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Analysis of Damage to Human Ciliated Nasopharyngeal Epithelium by *Neisseria meningitidis*

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We used an in vitro model of human nasopharyngeal tissue in organ culture to evaluate the effects of *Neisseria meningitidis* on human cilia and ciliary function. Encapsulated, viable meningococci damaged ciliated epithelium of nasopharyngeal organ cultures, whereas *Neisseria subflava*, a commensal species, did not. Meningococcus-induced ciliary damage was due to loss of ciliated cells to which meningococci were not attached. Damage was seen with piliated and nonpiliated meningococci and did not appear to require the presence of other specific meningococcal surface proteins. Meningococcal viability was a requirement for both ciliary damage and interactions of meningococci with microvilli of nonciliated epithelial cells. That is, filter-sterilized supernatants from meningococcus-infected organ cultures, heat-killed meningococci at high inoculum, and purified meningococcal or gonococcal lipopolysaccharide at concentrations of 100 μg/ml did not damage ciliary activity of nasopharyngeal organ cultures. In contrast, meningococcal lipopolysaccharide at 10 μg/ml markedly damaged ciliary activity of human fallopian tube organ cultures, suggesting a selective toxicity of lipopolysaccharide for specific human ciliated cells. Damage to nasopharyngeal ciliated epithelium by *N. meningitidis* may be an important first step in meningococcal colonization of the human nasopharynx, but meningococcal lipopolysaccharide does not appear to be directly responsible for this toxicity.

Neisseria meningitidis continues to cause worldwide health problems (25). The human nasopharynx is the major focus from which the organism is transmitted to other individuals (26), the primary site of colonization which leads to the development of systemic immunity (12), and possibly a site of mucosal invasion preceding systemic disease (28, 30). Most individuals who harbor the meningococcus in the nasopharynx are asymptomatic, but overt nasopharyngitis (3, 18, 26) has been reported in patients with meningococcal sepsis or meningitis.

Despite the importance of nasopharyngeal colonization in the pathogenesis of meningococcal infections, this area of the biology of the organism has been neglected. Only recently have studies focused on the events that occur in the upper respiratory tract leading to attachment and colonization of the human nasopharynx by meningococci (8, 27, 28, 30, 31). We have developed an experimental model for analysis of meningococcal interactions with human nasopharyngeal mucosa (30). This model provides an opportunity to study the first steps in the pathogenesis of *N. meningitidis* by utilizing mucosa from a site of proven importance in the initiation and transmission of meningococcal disease.

Previously, we examined the interactions of encapsulated, piliated *N. meningitidis* with ciliated columnar epithelium of the human nasopharynx (30). Ciliated columnar epithelium was studied because this composes the majority of the mucosa of the nasopharynx in young children (1), a group especially susceptible to complications of meningococcal colonization and invasion. We found that meningococci attached selectively to certain nonciliated columnar epithelial cells. After attachment, the microvilli of these nonciliated cells elongated and surrounded meningococci. At 6 to 12 h after infection, endocytic vacuoles containing meningococci were seen in the apical portion of some nonciliated columnar cells. Later, diplococci were seen in the subepithelial tissue adjacent to lymphoid tissue, suggesting that meningococci had penetrated the epithelial layer. In addition, we noted that meningococci damaged the ciliary vigor (CV) of human nasopharyngeal mucosa. In the current study, we investigated some of the requirements for meningococcus-induced damage to ciliary function and studied further the interactions of meningococci with columnar epithelial cells of human nasopharyngeal mucosa.

MATERIALS AND METHODS

Microorganisms. Four well-characterized group B meningococcal strains were used in these studies. Their antigenic designations are according to a newly proposed scheme for meningococcal strain designation which includes serogroup: serotype: class 1 subtype: LPS serotype: sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) type (10). Strains 3006 (B:2b:P1.2:L2,3) and H-355 (B:15:P1.15:L3,8) were kindly supplied by W. D. Zollinger, Walter Reed Institute of Research, Washington, D.C.; strain 2996 (B:2b:P1.2) was kindly supplied by Jan Poolman, Laboratorium Voor de Gonzondheidsleer, Amsterdam; strain 269B (B:NT:SDS-PAGE type IV), the characteristics of which have been previously reported (32), was also used. Outer membrane protein composition, colony type (opaque or transparent), and piliation of these isolates were determined as previously described (32). In addition, *Neisseria subflava* ATCC 19243 was used.

The solid medium used for cultivation of microorganisms consisted of chocolate agar plus 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.), designated ChocIso agar, or GC agar base (Difco Laboratories, Detroit,

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Mich.) plus 2% (vol/vol) IsoVitaleX, designated GCIso agar. The medium used for suspending microorganisms was Eagle minimal essential medium containing Earle salts and L-glutamine buffered with 0.05 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.45) (HEPES-MEM).

Preparation of human nasopharyngeal organ cultures. Nasopharyngeal tissue was obtained at the time of surgery from children undergoing adenoidectomy for medical indications. Nasopharyngeal tissue was collected and maintained (30) in HEPES-MEM containing colistin sodium methanesulfonate (3 μg/ml), vancomycin hydrochloride (5 μg/ml), and penicillin G (1 μg/ml) (HEPES-MEM-ATB). In some experiments, HEPES-MEM-ATB was further supplemented with heat-inactivated fetal bovine serum (20% [vol/vol] final concentration). Using sterile technique, the nasopharyngeal tissue was dissected into pieces (~3 by 3 mm), making sure that at least one side was a mucosal surface. The smooth, glossy mucosal surface can, with practice, be readily distinguished from the rough, cut surface. Each organ culture was placed in a tissue culture dish (Falcon 3001, 35 by 10 mm; Becton Dickinson Labware, Oxnard, Calif.) with 2.0 ml of HEPES-MEM-ATB. The dishes were placed in an incubator at 37°C in a humid atmosphere containing 3% CO_2 for 4 h to allow time to recover from trauma. Ciliary activity after this incubation was superior to that of tissue used immediately after dissection. After the 4-h incubation, HEPES-MEM-ATB was removed, the tissue was rinsed with HEPES-MEM, and HEPES-MEM or HEPES-MEM containing only vancomycin (5 μg/ml) was used as the medium for the organ cultures for the remainder of the experiment. Contamination by indigenous or exogenous bacteria was monitored by culturing ~0.01 ml of the medium before and at 6-h intervals after beginning each experiment. Data obtained from experiments were discarded if control or infected organ cultures were contaminated with bacteria other than the test strains.

Infection of human nasopharyngeal organ cultures. The tissue was infected or exposed to meningococcal components according to the experimental design. Microorganisms used to infect organ cultures were grown overnight on ChocIso agar or GCIso agar at 37°C in a humid atmosphere containing 3% CO_2. An inoculum was prepared as previously described (31). The inoculum was added to organ cultures to give a final concentration of $\sim 4 \times 10^7$ CFU/ml. In organ cultures infected with this inoculum, the number of viable meningococci increased with stationary incubation to between 2×10^8 and 5×10^8/ml by 20 h after infection (30).

In some experiments inocula containing $\sim 4 \times 10^8$ CFU/ml (as determined by optical density) were heated at 100°C for 15 min, and 1.0 ml of this heated preparation was added to organ cultures in 1.0 ml of HEPES-MEM containing fetal bovine serum (final concentration of 2×10^8 heat-killed meningococci per ml). Culture of this inoculum revealed no growth.

In other experiments filter-sterilized supernatants were applied to nasopharyngeal organ cultures. Media in human organ cultures infected with meningococcal strain H-355 or 269B were collected and sterilized by passage through 0.45-μm-pore-size membrane filters (Millipore Corp., Bedford, Mass.). The filter-sterilized organ culture supernatants were used fresh or stored at −70°C. Each sample was diluted 1:5 in fresh HEPES-MEM and used as the medium for recipient nasopharyngeal organ cultures.

Nasopharyngeal organ cultures were also exposed to inert latex particles, 1.09 μm in diameter (Dow Diagnostics, Indianapolis, Ind.), at a final concentration of $\sim 6 \times 10^8$ latex particles per ml. Tissue was incubated in a humid atmosphere for 18 h with gentle rotation. After incubation, organ cultures were washed three times in HEPES-MEM to remove extracellular latex, and the tissue was prepared for scanning electron microscopy (SEM).

Quantification of ciliary damage to human nasopharyngeal organ cultures. Ciliary damage to nasopharyngeal mucosa was quantified by using a modification of the procedure described by McGee et al. (21) for quantification of ciliary activity of human fallopian tube organ cultures. CV and the percentage of the periphery with ciliary activity (PPCA) remaining at each time of observation were determined. The organ cultures were monitored over at least a 24-h period. PPCA and CV in control and infected nasopharyngeal organ cultures were recorded at 0, 6, 12, 18, and 24 h and expressed as the percentage of the zero time value for that organ culture. In some experiments PPCA and CV were also assessed at 36 and 60 h.

Meningococcal and gonococcal LPS. Three lipopolysaccharide (LPS) preparations were used in these studies: (i) partially purified LPS from meningococcal strain 2070 (C:2:SDS-PAGE type IV), prepared by the phenol-water extraction method of Johnson and Perry (17) as modified by Gregg et al. (14); (ii) purified LPS from T_1 colonies of gonococcal isolate 2686 prepared by the same phenol-water extraction method; and (iii) LPS from a nonencapsulated meningococcal strain M986 NCV-1, prepared by sodium deoxycholate extraction, purified by Sephacryl S-300 column chromatography, precipitated from LPS-containing fractions with 4 volumes of ethanol, and washed with ethanol and then dissolved in distilled water (15). These LPS preparations were used to assess the effects of meningococcal and gonococcal LPS on ciliary activity of human nasopharyngeal organ cultures and human fallopian tube organ cultures. *Limulus* amoebocyte lysate assay (14) of these LPS preparations consistently gelled at ≧3 pg/ml concentrations.

The LPS preparations were further characterized by SDS-PAGE (19, 34). LPS was detected in the gel by the silver staining procedure of Tsai and Frasch (34).

Human fallopian tube organ cultures. Human fallopian tube organ cultures were established (21). Purified meningococcal or gonococcal LPS in concentrations of 10 and 50 μg were tested for their effects on ciliary activity by methods previously described (14). Mucosal ciliary activity was monitored before and 24 h after the addition of LPS, and damage was quantified as the percentage of the periphery of fallopian tube organ cultures with ciliary activity relative to zero time values.

Microscopy techniques. Tissues to be examined by light microscopy or SEM and transmission electron microscopy were prepared as previously described (30). SEM was performed (30) with a Hitachi model S-500 (Hitachi Scientific Instruments, Mountain View, Calif.) or an ISI model DS-130 (International Scientific Instruments, Santa Clara, Calif.) electron microscope. Transmission electron microscopy was performed with an EMU-4 (RCA, Camden, N.J.) or a model 300 (Phillips Electronic, Skokie, Ill.) electron microscope.

Analysis of data. Ciliary activity (PPCA and CV) of each organ culture was monitored over time and was expressed as the percentage of the zero time value. Control PPCA and CV values at each time interval were ≧60% of those at zero time. The results reported were derived from two to six experiments with each variable, and each variable was tested in at least six individual nasopharyngeal or fallopian

tube organ cultures. The significance of differences between the means of two variables was determined by Student's *t* test with a two-sided hypothesis. To determine the significance of differences between an experimental variable and a control group, the paired Student's *t* test was used. Tissue samples prepared for electron microscopy were coded and observations were made without knowledge of the experimental variable involved.

RESULTS

Microscopic assessment of ciliary function and structure in control and in meningococcus- or *N. subflava*-infected human nasopharyngeal organ cultures. Ciliary activity in control nasopharyngeal organ cultures was well maintained for the first 24 to 36 h of the experiment. In some instances excellent ciliary activity persisted for >60 h. Ciliary activity resulted in coordinated, unidirectional, wavelike motion. Particulate material in the medium was rapidly propelled along the mucosal surface.

SEM examination of control tissue at 0 to 24 h revealed ciliated cells in patches on the mucosal surface. Each ciliated cell contained 50 to 100 cilia (mean, 64 per cell) with microvilli 1 to 2 μm in length at the base of the cilia. Cilia were 0.3 μm in width and extended 3 to 6 μm outward from the mucosal surface. Occasional mucus globules were noted on both ciliated and nonciliated cells. Transmission electron microscopic studies showed abundant mitochondria in the apical portion of the ciliated cells.

Infection of nasopharyngeal organ cultures with meningococci resulted in damage to the ciliated epithelium. For example, Table 1 shows that infection with meningococcal strains 269B and H-355 reduced ciliary activity to <30% of zero time values by 18 h. Organ cultures showed progressive slowing of the rhythmic movement of cilia, ciliary dyskinesia, release of cells and cellular debris into the medium, accumulation of debris on the mucosal surface, and ballooning of mucosal cells. Ciliary activity was not noted on sloughing ciliated cells. Electron microscopic examination of tissue removed 6 to 12 h after infection showed vacuolization and loss of mitochondria in the apical portion of ciliated cells and sloughing of ciliated cells from the mucosal surface. Meningococci were not attached to mucous globules, cilia, microvilli of ciliated cells, or to sloughing ciliated cells.

By 18 to 24 h after infection with meningococci, there was a marked loss of ciliated cells. The appearance of the remaining ciliated cells was also altered. Cilia were bent and collapsed in an irregular fashion on the cell surface. Complete ciliostasis was observed in most meningococcus-infected organ cultures by 30 h after infection, and a marked increase in the number of sloughed cells present in the medium was also observed. Histologic studies revealed almost complete loss of ciliated cells from the mucosal surface. These results were obtained with each of the meningococcal strains used, although the rate at which damage occurred was different (see below). In contrast, ciliary activity of organ cultures infected with *N. subflava* remained at levels not significantly different from controls (Table 1).

TABLE 1. Effect on ciliary activity of human nasopharyngeal organ cultures exposed to viable piliated or nonpiliated meningococci, *N. subflava*, heat-inactivated meningococci, or HEPES-MEM containing 20% (vol/vol) filter-sterilized medium from meningococcus-infected donor nasopharyngeal organ cultures

Strain	PPCA[a]		*P*[b]
	Exptl	Control	
N. meningitidis[c]			
269B (B:NT:IV), p⁻	27.2 ± 20.5	97.4 ± 4.3	<0.02
269B, p⁺	9.7 ± 5.1	69.4 ± 5.8	<0.001
Heat-inactivated 269B	95.9 ± 15	97.4 ± 4.3	NS
Filter-sterilized supernatants of 269B	81.4 ± 15.9	61.5 ± 20.1	NS
H-355 (B:15:IV)	8.5 ± 4.2	75.3 ± 11.9	<0.001
Filter-sterilized supernatants of H-355	71.5 ± 7.9	82.4 ± 9.3	NS
N. subflava	76.1 ± 11.7	101.5 ± 5.2	NS

[a] PPCA at 18 h (relative to zero time value). Data are the mean ± standard error of the mean of two or more experiments with each variable, and each variable was tested in at least six individual nasopharyngeal organ cultures. Control organ cultures were incubated in HEPES-MEM.

[b] The significance of differences between the experimental variable and control was determined by a paired Student's *t* test with a two-sided hypothesis. NS, Not significant.

[c] Strain designation (serogroup:serotype:SDS-PAGE type); p⁻, nonpiliated by electron microscopy; p⁺, piliated by electron microscopy.

Effect of meningococcal viability on ciliary activity of human nasopharyngeal organ cultures and on interactions of meningococci with ciliated and nonciliated epithelial cells. Heat-killed meningococci of isolate 269B were used to examine the role of meningococcal viability in ciliary damage and in attachment of meningococci to nasopharyngeal organ cultures. As noted by SEM, the shape of heat-killed meningococci was irregular, with multiple folds on the surface. Often these meningococci were coated with an increased number of blebs. Pili were not seen in negatively stained preparations of heat-killed meningococci.

Ciliary activity (PPCA) was not affected by heat-killed meningococci (Table 1). After the addition of ~2×10^8 heat-killed meningococci, only a few organisms appeared attached to the microvilli of nonciliated mucosal cells. Attachment of heat-killed meningococci to ciliated cells was not observed. In contrast to experiments with viable piliated meningococci (Fig. 1a), there was little evidence of adherence to specific nonciliated cells or interaction of heat-killed meningococci with nonciliated cell microvilli (Fig. 1b). Results similar to those obtained with heat-killed meningococci were obtained in experiments with 1-μm latex spheres.

Role of pili in meningococcus-induced damage to ciliary activity of human nasopharyngeal organ cultures. Piliated and nonpiliated (by electron microscopy) colonies of strain 269B were used to assess the role of pili in damage to ciliary activity. Ciliary activity (PPCA and CV) decreased by 50% from zero time values by 6 h after infection with piliated colonies of strain 269B. By 18 h (Table 1), PPCA of organ cultures infected with piliated colonies of 269B was <10% of the zero time value.

The effect of the nonpiliated colony variant of strain 269B on ciliary activity is shown in Table 1 and in Fig. 2. In contrast to the damage observed with the piliated colony variant, PPCA at 6 h after infection was not significantly different from zero time values or control. However, by 18 h PPCA was <30% and by 24 h it was <10% of zero time values for the nonpiliated colony variant of 269B, values not significantly different from damage induced by the piliated colony variant. These data suggest that although damage may have been produced at a slower rate, meningococci which were nonpiliated induced a similar degree of

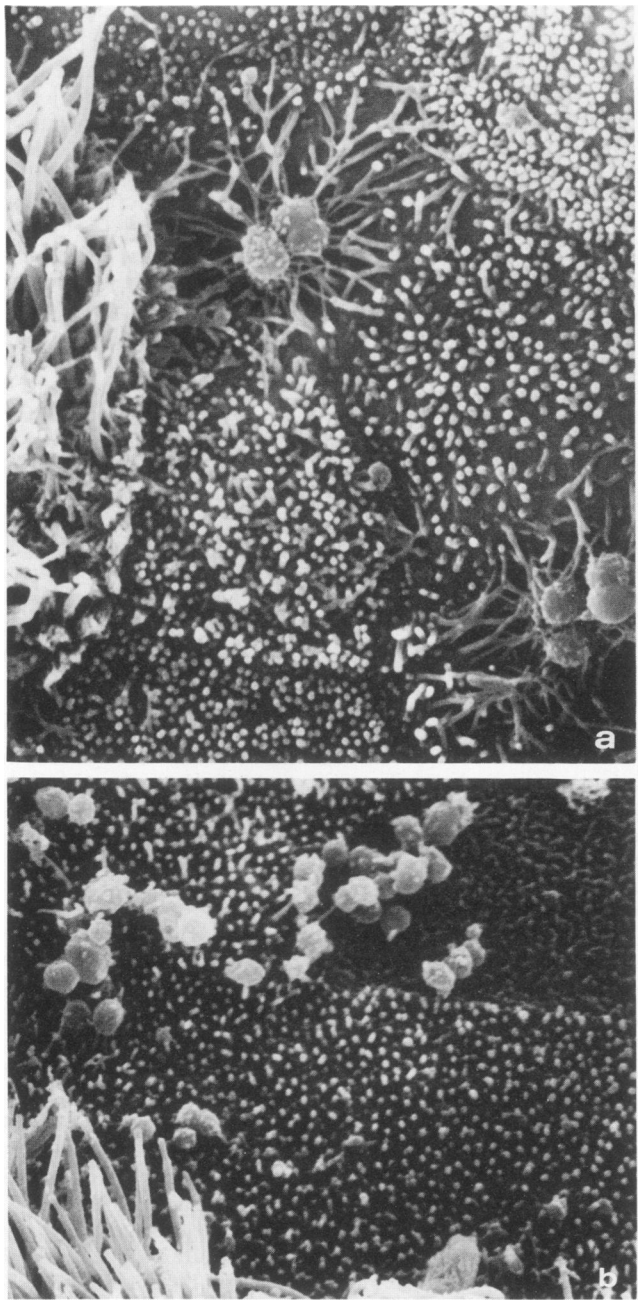

FIG. 1. Interaction of viable and heat-killed *N. meningitidis* with nonciliated cells of human nasopharyngeal organ cultures. (a) The microvilli of the nonciliated cells to which viable meningococci attached appeared to elongate and surround meningococci (×7,600). (b) In contrast, there was little evidence of interaction of heat-killed meningococci with nonciliated cell microvilli (×6,200).

ciliostasis when compared with a piliated colony variant of the same strain.

Effect of meningococcal outer membrane protein composition on ciliary activity of human nasopharyngeal organ cultures. Nonpiliated, serogroup B meningococcal isolates with defined outer membrane composition, as determined by serotype and SDS-PAGE type, were used to examine the possible role of surface proteins other than pili in meningococcal-induced damage to human nasopharyngeal organ cultures. The effect of the nonpiliated isolate H-355 (B:15:P1.16:SDS-PAGE type IV) on CV and PPCA is shown in Fig. 2. Six hours after infection with strain H-355, PPCA was <40% of zero time values ($P < 0.005$) and by 24 h it was <10% ($P < 0.001$). PPCA in organ cultures infected with nonpiliated clones of isolate 269B (B:NT:SDS-PAGE type IV) and isolate 3006 (B:2b:P1.2) decreased more gradually than PPCA in organ cultures infected with isolate H-355. However, for the nonpiliated clone of 269B, PPCA was <10% of the zero time value by 24 h ($P < 0.001$), and for strain 3006, PPCA was <20% of the zero time value by 30 h ($P < 0.01$). In contrast, nonpiliated isolate 2996 (B:2b:P1.2) of identical serogroup, serotype, and SDS-PAGE type (I) to 3006 produced complete ciliostasis in <18 h. The pattern of changes in CV was similar to that of PPCA for each meningococcal strain tested.

Thus, four serogroup B nonpiliated meningococcal isolates with different surface proteins damaged ciliary activity of nasopharyngeal organ cultures to approximately the same degree but at different rates. However, no relationship was noted between serotype or SDS-PAGE type and the degree or rate of ciliostasis.

Effect of sterile filtrates from meningococcus-infected human nasopharyngeal organ cultures on ciliary activity of recipient nasopharyngeal organ cultures. Filter-sterilized supernatants from gonococcus-infected fallopian tube organ cultures damage ciliary activity in recipient uninfected fallopian tube organ cultures (22). To explore whether meningococci might elaborate a toxic factor or factors which inhibit ciliary activity, filter-sterilized media from meningococcus-infected nasopharyngeal organ cultures were added to uninfected organ cultures. Table 1 shows that sterile filtrates from nasopharyngeal organ cultures infected with either isolate 269B or isolate H-355 at a 20% (vol/vol)

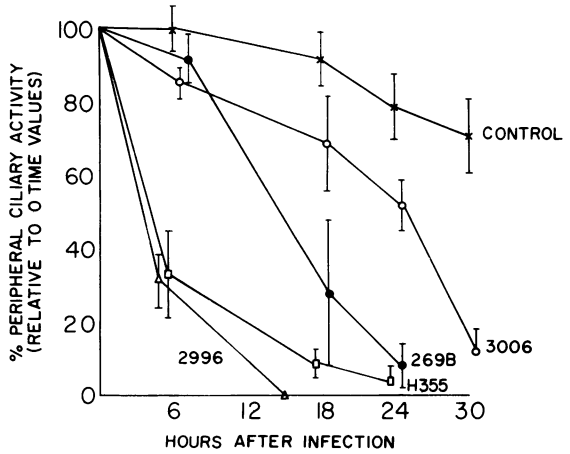

FIG. 2. Comparison of damage to the ciliated mucosa of human nasopharyngeal organ cultures by four strains of nonpiliated, serogroup B *N. meningitidis*. Four nonpiliated, serogroup B meningococcal isolates with defined outer membrane protein composition (see Materials and Methods) were used. Damage to nasopharyngeal organ cultures was assessed periodically by monitoring the PPCA (relative to zero time value) in infected and uninfected organ cultures. Each point represents the mean value ± the standard error of the mean (bars) of six or more organ cultures from two or more experiments. Each of the meningococcal isolates significantly damaged ciliary activity of nasopharyngeal organ cultures but at different rates. Shown are control and meningococcal strains 2996, H-355, 269B, and 3006.

concentration did not produce detectable damage to recipient organ cultures.

Effect of meningococcal LPS on ciliary activity of human nasopharyngeal organ cultures and human fallopian tube organ cultures. LPS from *Neisseria gonorrhoeae* has been shown to damage ciliary activity of human fallopian tube organ cultures (14). We examined the effect of three LPS preparations from *N. meningitidis* or *N. gonorrhoeae* on ciliary activity of human nasopharyngeal organ cultures and fallopian tube organ cultures. When compared with *Salmonella minnesota* and *Escherichia coli* standards, the meningococcal and gonococcal LPS preparations appeared to have rough-type LPS. Three major components were noted below 16,000 molecular weight without evidence of O-side-chain repeating units.

Concentrations of 100 μg of meningococcal LPS per ml (LPS prepared by sodium deoxycholate extraction [15]) had no effect on ciliary activity of nasopharyngeal organ cultures over 66 h of observation (Fig. 3). Similar results were obtained with HEPES-MEM, HEPES-MEM with fetal bovine serum, or HEPES-MEM with vancomycin as the medium. However, the same meningococcal LPS preparation in concentrations of 10 μg/ml (Fig. 3) and 50 μg/ml (data not shown) caused significant damage to fallopian tube organ cultures over 24 h. Damage to ciliary activity of fallopian tube organ cultures caused by meningococcal LPS was similar to damage induced by gonococcal LPS. Meningococcal LPS prepared by phenol-water extraction also damaged ciliary activity of human fallopian tube organ cultures, but neither meningococcal nor gonococcal LPS affected ciliary activity of nasopharyngeal organ cultures. Thus, both meningococcal and gonococcal LPS damage ciliary activity of human fallopian tube organ cultures but do not damage ciliary activity of human nasopharyngeal organ cultures.

DISCUSSION

The mucociliary blanket, composed of mucus glycoproteins and propelled by movement of cilia, is an initial barrier

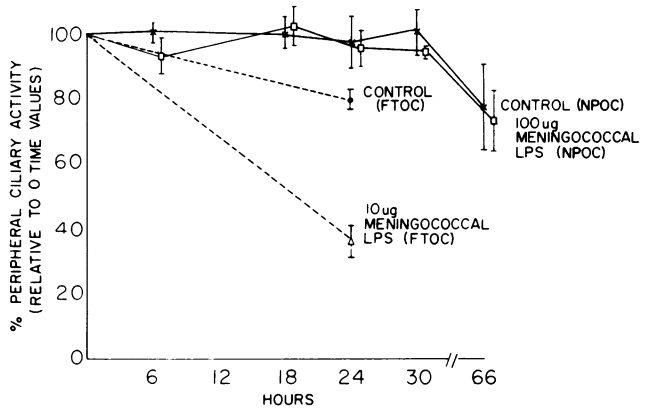

FIG. 3. Effect of meningococcal LPS on the ciliated mucosa of human nasopharyngeal organ cultures and human fallopian tube organ cultures. Results are expressed as the PPCA of nasopharyngeal (NPOC) or fallopian tube organ cultures (FTOC) (relative to zero time values). Each point represents the mean value ± the standard error of the mean (bars) of six or more replicates from two or more experiments. Meningococcal LPS (100 μg/ml) added to nasopharyngeal organ cultures at 0, 24, and 48 h (□) did not damage ciliary activity. However, at a concentration of 10 μg/ml (△), this same LPS preparation significantly damaged ciliary activity of human fallopian tube organ cultures within 24 h (×, NPOC controls; ●, FTOC controls).

to the colonization and invasion of mucosal surfaces by bacteria. Several bacterial species that colonize or infect the upper respiratory tract have been shown to inhibit ciliary activity of ciliated respiratory cells. *Bordetella pertussis* adheres preferentially to ciliated respiratory epithelial cells and inhibits ciliary activity (7). *Mycoplasma pneumoniae* attaches to sialoglycoprotein receptor sites on ciliated respiratory cells by means of a specialized attachment tip (6). An active component of the mycoplasma cell membrane (5) or release of superoxide anion and hydrogen peroxide by mycoplasmas may be responsible for damage to ciliary activity after attachment (2). Other bacterial species appear to induce ciliary damage without direct attachment to ciliated cells. Soluble toxins of *Haemophilus influenzae* (9) or diphtheria toxin (4) may cause ciliostasis.

Investigation of the effects of bacteria on mucosal surfaces has been hampered by the selectivity of some pathogens for specific hosts and for specific types of host cells. For example, Johnson et al. (16) found that *N. gonorrhoeae* adhered to the mucosa of human fallopian tube organ cultures but not to rabbit, porcine, or bovine fallopian tubes. We recently developed an experimental model of human nasopharyngeal mucosa in organ culture (30). This model allows study of exclusive human pathogens such as *N. meningitidis* by utilizing mucosa from a site of proven importance in the initiation and transmission of disease. In the current study, we evaluated the effects of *N. meningitidis* on nasopharyngeal cilia and ciliary function. We noted that infection of nasopharyngeal organ cultures by *N. meningitidis* resulted in loss of ciliary activity. In contrast, infection of nasopharyngeal organ cultures by *N. subflava*, a commensal species, did not result in ciliary damage. In similar studies with human fallopian tube organ cultures, McGee et al. (20) found that gonococci damaged ciliary activity but that *Neisseria mucosa* or *N. subflava* did not. Taken together, these data suggest that damage to human ciliated mucosa caused by *N. meningitidis* or *N. gonorrhoeae* may be an important virulence factor. Damage to ciliary function by *N. meningitidis* or *N. gonorrhoeae* may facilitate enhanced attachment, colonization, and subsequent mucosal invasion.

Meningococcal viability appears to be an important requirement for both meningococcus-induced ciliary damage and for certain interactions of meningococci with nonciliated epithelial cells. However, our method of killing meningococci by heat inactivation may have altered meningococcal surface components important for organism attachment and for meningococcus-induced ciliary damage. Whether ciliary damage requires attachment of viable meningococci to nonciliated cell microvilli remains uncertain, but both events appear to require metabolically active organisms.

We were unable to establish a role for pili or other specific surface components in meningococcus-induced damage of ciliary activity of nasopharyngeal organ cultures. Although produced at different rates, damage to ciliary activity appeared similar for piliated and nonpiliated colony variants of the same strain and for strains with different outer membrane proteins. However, we interpret these data with caution. The number of isolates examined was small, and we did not study surface components such as capsular polysaccharide. Furthermore, there is increasing evidence that pathogenic bacteria have the ability to undergo changes in surface components in response to environmental conditions. For example, both meningococci and gonococci may produce pilus subunits without assembly into intact pili (33). Selective pressure at the nasopharyngeal mucosal surface might

stimulate assembly of intact, functional pili on previously nonpiliated meningococci.

The loss of ciliary activity in meningococcus-infected nasopharyngeal organ cultures was caused by sloughing of ciliated cells to which meningococci were not attached. This action on ciliated cells from a distance suggested that damage is mediated by a toxin. Both meningococci and gonococci shed blebs of outer membrane (containing pili, outer membrane protein, and LPS), peptidoglycan, and, in the case of meningococci, capsular polysaccharide. McGee et al. (20, 21) noted that the toxic effect of gonococci on ciliary activity of human fallopian tube organ cultures was mediated by soluble factors released into the milieu by growing gonococci. Gonococcal LPS appears to be responsible for much of this toxicity (14). In view of these studies, we investigated the possibility that meningococcus-induced damage to ciliary activity of nasopharyngeal organ cultures was toxin mediated and that the toxin was meningococcal LPS.

Meningococcal and gonococcal LPSs contain lipid A, 2-keto-3-deoxyoctulosonic acid, and a core oligosaccharide without repeating polysaccharide (O antigenic) components (29). Lipid A appears to be the component of LPS responsible for toxicity of gonococcal LPS for ciliated cells of human fallopian tube organ cultures (14). We found that meningococcal LPS in concentrations of 10 μg/ml damaged ciliary activity of human fallopian tube organ cultures. Because of the conserved structure of lipid A among gram-negative bacteria (24), the mechanism of damage to ciliary activity of fallopian tube organ cultures by meningococcal LPS is probably similar. In contrast, neither gonococcal nor meningococcal LPS in concentrations up to 100 μg/ml damaged ciliary activity of human nasopharyngeal organ cultures. The toxic effects of LPS may have been neutralized by binding of LPS to nonspecific proteins, antibody, or other protective factors present in the nasopharyngeal organ culture system.

Alternatively, ciliated cells from different sources may differ in their susceptibility to the toxicity of LPS. For example, gonococcal LPS does not produce detectable damage to ciliary activity of rabbit, pig, or cow oviducts despite a profound effect on ciliary activity of human fallopian tube organ cultures (13). In addition, a ciliostatic substance, probably endotoxin, of *H. influenzae* causes marked damage at low concentrations in rat tracheal organ cultures, but chicken and some human fetal tracheal organ cultures are less sensitive or insensitive (9). Our data suggest that this selectivity of LPS damage may extend to human ciliated cells from different anatomic locations. Lack of cell membrane receptors for LPS, alteration in the hydrophobic characteristics of the cell membrane, or other intrinsic cellular differences may lessen the toxic interactions of human nasopharyngeal ciliated cells with LPS.

Although LPS does not appear to be a direct contributor to meningococcus-induced ciliary damage of human nasopharyngeal organ cultures, other toxins released by meningococci may be important in producing ciliary damage. For example, monomers of gonococcal peptidoglycan have recently been shown to damage fallopian tube organ culture ciliary activity (23), and peptidoglycan subunits of *B. pertussis* containing additional amino acids damage ciliated hamster tracheal cells (11). We found no direct evidence of a soluble toxin in our studies, but heat, filtration, or freezing may have inactivated toxin(s) in our preparations.

In summary, the nasopharyngeal organ culture model provides opportunities to study the initial steps in the pathogenesis of *N. meningitidis* and, potentially, other pathogens at a site of proven importance in the initiation and transmission of disease. Using this model, we have defined some of the requirements for ciliary damage caused by *N. meningitidis*. Damage to ciliary activity may be an important first step in bacterial colonization and invasion of this mucosal surface by *N. meningitidis* and other bacterial pathogens.

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