INFECTION AND IMMUNITY, July 1983, p. 106–113
0019-9567/83/070106-08$02.00/0

Vol. 41, No. 1

Differences in the Adhesive Properties of *Neisseria meningitidis* for Human Buccal Epithelial Cells and Erythrocytes

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Received 7 October 1982/Accepted 8 April 1983

The ability of clinical and carrier isolates of *Neisseria meningitidis* to adhere to human buccal epithelial cells and erythrocytes was investigated. Four of the 10 fimbriated strains were able to hemagglutinate. Serial subculture of three of these strains resulted in a loss of ability to hemagglutinate and was coincident with a loss of fimbriation. Other fimbriated strains were unable to hemagglutinate but did adhere to buccal epithelial cells. Subculture of one of these strains for as many as 42 passages did not result in loss of fimbriation or ability to adhere to buccal epithelial cells. The attachment of this strain to buccal epithelial cells was inhibited by glycoconjugates. Further, pH exerted different influences on the attachment of hemagglutinating and non-hemagglutinating fimbriated strains to buccal epithelial cells and erythrocytes. The results suggest that different fimbrial mechanisms are involved in the attachment of *N. meningitidis* to different cell types and that hemagglutination is not an absolute test for fimbriae.

Neisseria meningitidis is carried asymptomatically in the nasopharynx of 5 to 10% of the population during nonepidemic periods (10). This carrier state and presumably meningococcal disease result from the ability of *N. meningitidis* to adhere to nasopharyngeal mucous membranes (21). Fimbriae are normally present on freshly isolated meningococci from both the nasopharynx of carriers and the blood and cerebrospinal fluid of patients with meningococcal disease (7, 8) and are undoubtedly important in the interaction of these microorganisms with human cells. Recent studies have demonstrated a role for these filamentous protein appendages in the attachment of meningococci to human cells (5, 6, 23, 24, 26). Indeed, Salit (23) has suggested the use of an attachment assay, hemagglutination, as a test for monitoring the degree of fimbriation of meningococcal cultures and fimbrial purification.

Fimbriae are also important in the attachment of *Neisseria gonorrhoeae* to human cells (19, 26, 27). The reported subunit molecular weights and amino acid compositions for gonococcal fimbriae suggest significant structural variation in the fimbriae isolated from different strains, and this is reflected in marked antigenic variation (3, 4, 22). Most recently, Lambden et al. (16) have shown that a single strain of gonococcus can produce as many as four different fimbrial types (designated α, β, γ, and δ). Two of these fimbrial types, α and β, have been shown to differ in their ability to attach to buccal epithelial cells and human erythrocytes (15, 17), α fimbriae binding specifically with a receptor on buccal cells (15, 17). Brener et al. (2) have also presented morphological evidence for the production of three fimbrial types by a strain of *N. meningitidis*, but there is little other information on differences among meningococcal fimbriae or indeed on the role that these fimbrial types might play in meningococcal attachment.

In this study we have examined the adherence of case and carrier strains of meningococci to human buccal epithelial cells and to erythrocytes. The results suggest that, like the case of *N. gonorrhoeae*, at least two different fimbrial mechanisms are involved in the attachment of *N. meningitidis* to different human cell types.

MATERIALS AND METHODS

Bacterial strains. The strains of *N. meningitidis* used during the course of this study are listed in Table 1, with their serogroups and sources. Both disease strains (i.e., strains obtained from cases of overt meningitis) and carrier strains (i.e., strains obtained by nasopharyngeal swabbing of asymptomatic carriers) were included. Stock cultures were stored at −80°C in 15% (vol/vol) glycerol–trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.). Bacteria were grown on the clear gonococcal typing medium of Swanson (27) to which vancomycin-colistin-nystatin inhibitor (BBL Microbiology Systems) and 20 μg of iron dextran (Imferon; Fisons Corp. Ltd., Scarborough, Ontario) per ml were added. The incubation

TABLE 1. Bacterial strains, sources, and serogroups

Strain	Source[a]	Serogroup
DRES-30A	Blood, L. A. White	A
SP3428	CSF, I. W. DeVoe	A
SP3428-P	L. A. White, derivative of SP3428	A
SP3428-NP	L. A. White, derivative of SP3428	A
SP3428.16	This laboratory, subcultured derivative of SP3428	A
VC7	Carrier, this laboratory	B
VC7.42	This laboratory, subcultured derivative of VC7	B
VC10	Blood, W. Black	W-135
VC11	Carrier, W. Black	W-135
VC12	Carrier, W. Black	X
VC13	Carrier, W. Black	B
VC14	Carrier, W. Black	X
VC15	Carrier, W. Black	NG[b]
VC15.15	This laboratory, subcultured derivative of VC15	NG

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[b] NG, Nongroupable.

conditions were 37°C for 18 h in an atmosphere containing 5% (vol/vol) CO_2 in air. All strains studied produced transparent colonies under these growth conditions. All strains were identified as meningococci by oxidase testing, Gram stain, and sugar utilization in Mueller-Hinton broth and were serogrouped by slide agglutination to detect capsular antigens.

^{3}H-labeled meningococcal suspensions free of aggregates for use in attachment assays were prepared according to the criteria established by Stephens and McGee (26) by a modification of their procedure. Cells were grown on plates which had been spread with D-[1-^{3}H]glucose (Amersham Corp., Oakville, Ontario), harvested by swab, and gently suspended in 5 ml of minimal essential medium (Eagle with Hanks balanced salts (TCM; Flow Laboratories, Inc., Rockville, Md.]), pH 7.4 or appropriate pH, and aggregates were pelleted by centrifugation at 150 × *g* for 1 min. A small volume (normally 100 to 300 μl) was removed from the top 1 ml of the resulting meningococcal suspension and diluted to a cell density of 10^7 CFU/ml. At this cell density the ^{3}H-specific activity of all strains examined normally displayed >85% similarity. Suspensions were also examined by Gram stain or phase-contrast microscopy, and as shown by Stephens and McGee (26), this procedure normally resulted in >97% of the bacterial units containing one to three bacteria.

Determination of meningococcal attachment. Meningococcal attachment to host cells was quantitated by a modification of the method first described by Lambden et al. (1, 15) for gonococci. Buccal epithelial cells and group A erythrocytes were collected from volunteers, washed several times in phosphate-buffered saline (PBS) at the appropriate pH, and finally suspended at a packed cell volume of 3% (buccal) or 5% (blood) in TCM at the appropriate pH. In some experiments erythrocytes from other blood groups or species were used (Flow Laboratories). Cells were washed three times and resuspended in TCM. In other experiments a 10% (vol/vol) suspension of octyl-Sepharose (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) was used (19, 29). A 1-ml volume of ^{3}H-labeled meningococci (16,000 dpm) and a 1-ml volume of host cells or octyl-Sepharose were incubated together with constant gentle shaking at 37°C for 1 h unless otherwise specified. The extent of fimbriation of suspensions of meningococci used in attachment assays was normally confirmed by electron microscopy immediately before the assay. Control tubes contained only the tritiated bacteria and 1 ml of TCM. After the incubation period, unattached bacteria were separated from bacteria attached to host cells (or octyl-Sepharose) by layering 500 μl of reaction mixture onto each of three 10-cm columns of Ficoll 400 (57.6 g/liter in PBS; Sigma Chemical Co., St. Louis, Mo.) in a Nunclon plastic tube (Nunc, Roskilde, Denmark) and centrifuging at 500 × *g* for 3 min in a swing-out head. This Ficoll isokinetic centrifugation procedure has been shown to efficiently separate unattached bacteria which remain at the top of the Ficoll 400 shelf from attached bacteria which pellet along with the host cell (or octyl-Sepharose) (1, 28). The pellet was recovered after aspiration of the Ficoll 400 and unattached meningococci and counted in a Beckman LS 1800 counter with the automatic quench correction mode. A second control was used to estimate the amount of extracellular radioactivity that would associate with the eucaryotic cells. In this control, the bacterial suspension was incubated by itself for 1 h under the test assay conditions. Bacterial cells were then removed by recentrifugation at 500 × *g* for 10 min. The cell-free supernatant was removed and added to a buccal epithelial or erythrocyte suspension, and the reaction was allowed to proceed as for the test assay. This control was then subjected to the Ficoll separation procedure. In the case of fractions containing erythrocytes, blood cells were first lysed with 100 μl of 1.0 M NaOH for 5 min and then decolorized for 3 h at 37°C after the addition of 100 μl of 30% H_2O_2. The degree of attachment was calculated from the ratio of radioactivity associated with the Ficoll-pelleted eucaryotic cells (or octyl-Sepharose) to the total radioactivity in the reaction mixture, after correction for the control assay values which accounted for any unattached bacteria or non-cell-associated radioactivity which may have sedimented through the Ficoll 400 shelf. Normally this was <2% of the total radioactivity in the reaction mixture.

Experiments were performed to study the effect on meningococcal attachment of: (i) time; (ii) pH (determined in TCM adjusted to the desired pH by the addition of 1 M Tris or 1 M maleic acid); (iii) the sugars D-glucose, α-L-fucose, D-fucose, D-+-mannose, L-mannose, D-mannosamine, yeast mannan (0.5%), α-methyl-D-mannose, D-galactose, L-galactose, arbutin, D-+-talose, *N*-acetyl neuraminic acid, and *N*-galactosamine; (iv) the glycoconjugates heparin, mucin type I (from bovine submaxillary glands), fetuin type IV (from fetal calf serum), ganglioside types II and III (from bovine brain). In these experiments, monosaccharides or glycoconjugates were added to the reac-

tion mixture immediately before addition of bacteria. All chemicals were of the highest grade available (Sigma). In other experiments designed to evaluate the role of surface carbohydrates in attachment, meningococci were subjected to periodate oxidation with 20 mM sodium periodate followed by treatment with 10 mM sodium borohydride in the dark at 4°C as previously described (29).

Hemagglutination. Human group A blood was collected by venipuncture, cells were washed three times in complete Dulbecco PBS, pH 7.4 (Oxoid, Basingstoke, England), and 3% (vol/vol) suspensions were prepared in this saline. Plate-grown culture suspensions of bacteria were prepared in PBS to yield approximately 1.5×10^9 bacteria per ml by McFarland turbidity standards. Slide hemagglutinations were performed at room temperature by mixing 20 μl of the blood suspension with 20 μl of bacterial suspension on a slide and gently rocking the slide by hand. A PBS-erythrocyte control was always included. Strains were considered negative hemagglutinators if hemagglutination had not occurred within 10 min. The inability to hemagglutinate was confirmed in microtiter plates according to the procedures of Salit (23). The minimum hemagglutinating dose was measured as the smallest number of bacterial cells per milliliter that gave visible hemagglutination in 10 min, and the hemagglutinating power of a culture was calculated as 10^{11} per minimum hemagglutinating dose (1).

Salt aggregation. The concentration of $(NH_4)_2SO_4$ required to cause aggregation of meningococcal cells was determined according to the procedure of Lindahl et al. (19). Strains of *Escherichia coli* used by Lindahl et al. (19) were used as controls.

Electron microscopy. Fimbriation was quantitatively assessed by electron microscopy of negatively stained preparations. A total of 1 to 2 μl of meningococcal suspensions used in attachment assays, or directly from plates and gently suspended in PBS, were allowed to stand on Formvar-carbon grids for 1 to 2 min. Excess suspension was removed, and the remaining sample was negatively stained by adding an equivalent volume of 1% (wt/vl) ammonium molybdate, pH 7.2. After a further 1 to 2 min, the excess ammonium molybdate was removed and the grids were dried in air before examination with a Philips EM300 electron microscope. Each strain was examined on three separate occasions by three different observers. On each occasion, 25 meningococci were examined and the percentage of cells with fimbriae was estimated. The numbers of fimbriae per cell were also estimated, as was fimbrial width.

RESULTS

Meningococcal attachment and fimbriation. Two techniques were applied to the quantitation of meningococcal attachment to human cells. One was the hemagglutination technique, which has been extensively used to determine attachment to erythrocytes. The other technique allowed the attachment to buccal epithelial cells to be quantitated. The radiolabel technique chosen has previously been successfully used to quantitate gonococcal attachment and to study the attachment of purified gonococcal fimbriae (15, 17, 18). Indeed, the accuracy and reproducibility of this technique allowed the roles of different outer membrane proteins and different fimbrial types in the adhesive capacity and specificity of *N. gonorrhoeae* to be defined (12, 15, 17, 18).

TABLE 2. Fimbriation and adhesive ability of *N. meningitidis* cultures

Strain	% Fimbriation	% Attachment to buccal cells[a]	Hemagglutinating power[b]
DRES-30A	>90	16 ± 3 (5)	N
SP3428	90	25 ± 3 (5)	N
SP3428-P	50	6 ± 2 (7)	N
SP3428-NP	50	14 ± 5 (6)	N
VC7	>90	27 ± 3 (9)	1.7×10^5
VC10	70	22 ± 3 (4)	N
VC11	90	28 ± 2 (6)	5.6×10^3
VC12	50	24 ± 5 (6)	N
VC13	50	11 ± 5 (6)	1.1×10^4
VC14	0	16 ± 4 (3)	N
VC15	>90	36 ± 4 (6)	8.5×10^4

[a] Percent attachment = (^{3}H associated with Ficoll-pelleted eucaryotic cells/^{3}H total in reaction mixture) × 100. Arithmetic mean ± standard deviation (number of estimates).

[b] Hemagglutinating power is 10^{11} per minimum hemagglutinating dose (MHD), where MHD is the smallest number of bacterial cells per milliliter giving visible hemagglutination in 10 min. N, No hemagglutination.

The results in Table 2, using these two attachment assay techniques, clearly show that the ability to attach to buccal epithelial cells was not correlated with the ability to attach to erythrocytes as measured by hemagglutination. Indeed, although all but one strain produced fimbriae, only four strains were able to hemagglutinate. These strains, VC7, VC11, VC13, and VC15, gave 38 ± 4, 23 ± 3, 23 ± 4, and 35 ± 4% attachment (mean ± standard deviation) to erythrocytes, respectively, when assayed with the radiolabel technique. All other strains gave <14% attachment to erythrocytes. Since other fimbriated strains which were able to attach to buccal epithelial cells failed to hemagglutinate, even in microtiter assays, the results suggested that different fimbrial binding mechanisms were involved in the interaction of *N. meningitidis* with different human cells. Only one of the strains tested did not produce fimbriae, VC14; however, this strain did attach to buccal epithelial cells. This result suggested that, like some strains of *N. gonorrhoeae*, other surface components of the meningococcus likely participate in attachment to host cells.

Effect of subculture on fimbriation and adhesive ability. Although most workers have reported the failure of meningococci to maintain fimbriation after several passages in vitro (9, 23),

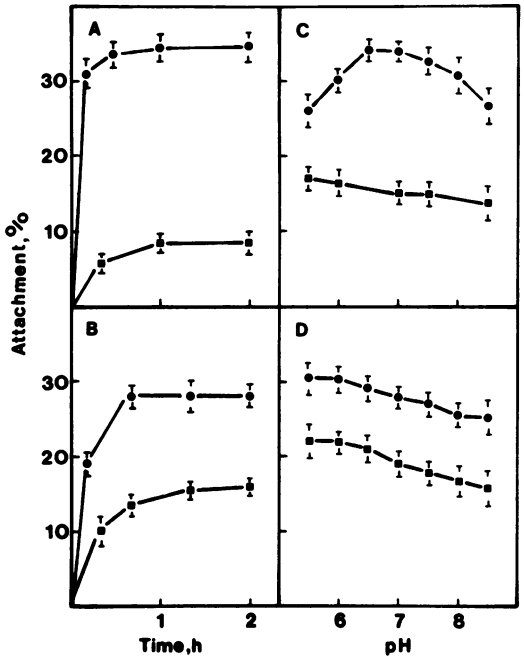

FIG. 1. Attachment of fimbriated hemagglutinating *N. meningitidis* strain VC7 (●) and nonfimbriated variant VC7.42 (■) to human cells. Kinetics of attachment to (A) human erythrocytes and (B) buccal epithelial cells, and effect of pH on attachment to (C) human erythrocytes and (D) buccal epithelial cells. Each point represents the mean ± standard deviation (vertical bars) of triplicate determinations.

there are reports of maintenance of fimbriation during in vitro passage (20). We therefore tested the stability of fimbriation of a representative piliated non-hemagglutinating strain, SP3428, and a fimbriated hemagglutinating strain, VC7. The fimbriae on both strains had a mean diameter of 5.6 nm, were longer than 2 μm, and could not be distinguished morphologically. The average number of fimbriae per cell in the case of SP3428 was five, whereas VC7 had an average of seven fimbriae per cell. The other meningococcal strains used in the study had similar numbers of fimbriae per cell. However, although fimbriation was a stable property in the case of SP3428, this was not the case with VC7. After 42 subcultures, the ability of SP3428 to attach to buccal epithelial cells remained unchanged, as did the degree of fimbriation. In contrast, serial subculture of VC7 resulted in a loss of ability to produce fimbriae, and this coincided with a loss of ability to hemagglutinate and a reduced ability to attach to buccal cells. After nine subcultures, only 50% of the cells of VC7 were fimbriated, and the hemagglutinating power had fallen from 1.7×10^5 to 2.2×10^4. After 22 subcultures, VC7 could no longer hemagglutinate, and no fimbriated cells were detected. Attachment to buccal epithelial cells had also been reduced by 30%. When this result was obtained, two other hemagglutinating strains were serially subcultured and examined for stability of fimbriation. In the case of VC13, ability to hemagglutinate was lost after 4 subcultures, whereas in the case of VC15 hemagglutination was not seen after 10 subcultures. Electron microscopic examination of the non-hemagglutinating variants confirmed the absence of fimbriae, and two nonfimbriated variants, VC7.42 and VC15.15, derived in this manner were used in subsequent studies.

Characteristics of attachment. The adhesive advantage afforded by possession of fimbriae was clearly demonstrated when the kinetics of attachment to buccal epithelial cells and erythrocytes was measured. The results in Fig. 1 show that the fimbriated hemagglutinating strain VC7 attached rapidly to both buccal epithelial and blood cells, whereas the nonfimbriated variant VC7.42 displayed markedly reduced attachment to erythrocytes and a slower attachment to buccal epithelial cells. The non-hemagglutinating fimbriated strain SP3428 also attached quite rapidly to buccal epithelial cells, but did not attach significantly to erythrocytes in the 3-h incubation period (data not shown).

The adhesive advantage offered by possession of fimbriae was further revealed by UV irradiation of whole cells. This treatment has been shown to produce fimbrial denaturation (3, 29), and the results in Table 3 shown that a relatively mild UV irradiation of SP3428 and VC7 markedly reduced their ability to attach to buccal epithelial cells, whereas only a small reduction in adhesive ability was shown by the nonfimbriated strain VC7.42. In contrast, modification of surface carbohydrates by periodate oxidation followed by borohydride reduction did not affect the binding to buccal epithelial cells (Table 3). This provided evidence that capsular carbohydrate or lipopolysaccharide was not participating in the attachment of the strains tested.

Differences in the mechanism of attachment of meningococci were revealed when the influence of pH was tested. The attachment of the non-hemagglutinating fimbriated strain SP3428 was not strongly influenced by pH, although an optimum at pH 7.0 was demonstrated (data not shown). Striking differences were seen, however, between the fimbriated hemagglutinating strain VC7 and the nonfimbriated variant VC7.42 when attachment to erythrocytes was measured over the broad pH range (Fig. 1). The attachment of the fimbriated strain was strongly influenced by pH, with a pronounced optimum between pH 6.5 and 7.0. In contrast, the binding of the nonfimbriated variant showed a steady decline over the pH range 5.5 to 8.5. The influ-

ence of pH on the attachment of fimbriated VC7 was markedly different with buccal epithelial cells compared with erythrocytes. In this case, whereas more fimbriated cells bound to buccal epithelial cells than nonfimbriated cells, the binding of both VC7 and VC7.42 declined steadily over the pH range 5.5 to 8.5.

The specificity of the attachment of hemagglutinating strains VC7 and VC15 to erythrocytes was investigated by comparing erythrocytes from various species and from the major human blood groups. Strain VC15 gave good attachment to rabbit, guinea pig, and horse erythrocytes, with values (mean ± standard deviation) of 21 ± 3, 38 ± 4, and 36 ± 4% attachment, respectively. This strain attached poorly to bovine and sheep cells (<9%), but gave good attachment values with cells of each of the major human blood groups: group A, 38 ± 4%; group B, 45 ± 4%; group AB, 43 ± 4%; group O, 38 ± 3%. Strain VC7 was more restricted in its attachment ability, with attachment values of <9% with each of the animal erythrocyte species, but did attach well to human erythrocytes: group A, 27 ± 3%; group B, 31 ± 3%; group AB, 30 ± 2%; group O, 28 ± 2%. The nonfimbriated variants VC15.15 and VC7.42 and the fimbriated nonhemagglutinating strain SP3428 gave <11% attachment with all erythrocyte species.

Attachment specificity was further investigated by sugar inhibition studies. The attachment of SP3428 to buccal epithelial cells and of VC7 to human erythrocytes was not inhibited by the following simple carbohydrates at final concentration of 1 mM: D-glucose, α-L-fucose, D-fucose, D-+-mannose, L-mannose, D-mannosamine, yeast mannan (0.5%), α-methyl-D-mannose, D-galactose, L-galactose, arbutin, D-+-talose, *N*-acetyl neuraminic acid, *N*-acetyl galactosamine. Differences in the specificities of attachment were revealed, however, when the ability of more complex carbohydrates to inhibit attachment was tested. The results in Table 3 show that, whereas the attachment of VC7 and VC7.42 to buccal cells and erythrocytes was not inhibited by the glycoconjugates tested, the attachment of fimbriated strain SP3428 to buccal epithelial cells was strongly inhibited. Indeed this inhibition occurred with heparin, mucin, fetuin IV, and with quite low concentrations of mixed gangliosides.

The extent to which nonspecific hydrophobic interactions influence the binding of fimbriated strains of meningococci was then measured with octyl-Sepharose (7, 19, 29). At pH 7.4, under ionic concentrations comparable to those on mucosal surfaces, fimbriae did not appear to influence the binding to this alkyl-substituted agarose. Fimbriated strain VC7 gave 8 ± 1% attachment to octyl-Sepharose, whereas the nonfimbriated variant VC7 gave 10 ± 1% binding. In the case of VC15 and VC15.15, 8 ± 1% attachment was obtained. Strain SP3428 gave 11 ± 1% attachment. Control values with butyl-substituted Sepharose were <2%, and those with unsubstituted Sepharose were all <1%. The effect of fimbriae on the surface hydrophobicity of meningococcal cells was also evaluated by using the salting-out procedure recently developed by Lindahl et al. (19). The method is based on precipitation (aggregation) of cells by $(NH_4)_2SO_4$, with the concentration of $(NH_4)_2SO_4$ at which the cells are precipitated providing a measure of their surface hydrophobicities. In this procedure, fimbriae did not appear to significantly influence the surface hydrophobicity of meningococcal cells since both VC7 and VC7.42 aggregated at 2.0 M $(NH_4)_2SO_4$, and both VC15 and VC15.15 aggregated at 1.2 M. Strain SP3428 aggregated at 2.0 M $(NH_4)_2SO_4$.

TABLE 3. Effect of modifying meningococcal surface components and glycoconjugates on binding of ^{3}H-labeled fimbriated (F^+) and nonfimbriated (F^-) variants of *N. meningitidis* to buccal epithelial cells

Treatment	% Attachment[a]		
	F^+		F^-
	SP3428	VC7	(VC7.42)
None	22 (±3)	30 (±2)	10 (±1)
UV irradiation[b]	7 (±2)	10 (±1)	8 (±2)
Periodate/$NaBH_4$	21 (±4)	31 (±2)	14 (±2)
$NaBH_4$ control	22 (±2)	33 (±2)	14 (±2)
Glycoconjugate			
Heparin (2 mg/ml)	7 (±2)	39 (±5)	8 (±1)
Mucin (2 mg/ml)	5 (±1)	33 (±4)	8 (±2)
Fetuin IV (1 mg/ml)	8 (±2)	33 (±4)	8 (±1)
Gangliosides II (1 μg/ml)	16 (±2)	35 (±5)	9 (±1)
Gangliosides III (1 μg/ml)	10 (±1)	40 (±4)	9 (±2)

[a] Percent attachment = (^{3}H associated with Ficol-pelleted eucaryotic cells/^{3}H total in reaction mixture) × 100. Arithmetic mean (± standard deviation) for three or more estimates.

[b] UV irradiation = 254 nm at 4.5 cm (8 μW cm^{-2} at 48 cm) for 10 min.

DISCUSSION

The mechanism of attachment of *N. gonorrhoeae* to human cells and the role of fimbriae in this attachment has commanded much attention. These studies received impetus because of the correlation between fimbriation and virulence of the gonococcus. Further, gonococcal fimbriation can be correlated with colonial type, and because of the ease with which nonfimbriated isogenic variants can be identified and isolated, the role of fimbriae in adhesion was quickly established (14, 26–28). The picture of gonococ-

cal attachment that has emerged is complex, involving production of a variety of fimbrial types, some of which are selected for during in vivo growth (16, 17, 18). There is also evidence that these fimbrial types have different specific receptors on human cells, the recognition and binding to which may contribute to the tissue specificity of the gonococcus. Further, the mechanisms of attachment of these various fimbrial types are clearly different (16, 17, 18).

The attachment mechanism of *N. meningitidis* and the role of fimbriae in this attachment has received less attention. In part this has been attributed to lack of differences in colonial morphologies between fimbriated and nonfimbriated variants (2, 7, 9, 20). Moreover, considerable variability in meningococcal fimbriation has been reported, and the regulation of fimbrial production by nutrients during in vitro culture is not understood (2, 8, 9, 20, 23). This is illustrated by the case of one of the strains used in this study, SP3428. DeVoe and Gilchrist (8) have reported that SP3428 can dissociate into two clones, one constitutively fimbriated and one lacking fimbriae. These substrains were included in this study and were SP3428-P and SP3428-NP, respectively. On Mueller-Hinton agar the parent SP3428 has been reported to be nonfimbriated, and when first grown on Trypticase soy agar this strain became fimbriated, losing its fimbriae upon subsequent transfers on the same medium (7). In our hands, using serial subculture, the fimbriation of the parent SP3428 was clearly stable. Furthermore, strain SP3428-NP was also fimbriated.

In contrast to strain SP3428, the fimbriation of hemagglutinating strains was unstable under the subculture conditions used in this study. This loss of fimbriae and ability to hemagglutinate was also shown by Salit (23). Indeed, Salit utilized an affinity culturing technique to maintain the fimbriation of hemagglutinating strains. Salit also suggested hemagglutination as a technique to replace electron microscopy for the detection of meningococcal fimbriae. However, our demonstration that not all fimbriated strains hemagglutinate, in addition to suggesting the occurrence of different fimbrial types, indicates that the hemagglutination test should not replace electron microscopy for the detection of meningococcal fimbriae, but rather should supplement it.

Fimbriae clearly can enhance attachment of the meningococcus to human cells. However, the results obtained in this study show that meningococci display specificity in this enhanced attachment. In the case of SP3428, the meningococcal cells attached rapidly to buccal epithelial cells, but did not attach at all to erythrocytes. Furthermore, attachment to buccal cells was not inhibited by a range of single monosaccharides known to be constituents of cell surface glycolipids and glycoproteins, but attachment was inhibited by glycoconjugates. This suggests that an oligosaccharide may be involved in the receptor for this fimbrial type.

In contrast to SP3428, cells to strain VC7 attached to both buccal epithelial cells and human erythrocytes. Further, the attachment mechanisms used with these two cell types differed. Attachment to erythrocytes was clearly specific, with a distinct pH optimum for the fimbriae-mediated attachment. Binding of this strain to buccal epithelial cells involved a different mechanism, one with no defined pH optimum. Indeed, although fimbriae enhanced attachment of strain VC7 to buccal cells, the marked similarity of the pH curve for the nonfimbriated variant VC7.42 suggests that the enhanced binding could involve nonspecific factors. Certainly buccal epithelial cells differ from erythrocytes in lacking a specific binding site for hemagglutinating fimbriae. Interestingly, in the case of *N. gonorrhoeae* an analogous picture is presented (17, 18) since isolated α fimbriae and bacteria bearing α fimbriae bind to buccal cells with a distinct pH optimum, whereas their binding to erythrocytes shows no pH optimum, but rather a steady decline with increasing pH (17, 18). A similar steady decline is shown by β fimbriae with both buccal cells and erythrocytes. This parallel between the two species of *Neisseria* clearly points to the occurrence of different fimbrial types in *N. meningitidis*.

To date, the fimbrial protein of only one strain of *N. meningitidis* has been characterized (13). Hermondson et al. (13) reported that the meningococcal protein sequence was identical with the amino acid sequence of fimbrial proteins from four antigenically dissimilar strains through the first 29 residues. More recent studies by Lambden et al. (16, 17, 18) have shown that, although there is a high degree of structural similarity among the α, β, γ, and δ fimbriae of *N. gonorrhoeae*, there are significant differences. For example, although α and β fimbriae share some 25 peptides, each fimbrial protein possesses a smaller number of unique peptides. One or more of these unique peptides presumably provides α fimbriae with the ability to specifically bind to a receptor on buccal epithelial cells. Our findings clearly indicate that similar detailed attention needs to be directed at the structural differences between meningococcal fimbrial proteins.

The ability of the nonfimbriated strain VC14 to adhere to buccal epithelial cells, together with the ability of nonpiliated variant VC7.42 to adhere to buccal epithelial cells, albeit relatively poorly, suggests a role for surface components other than fimbriae in the adherence of meningo-

cocci. In the case of *N. gonorrhoeae*, outer membrane protein II has been shown to play an important role in adhesion. Indeed, Heckels (12) has recently provided evidence that gonococcal cells producing a 28.5K protein II show enhanced attachment to leukocytes, whereas strains which produce a 28.0K protein II display enhanced attachment to buccal epithelial cells. Clearly, one or more of the surface-exposed outer membrane proteins of the meningococcus could also have an important role in attachment and the best candidate may be protein e, which is surface exposed and has a molecular weight of approximately 28,000 (10).

Nonspecific hydrophobic interactions may contribute to the adhesion of meningococci. Certainly the strains tested here did bind better to octyl-Sepharose than to butyl-Sepharose, and the binding obtained with the octyl-Sepharose was of the same magnitude as that reported for gonococci (29). Fimbriae, however, did not confer an advantage on the binding of meningococci to octyl-Sepharose. This was also the case in *N. gonorrhoeae*, but is in marked contrast to the high degree of binding to amphipathic gels shown by strains of *E. coli* bearing K88 and other fimbriae-like antigens (19, 25). The simplest explanation of this is that, by virtue of the small numbers of fimbriae per meningococcal cell, the fimbriae do not make a significant contribution to surface hydrophobicity. This would account for our observation that both fimbriated and nonfimbriated variants aggregated at the same $(NH_4)_2SO_4$ concentration. In contrast, Lindahl et al. (19) demonstrated that in the case of *E. coli* the large numbers of fimbriae present per cell clearly contribute to surface hydrophobicity. For example, a strain bearing CFAII antigen aggregated in 0.06 M $(NH_4)_2SO_4$, whereas an isogenic nonfimbriated variant aggregated in 1.9 M $(NH_4)_2SO_4$.

In summary, this report provides evidence for the occurrence of different fimbriae types on *N. meningitidis* which allow enhanced specific attachment to human cells. One of these fimbriae types has a specific receptor on human erythrocytes, whereas another fimbriae type lacks a receptor on human erythrocytes. The binding of this latter fimbriae type to buccal epithelial cells is inhibited by glycoconjugates. Studies are now under way to isolate and characterize these fimbriae proteins and to define their adhesive mechanisms.

ACKNOWLEDGMENTS

Dedicated technical assistance was provided by A. G. Khouri and M. H. Knodel.

This study was supported by a contract grant from the Defence Research Establishment, Suffield, Alberta, and the Department of National Defence.

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