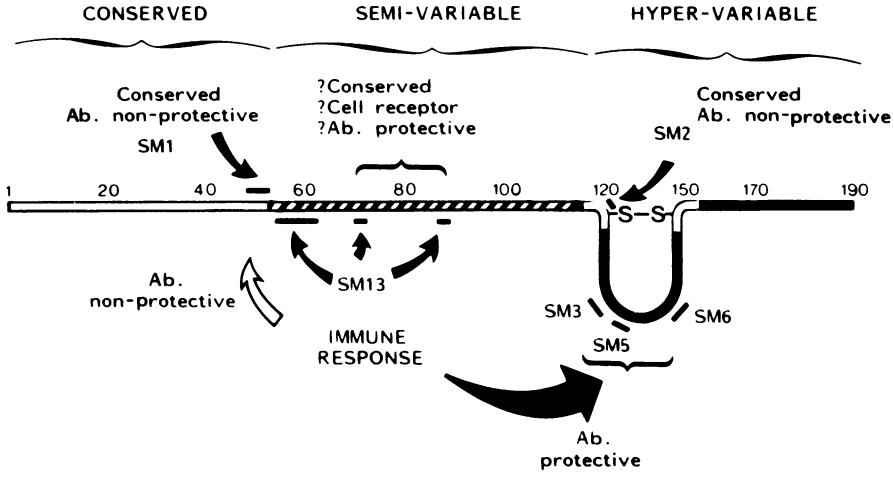

FIG. 1. Schematic diagram of the pilin molecule produced by gonococci and many strains of meningococci, showing conserved (▭), semivariable (▨), and hypervariable (▬) regions. The normal immune response is directed primarily against epitopes in the hypervariable region (➧), although low levels of antibodies (Ab.) are directed against conserved determinants (⇨). Epitopes recognized by particular monoclonal antibodies are denoted (——), and with their potential protective effect is given.

(SM1) was obtained which reacted equally well with all gonococci tested, whereas a second (SM2) reacted with all of them, but to variable extents. The type-specific antibodies showed limited and variable reactivity with other strains. Four antibodies that all reacted with α-pili were tested against the pili expressed by eight variants from three groups of clinically related isolates. Only one isolate reacted, and then with a single antibody, suggesting that the antigenic specificity of a pilus is due to a particular combination of different epitopes, some of which may be independently shared with individual variants of other strains.

Comparison of the predicted amino acid sequence of cloned pilin genes with the immunological reactivity of the encoded pilins has allowed the amino acid sequence to be correlated with monoclonal antibody reactivity (32). The epitopes for three type-specific antibodies were found to depend on the presence of specific sequences in discrete regions within the disulfide loop, between residues 127 and 140. Each putative epitope was located in a hydrophilic domain with high β-turn probability. Reactivity with one type-specific antibody, however, was associated with the presence of three well-separated sequences (residues 56 to 63, 69 to 71, and 92 to 95). Each of the three domains had high β-turn potential, suggesting that the epitope was formed on the surface of the pilin molecule from the combination of three discontinuous regions all occurring within the semivariable region (Fig. 1).

The localization of each of the putative type-specific epitopes in hydrophilic domains with high turn potential would be in accord with their exposure and hence their immunogenicity. In contrast, the weakly immunogenic conserved epitopes recognized by antibodies SM1 and SM2, which have been identified by using synthetic peptides (J. E. Heckels and M. Virji, unpublished observations) (Fig. 1), are located between residues 49 and 53 and residues 118 and 127, respectively, in regions of moderate hydrophilicity but low turn potential.

PROSPECTS FOR A PILUS VACCINE

Biological Role of Anti-Pilus Antibodies

The association of pili with virulence has prompted many studies to determine the potential of pili for vaccination against gonorrhea. Antisera raised in laboratory animals by immunization with purified or partially purified pili have been shown to have a protective effect in a variety of biological systems. Anti-pilus antisera reduce the adhesion of both piliated gonococci (60) and purified pili (35) to human buccal epithelial cells. Antibodies to pili opsonize pili for phagocytosis by PMN (36) and macrophages (19) and protect tissue culture cells from the cytotoxic effect of challenge with gonococci (67). Anti-pilus monoclonal antibodies also inhibit adhesion, opsonize, and protect against infection (70, 71), and immunization with pili can also protect guinea pigs against infection following gonococcal challenge of subcutaneously implanted plastic chambers (24). However, in many of the above studies, the test and immunizing strains were identical, and little or no protection was observed with heterologous strains.

Anti-pilus antibodies can be detected in genital secretions from patients with gonorrhea, and such antibodies inhibit the attachment of the infecting strain to buccal epithelial cells (61, 63). Human volunteers immunized with pili produce detectable anti-pilus antibodies in both serum and genital secretions; these antibodies are also able to inhibit epithelial cell attachment (28, 64). Challenge of immunized volunteers with the homologous strain has shown statistically significant protection, as revealed by the size of the dose required to produce subsequent infection (2). These studies also suggested that the human immune response to pili might be less type specific than that seen in laboratory animals and so provide useful protection against infection. However, in large-scale field trial with male volunteers, no difference in protective effect was seen between the vaccine and placebo groups (62). Vaccinated volunteers developed gonorrhea despite high levels of serum antibody directed against their infecting strain. Thus, it seems likely that the levels of antibody present in genital secretions either were insufficient or perhaps reflect an immune response directed against a conserved but nonprotective epitope (see below).

The Problem of Antigenic Shift

One possible strategy to overcome the problems posed by the antigenic variability of pili is to design vaccination regimens to boost the immune response to conserved rather than type-specific epitopes. The protective effect of anti-

pilus antibodies directed against different pilus epitopes has been investigated by using a panel of monoclonal antibodies raised against pili from strain P9. The binding of ^{125}I-labeled variant pili to buccal epithelial cells was inhibited by the appropriate type-specific antibodies but not by the cross-reacting antibodies SM1 and SM2 (70), and the virulence of variants expressing different pili was also inhibited by the type-specific but not by the cross-reacting antibodies. Similarly, the type-specific antibodies promoted opsonization and killing by PMN, but the two cross-reacting antibodies had little effect (71). The cross-reacting antibody SM1 and one of the type-specific antibodies had the same isotype and bound to native pili in similar numbers and with similar avidity, demonstrating that the difference in biological activity was directly related to the nature of the epitope recognized. Antibody SM1 recognizes an amino acid sequence within the weakly immunogenic region encompassed by amino acid residues 48 to 60 (see above). Thus, it appears that even the low levels of cross-reacting antibodies which are seen on immunization with intact pili are directed against nonprotective antigenic determinants.

An alternative strategy to immunization with intact pili has been suggested by Rothbard et al., who reported that antibodies raised against the conserved CNBr-2 fragment produced antibodies which were more cross-reactive than those raised against intact pili and reacted with a peptide corresponding to residues 69 to 84 rather than 48 to 60 (39). Synthetic peptides corresponding to one variant pilus of strain MS11 were conjugated to carrier proteins and used to produce specific anti-peptide antibodies. The antibodies were tested for their ability to inhibit the adhesion to a human endometrial carcinoma cell line of a variant of the heterologous strain F62. Antibodies directed against residues 48 to 50 and 69 to 84 showed significant inhibition of attachment when used at high concentration, whereas antibodies directed against residues 48 to 60 were without effect (40). The authors suggested that synthetic peptides could be used to direct the immune response to normally nonimmunogenic epitopes, and since they could elicit cross-reacting, receptor-blocking antibodies, the peptides were promising candidate immunogens for the prevention of gonorrhea. The studies, however, showed a protective effect with a single heterologous strain and did not explore the possible consequences of antigenic variation within a strain. In subsequent experimental infections of human volunteers, Swanson et al. have demonstrated that significant antigenic variations in strain MS11 variants can occur at residues 69 to 84 and that these destroy the epitope recognized by a monoclonal antibody that inhibits epithelial-cell adhesion (55). They suggested that these data indicate that a peptide of residues 69 to 84 would not be effective as a gonorrhea vaccine. Comparative analysis of published sequences (15, 32, 55) does, however, suggest that a central portion of the peptide, residues 73 to 78, may be more highly conserved; unfortunately, it is not known whether antibodies directed against this region would have any inhibitory effect.

CONCLUSIONS

Pili appear to play an important role in the pathogenesis of gonococcal infections, and presumably also meningococcal infections, through their ability to promote adhesion to epithelial-cell surfaces. Although the molecular basis of the pilus-host cell interaction remains to be fully defined, the attraction of inducing anti-pilus antibodies to prevent initial colonization of mucosal surfaces remains. However, the potential use of pili for vaccination against *Neisseria* infections has been frustrated by their antigenic heterogeneity, since they have evolved so that the main immune response is directed against variable determinants and even the low levels of cross-reacting antibodies produced are directed against nonprotective epitopes. It remains to be seen whether alternative strategies involving selected fragments of the pilin molecule will enable the stimulation of an effective immune response which would protect against the almost limitless number of distinct antigenic types that appear possible.

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