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Morphological Differences in *Neisseria meningitidis* Pili

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Disease and carrier isolates of *Neisseria meningitidis* were examined for their ability to adhere to human buccal epithelial cells and human cell lines and to hemagglutinate human erythrocytes, properties thought to be associated with the presence of pili. Seventy percent (7 of 10) of carrier isolates were found to be highly adherent to human buccal epithelial cells and to agglutinate human A, B, O, Rh^-, and Rh^+ erythrocytes. In contrast, 60% of the disease isolates adhered poorly to human buccal epithelial cells and 80% failed to agglutinate human erythrocytes. No adherence of either disease or carrier isolates was observed when several human cell lines were tested. When the meningococcal strains were examined by electron microscopy, 7 of 10 disease isolates were found to possess large bundles of aggregated pili (alpha-type pili), while 7 of 10 carrier isolates were found to have numerous unaggregated pili (beta-type pili). A monoclonal antibody against meningococcal pili and one against gonococcal pili reacted with 6 of 10 piliated carrier isolates and 4 of 10 piliated disease isolates. These results suggest that meningococci, like gonococci, possess different types of pili which differ in morphological, antigenic, and binding properties. In addition, antigenic and morphological differences between pili from carrier and disease isolates were observed as well as differences in adherence and hemagglutinating properties.

Pili are filamentous, nonflagellar surface appendages which play an important role in the adherence of many bacteria to mucosal surfaces (1, 3, 7, 18, 26, 33). The presence of pili frequently correlates with virulence and has been shown in many cases to be necessary for colonization and subsequent infection of the host (1, 5, 19, 29). Among encapsulated meningococci, pili have been shown to be important in adherence to mucosal surfaces and erythrocytes (7, 20, 21, 24, 31). Differences in adherence between disease and carrier isolates have been observed, with carrier isolates being more highly adherent to nasopharyngeal and buccal epithelial cells than disease isolates are (7, 14). Studies have shown the importance of pili in the pathogenesis of gonococci (5, 19); however, the role of pili in the pathogenesis of meningococci remains unclear. The studies of meningococcal pili have been hampered somewhat by the lack of an association between piliation and colonial morphology such as that seen with gonococci (5, 9).

Although many studies have revealed morphological, antigenic, and physiochemical differences among gonococcal pili (4, 12, 13, 16, 27, 28, 30, 32), less is known about meningococcal pili. Morphological differences (2, 9, 25) and antigenic and pilin subunit molecular weight differences (10) have been reported among meningococcal pili. However, no studies to date have attempted to correlate adherence or virulence with differences in pilus morphology. This report describes two morphologically different types of pili of *Neisseria meningitidis* and examines several properties of meningococci possessing each pilus type. The frequency of occurrence of pilus type among carrier and disease isolates and among hemagglutinating strains and human buccal epithelial cell (HBEC)-adherent strains is reported. Antigenic differences among meningococcal pili are also described.

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MATERIALS AND METHODS

Meningococcal strains. Strains of *N. meningitidis* were isolated from the cerebrospinal fluid, blood, and throats of patients with meningitis and from the throats and nasopharynxes of healthy carriers. The organisms were recovered on 5% sheep blood agar or chocolate agar and subsequently grown on brain heart infusion agar supplemented with 1% normal horse serum (BHIA-HS). The characteristics of the strains studied are shown in Table 1. Strains with the prefix BB, CL, or M were obtained from the Office of Biologics Research and Review collection. Strains with the prefix S were obtained from the late Harry Feldman (Upstate Medical Center, Syracuse, N.Y.). The nonpiliated strains (M986, M981, and S7290) are laboratory strains which have undergone numerous subcultures and were selected by electron microscopy for the absence of pili. Most other strains were subcultured less than six times and were considered fresh isolates. All strains were encapsulated and were of group A, B, C, or Y. All strains were stored frozen at −70°C in Greaves solution.

Adherence assay. The meningococcal adherence assay was a modification of that reported by Craven et al. (7). HBECs were obtained from normal volunteers by scraping the inner cheek with a tongue depressor. The HBECs were washed three times by suspension in phosphate-buffered saline (PBS; pH 7.4) and centrifugation at 200 × *g* for 10 min. The cells were counted in a hemacytometer and diluted to a concentration of 10^5 HBECs per ml. Meningococci were grown for 14 to 16 h on BHIA-HS at 37°C in 5% CO_2. They were subcultured for 4 h on fresh BHIA-HS plates and suspended in PBS containing 1% bovine serum albumin (BSA). Clumps were removed by centrifugation for 1 min at 400 × *g*. The supernatant containing unclumped bacteria was placed in screw-cap tubes (100 by 15 mm) and diluted with PBS containing 0.1% BSA to 80% transmission at 540 nm in a Coleman Jr. spectrophotometer. This suspension contained approximately 10^8 viable bacteria per ml. To plastic screw-cap tubes (13 by 100 mm; Becton Dickinson

TABLE 1. Summary of strains examined and their characteristics[a]

Strain set	Strain	Group:type	Source	Pilus type[b]	Adherence[c] (GNDC/HBEC)	HA titer	Reactivity with monoclonal antibody	
							2-1-Fc	02
Nonpiliated	M986	B:2	LAB		Low (1)	<1:2	−	−
	M981	B:4	LAB		Low (2)	<1:2	−	−
	S7290	C:2	BLD		Low (5)	<1:2	−	−
Carrier	CL6	B:NT	THR	B	High (199)	1:32	+	+
	BB45	Y:5	THR	B	High (140)	1:32	+	+
	BB64	B:5	THR	A	High (112)	1:128	+	+
	BB98	B:14	THR	B	High (179)	1:8	+	+
	M992	B:5	THR	B	Medium (54)	1:16	+	+
	BB199	B:NT	NP	B	Medium (26)	1:4	−	−
	BB261	C:2	THR	B	Medium (51)	1:8	−	−
	BB272	C:2	THR	B	Low (17)	1:8	−	−
	BB264	C:2	THR	A	Low (11)	<1:2	+	+
	BB259	C:2	THR	A	Low (2)	<1:2	−	−
Disease	S366	A:21	CSF	A	High (103)	1:8	−	−
	S6063	C:2	CSF	A	High (148)	<1:2	−	−
	BB269	C:2	CSF	B	Medium (63)	<1:2	−	−
	S7297	B:NT	CSF	B	Medium (30)	<1:2	+	+
	S472	A:21	CSF	A	Low (19)	<1:2	−	−
	BB11	C:NT	BLD	B	Low (20)	<1:2	+	+
	BB252	B:4	CSF	A	Low (5)	1:8	+	+
	S6056	C:2	CSF	A	Low (12)	<1:2	−	−
	BB1	B:1,8	CSF	A	Low (6)	<1:2	+	+
	S6055	C:2	BLD	A	Low (14)	<1:2	−	−

[a] Abbreviations: HA, hemagglutination; NT, nontypable; THR, throat; NP, nasopharynx; BLD, blood; LAB, laboratory; CSF, cerebrospinal fluid.
[b] A, Alpha pili (aggregated); B, beta pili (single filament).
[c] Adherence was measured by counting the number of GNDC on 40 consecutive HBECs and scoring them as low (0 through 25 GNDC per HBEC), medium (26 through 100 GNDC per HBEC), or high (>100 GNDC per HBEC).

Labware, Oxnard, Calif.) were added 0.3 ml of HBECs and 0.3 ml of meningococcal suspension. The mixture was incubated at 37°C with shaking and after 30 min was layered over lymphocyte separation medium (Litton Bionetics, Kensington, Md.) and centrifuged for 10 min at 400 × *g*. The supernatant was removed, and the pellet was suspended in 30 μl of PBS, placed on a microscope slide, air dried, fixed in methanol, and Gram stained. The number of gram-negative diplococci (GNDC) per HBEC was determined by differential interference contrast microscopy, and the number of bacteria on 40 consecutive nucleated, undamaged HBECs was determined. A low-adherence strain and a high-adherence strain were included in all experiments as controls. Adherence levels were defined as low (0 through 25), medium (26 through 100), and high (over 100), according to the criteria of Craven et al. (7).

Hemagglutination assay. Meningococci were cultured overnight on BHIA-HS plates and subcultured in the morning to fresh plates. Then the bacteria were suspended in PBS with 0.1% BSA to 50% transmission at 540 nm in a Coleman Jr. spectrophotometer (~10^9 bacteria per ml). Fresh human erythrocytes of blood group A Rh^+ were diluted to 1% in PBS with 0.1% BSA (~10^9 erythrocytes per ml).

A 100-μl volume of the erythrocyte suspension was added to 100 μl of a doubling dilution of bacterial suspension in V-well microdilution plates (Linbro Chemical Co., New Haven, Conn.) and incubated overnight at 4°C. The hemagglutination titer was read as the highest dilution of bacteria giving hemagglutination. Binding of erythrocytes to meningococci was also confirmed by nitrocellulose hemadsorption as described by Conner et al. (6). Briefly, meningococci were grown on BHIA-HS plates as described above. The growth from a 4-h subculture was removed with a cotton-tipped swab and blotted onto a nitrocellulose disk (Schleicher & Schuell, Inc., Keene, N.H.) premoistened with PBS. The disk was washed with PBS containing 3% BSA for 10 min, and a 5% suspension of fresh human A^+ erythrocytes was added and agitated for 30 min at room temperature. After two PBS washes, the disk was examined for binding of erythrocytes.

Immunoblotting. Dot immunoblots were prepared by the method of Hawkes et al. (11) with a meningococcal pilus monoclonal antibody (2-1-Fc) which was a gift from Wendell Zollinger (Walter Reed Army Institute of Research, Washington, D.C.). The growth from a 4-h subculture on BHIA-HS was removed with a cotton-tipped swab and blotted onto a nitrocellulose disk. The disk was placed in Tris-buffered saline (TBS) containing 3% gelatin for 30 min. The TBS was removed, and 10 ml of a 1:100 dilution of monoclonal antibody 2-1-Fc in TBS with 1% gelatin was then added.

Whole-cell lysates of the different meningococcal strains were separated by Tris-glycine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electroblotted onto nitrocellulose by using 20 mM sodium phosphate buffer, pH 7.5. The nitrocellulose sheets were blocked in TBS–3% gelatin as for the dot blot, and then a 1:500 dilution of a gonococcal pilus monoclonal antibody (02), a gift from John Swanson (Rocky Mountain Laboratory, Hamilton, Mont.), was added.

The nitrocellulose preps were shaken with antibody for 8 h at room temperature. After the antibody solution was removed, the nitrocellulose was rinsed with distilled water and then washed two times (10 min each) with TBS containing 0.05% Tween 20. The nitrocellulose was then transferred

to a 1:2,000 dilution of horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G (whole molecule) (Sigma Chemical Co., St. Louis, Mo.) in TBS containing 1% gelatin. It was then incubated at room temperature overnight with agitation. The antibody was then removed, and the nitrocellulose was washed two times with TBS containing 0.05% Tween 20. A 0.006% horseradish peroxidase color development reagent (Bio-Rad Laboratories, Richmond, Calif.) containing 0.015% hydrogen peroxide was added. Color development was allowed to proceed for 15 min and was stopped by washing with water.

Preparation of pili. Meningococcal pili were prepared by methods similar to those used to prepare gonococcal pili (3). Meningococcal strains were grown in a dialysate of tryptic soy broth (Difco Laboratories, Detroit, Mich.) for 14 h at 37°C on a gyratory shaker at 125 rpm. The bacteria were harvested by centrifugation at 10,000 × *g* for 15 min, and the supernatant was discarded. For each 20 g of packed cells, 50 ml of 15 mM ethanolamine buffer (pH 10.5) was added and mixed in an Omnimixer (Dupont Sorvall, Newtown, Conn.) at setting 4 for 10 min. The bacteria were removed by centrifugation at 10,000 × *g* for 10 min. The supernatant was then centrifuged at 50,000 × *g* for 1 h to pellet the pili. The pelleted pili were resuspended in 9 ml of ethanolamine buffer and precipitated overnight at 4°C with 10% ammonium sulfate. After centrifugation at 35,000 × *g*, the sedimented pili were resuspended in ethanolamine buffer (pH 10.5). Isopycnic centrifugation was also performed in 20 to 40% gradients of potassium tartrate. Slight modifications of the purification procedures, such as changes in the *g* forces, duration of centrifugation, and ionic strength of buffers, were made depending on the bacterial strain used (3).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Electrophoresis of pili was performed in a slab gel apparatus (Bio-Rad) by the method of Laemmli (15) with 4 M urea and a 12% separating gel. The gel dimensions were 140 by 100 by 1.2 mm. Pilus preparations were mixed with an equal volume of 0.1 M Tris hydrochloride buffer (pH 6.8) containing 2% (wt/vol) sodium dodecyl sulfate, 20% sucrose (wt/vol), 1% 2-mercaptoethanol (vol/vol), and 0.001% bromphenol blue (wt/vol). The mixture was heated at 100°C for 5 min before 20-μl samples were applied to 6mm-wide sample wells. The samples were electrophoresed at 20 mA per slab gel until the bromphenol blue migrated 10 cm. Proteins were visualized by Coomassie brilliant blue staining.

Electron microscopy. Examination of meningococcal strains for the presence and appearance of pili was performed by electron microscopy as described by Craven et al. (7). Meningococci were grown overnight on BHIA-HS plates and subcultured on fresh plates. Bacteria from a 4-h growth were suspended in PBS containing 1% BSA, and Parlodion (Mallinckrodt, Inc., St. Louis, Mo.)-coated grids were floated on a drop of bacterial suspension for 2 min, fixed for 2 min in 0.1 M cacodylate buffer (pH 7.4) containing 2% glutaraldehyde, blotted with filter paper, and washed two times with distilled water. The grids were stained with 1% sodium phosphotungstic acid (pH 6.1) for 15 s. The specimens were observed and photographed with an Hitachi H-500 transmission electron microscope.

RESULTS

Morphology of pili. When *N. meningitidis* strains were examined by electron microscopy, two morphologically distinct types of pili were observed (Fig. 1). Figure 1A shows a carrier isolate (M992) which has numerous pili which radiate from the surface as distinct single filaments. Partially purified pili from strain M992 are shown in Fig. 1B and, like pili from the bacterial strain from which they were taken, appear as distinct single filaments. Earlier studies in our laboratory (7) indicated that meningococci that elaborated unaggregated pili had 5 to 10 pili per diplococcus. In contrast, *N. meningitidis* S366, a disease isolate, contained a single thick aggregated bundle of pili (Fig. 1C). Strains with aggregated pili usually had one and up to three pilus bundles per diplococcus. A partially purified pilus preparation from S366 shows that the isolated pili also appeared as aggregated bundles (Fig. 1D). We have designated the aggregated pili alpha pili and the unaggregated single filaments beta pili in accordance with the systems of Lambden and Watt (16) and Watt and Heckels (32), who described a similar pilus morphology among strain P9 variants of gonococci.

Subunit molecular weight. Purified pilus preparations from four strains (two alpha and two beta) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and molecular weight determinations were made by measuring the migration distances relative to those of molecular weight standards (data not shown). The relative molecular sizes were also examined by Western blots (immunoblots) with monoclonal antibody 02 (Fig. 2). The molecular weights of the four purified preparations (two alpha and two beta) ranged from 15.3 to 19.0 kilodaltons. The Western blot compared four alpha and four beta pilus-elaborating strains. No distinction between alpha and beta pili could be made on the basis of molecular weight.

Association of pilus type with carrier and disease isolates. The carrier and disease isolates were examined by electron microscopy to determine whether they possessed alpha or beta pili. The frequency of occurrence of alpha and beta pili among disease and carrier isolates can be seen in Table 1. Alpha pili were observed more frequently among disease isolates, with 70% of the disease isolates examined having alpha pili. The opposite was found for beta pili, with 70% of the carrier isolates examined having beta pili.

Reactivity with monoclonal antibodies. The reactivity of meningococci with monoclonal antibody 2-1-Fc was examined by a dot immunoblot technique. Of 10 meningococci with beta pili, 6 reacted with the monoclonal antibody, while 4 of 10 meningococci with alpha-type pili reacted with this antibody (Table 1). As expected, none of the three nonpiliated strains reacted with the monoclonal antibody. Monoclonal antibody 02 showed identical reactivity when Western blot rather than dot blot analysis was used (Table 1 and Fig. 2).

Association of pilus type with meningococcal adherence to HBECs. Twenty piliated and three nonpiliated meningococcal strains were examined for adherence to HBECs (Table 1). We found that 70% of strains with alpha pili had low adherence levels. Among those strains with beta pili, 80% had high or medium adherence levels. The three nonpiliated strains all had low adherence levels. The presence of alpha pili on a meningococcal strain does not necessarily mean that the strain will be low adherent, since 30% of meningococcal strains with alpha pili had high adherence levels, and they were some of the most highly adherent strains observed.

A number of low-adherence and high-adherence meningococcal strains were examined for adherence to several human epithelial cell lines, including FaDU, WIDR, MRC-5, and Detroit 562 (data not shown). There was no detectable adherence to any of these cell lines.

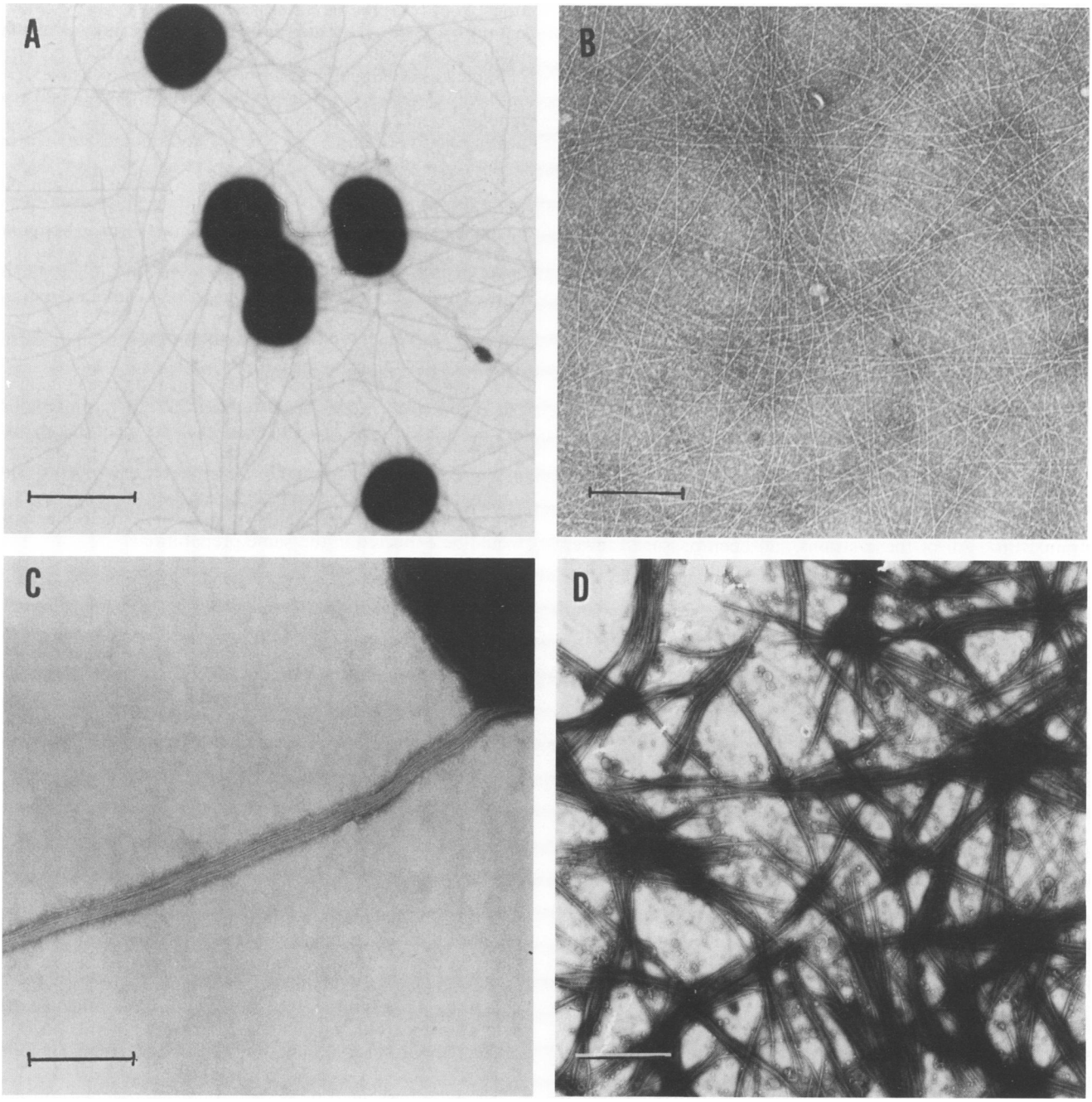

FIG. 1. Electron micrographs of whole meningococci and partially purified meningococcal pili. (A) Strain M992 with single filaments of unaggregated pili; (B) partially purified pili from strain M992 demonstrating unaggregated pilus filaments; (C) strain S366 whole bacteria with aggregated pili; (D) partially purified pili from strain S366 demonstrating aggregated pili. (A and D) Bar = 1.0 μm; (B and C) bar = 0.25 μm.

Association of pilus type with hemagglutination. Meningococci with beta pili were more likely to hemagglutinate human A^+ erythrocytes than were strains possessing alpha pili (Table 1). Of the strains tested, only 20% of the strains with alpha pili hemagglutinated human erythrocytes, compared with 80% of the strains with beta pili. No exclusive relationship exists between buccal cell adherence and hemagglutination. Although medium- and high-adherence strains tended to hemagglutinate, hemagglutinating strains were also found among meningococci which had low HBEC adherence levels. Binding of erythrocytes to meningococci was confirmed by nitrocellulose hemadsorption. Similar patterns of hemagglutination were observed with human B and O Rh^+ and Rh^- erythrocytes.

Pilus phase shifts. We examined multiple isolates from the same patient for pilus morphology, antigenic characteristics, epithelial cell adherence, and hemagglutinating properties. Strain S6058 was a throat isolate from a meningitis patient infected with strain S6056. Strain BB82 was obtained from the throat of a chronic meningococcal carrier 6 weeks after

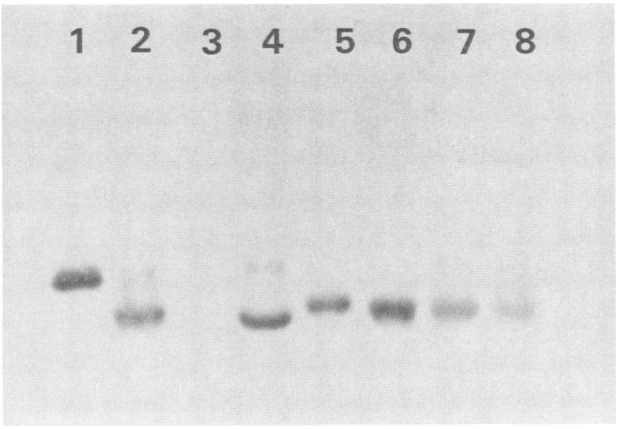

FIG. 2. Immunoblot of whole-cell lysates of meningococci expressing alpha and beta pili. Lanes 1 through 4 and 5 through 8 contain strains with beta and alpha pili, respectively. Lanes: 1, CL6; 2, BB45; 3, BB269; 4, BB98; 5, BB1; 6, BB64; 7, BB252; 8, BB264. The monoclonal antibody used was 02.

strain BB64. All of these strains had been passaged fewer than six times in the laboratory. Strains S6058 and BB82 had characteristics identical to those shown in Table 1 for S6056 and BB64, respectively. These results suggest that meningococci may not undergo pilus phase shift variation as readily as do gonococci.

DISCUSSION

Morphological differences among pili from *Neisseria* species have been described by others. Lambden and Watt (16) and Watt and Heckels (32) described a variant of gonococcal strain P9 having aggregated pili, which they termed alpha type, in contrast to unaggregated single pilus filaments as seen on the parent strain (beta-type pili). When purified, these pilus variants also had subunit molecular weight and antigenic differences. Other workers have also described aggregated pili in gonococcal cell and gonococcal pilus preparations (9, 13, 16, 27, 30). The aggregated pili on gonococci seem to be morphologically similar to the pili we have described in this report. We have therefore used the same designation, alpha, that Lambden and Watt (32) used to describe aggregated pili and the term beta to designate single pilus filaments observed on meningococci. The presence of aggregated pili on meningococci has also been noted by others. DeVoe and Gilchrist (8) reported that one group Y strain of meningococci had intertwined pili, and Poolman et al. (17) have observed aggregated pili in purified meningococcal pilus preparations.

The physiochemical differences between alpha and beta pili resulting in the aggregation of one pilus type and not the other remain unclear. Though pilus appearance can be affected by the preparative techniques used in electron microscopy (30), the data presented in this report suggest that some physiochemical differences exist between pili which radiate from the surface as single filaments and those observed as aggregated bundles. The strains described here were examined repeatedly on several occasions by electron microscopy, and preparations of meningococci have alpha and beta pili were included in all electron microscope studies as controls. In no case were alpha pili seen on meningococci classified as having beta pili. Furthermore, pilus preparations from bacteria classified as having beta pili by examination of whole cells were always seen as individual filaments when purified, and purified pili isolated from bacteria classified as having alpha pili by examination of whole cells were always seen as aggregated bundles. Alpha pili also tended to pellet at much lower speeds than beta pili, suggesting that their appearance is not determined by electron microscopy techniques.

A possible explanation for the aggregation of alpha pili may be found in the results of Schoolnik et al. (22). They found highly hydrophobic regions which form a nonpolar surface along one side of the pilus helix in gonococcal pili. They suggested that this topography favors the interaction of one subunit with the identical region of a subunit from an opposing strand. It is possible that alpha pili could have more hydrophobic regions than beta pili, resulting in aggregation in aqueous solution.

Todd et al. (30) proposed a model for the arrangement of pili in gonococci. They suggested that pili exist as individual threads on the bacterial surface but form thick aggregated bundles as they leave the surface to form a supporting network to interconnect colony members. Our results with meningococci would seem to disagree with their gonococcal pilus model, since aggregated bundles and single filaments would be expected in the same preparations.

Alpha pili were observed more frequently among disease isolates, and alpha-piliated strains were more likely to have low HBEC adherence levels and not to hemagglutinate human erythrocytes. However, alpha-piliated strains were occasionally observed among carrier isolates, and some alpha strains had high adherence levels. Craven et al. (7) and Stephens and McGee (24) have reported that in encapsulated strains, pili are the dominant factors in adherence to HBECs, although outer-membrane proteins may play some role in adherence.

Pili also play a role in hemagglutination, and Schoolnik et al. have demonstrated an erythrocyte-binding domain in gonococcal pili (23). With meningococci, Salit (20, 21) has reported that piliated strains hemagglutinate and that loss of hemagglutinating activity is associated with loss of pili. He could find no change in outer-membrane proteins associated with loss of hemagglutination but could not rule out the possibility of small changes in the structure of outer-membrane proteins. In our studies, nonpiliated, encapsulated strains all had low adherence levels and did not hemagglutinate or bind to erythrocytes, providing further evidence that at least in encapsulated meningococcal strains, pili appear to play an important role in both adherence and hemagglutination.

The data presented here show that heterogeneity in molecular size and expression of the monoclonal 2-1-Fc epitope exist within both the alpha- and beta-type pili. As expected, we found variability in the molecular sizes of the different pili but no consistent differences in size between alpha and beta pili. The monoclonal antibodies 2-1-Fc and 02 showed essentially identical reactivity, although one was made to a meningococcus and the other to a gonococcus. The antibodies reacted with more strains having beta pili than strains having alpha pili. We found no differences by electron microscopy in the degree of piliation between antibody-positive and -negative strains. The epitope(s) recognized by these two monoclonal antibodies was not present in 40% of the carrier strains and 60% of the disease isolates examined.

Our preliminary results with multiple isolates from the same patient demonstrated that these isolates had similar pilus morphology and antigenic characteristics as well as

similar HBEC adherence and erythrocyte-binding properties. Kristiansen et al. (14) examined numerous isolates of meningococci from different sites in individual patients. These multiple isolates were of the same group, type, and polyacrylamide gel electrophoresis-type and had identical DNA restriction endonuclease patterns. However, they differed significantly in adherence and degree of piliation, suggesting that phase shift variation among meningococcal pili is not uncommon, although, it was not observed in our study. Unless we had performed repeated adherence assays, it is doubtful that we would have observed the small but significant differences in adherence that were observed in their study. Another significant finding of our study is that strains having high adherence to HBECs did not necessarily hemagglutinate. We observed strains which readily hemagglutinated but had low adherence to HBECs. Similar results have been reported by Trust et al. (31). One interpretation of this observation is that the strains may be binding to different receptors. For example, a strain which is both hemagglutinating and highly adherent to HBECs may be binding to a different receptor than a strain which has high adherence to HBECs and is nonhemagglutinating. The possible significance of this in the disease process is unknown but suggests differing tissue specificities for different strains of meningococci. Gonococcal isolates have also been shown to have different tissue-binding specificities and to bind to different receptors (31).

In conclusion, we have observed two different pilus morphology types among isolates of meningococci, i.e., aggregated bundles (alpha pili) and unaggregated single filaments (beta pili). Alpha pili were found more frequently among disease isolates, which are strains with predominantly low adherence to HBECs and negative hemagglutination. In contrast, beta pili were found more frequently among carrier isolates, which are strains with predominantly high to medium adherence to HBECs and positive hemagglutination.

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