CLINICAL MICROBIOLOGY REVIEWS, Apr. 1989, p. S104–S111
0893-8512/89/0SS104-08$02.00/0
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Vol. 2, Suppl.

Gonococcal and Meningococcal Pathogenesis as Defined by Human Cell, Cell Culture, and Organ Culture Assays

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Neisseria gonorrhoeae and *N. meningitidis* are exclusive human pathogens. This important fact has limited the use and the relevance of animal models (30) in studies of the pathogenesis of these organsims. The potential severity of infections caused by gonococci and meningococci has limited direct human experimentation. Only studies of gonococcal urethritis in men (29, 63) and testing of potential vaccine candidates have been possible. Human tissue specimens obtained from infected individuals has provided important information (12, 14, 22), but these specimens are limited in availability and are often obtained late in the course of infection.

To address these problems, in vitro cell and organ culture assays involving predominantly cells and tissues of human origin have been developed. These assays have been used to identify important mechanisms by which gonococci and meningococci cause disease. They have provided much of the experimental data about three major pathogenic events: (i) mucosal cytotoxicity, (ii) attachment of gonococci and meningococci to epithelial cells, and (iii) mucosal invasion. This review will focus on the data derived from the use of human cells, cell lines, and organ cultures in defining these events.

DESCRIPTION OF HUMAN CELLS, CELL LINES, AND ORGAN CULTURES

The following human cells and cultures are used in studies of gonococcal and meningococcal pathogenesis: isolated buccal, pharyngeal, endocervical, vaginal, urethral, sperm, and fetal tonsil cells; cervical carcinoma (HeLa), Chang conjunctival epithelial, endometrial carcinoma (HEC-I-B, ENCA-4), and larynx carcinoma (HEp-2) cell lines; primary embryonic lung, fibroblast, amnion, endometrial, endocervical, and foreskin cell cultures; and fallopian tube, nasopharyngeal, and ectocervical organ cultures. Animal cells, cell lines, and organ cultures have also been used (1, 20, 35). However, when they are compared with human tissue from the same site, major differences are reported. For example, gonococci attach to nonciliated mucosal cells of the human fallopian tube and damage the ciliary function of human fallopian tube organ cultures but not fallopian tube organ cultures of rabbits, pigs, and cows (17, 27, 28).

Human buccal epithelial cells have been favored for use in attachment assays and are easily obtained (Fig. 1). Buccal cells are squamous epithelial cells obtained by scraping the buccal mucosa. Gonococci and meningococci attach to but do not invade these cells. However, the availability of buccal cells is offset by several factors. Cells obtained are not uniform in the degree of maturation, viability, and size, and their structure is influenced by hormonal factors and other variables that are not easily controlled. In addition, many of these cells already have bacteria (oral flora) and variable amounts of mucus attached to their surfaces. Furthermore, the buccal mucosa is not normally colonized by pathogenic *Neisseria* species. Despite these drawbacks, buccal cells have been extensively used in studies of gonococcal and meningococcal attachment (23, 44, 45, 56, 58, 61, 62, 64, 65).

Isolated squamous epithelial cells from mucosal surfaces closer to natural sites of meningococcal and gonococcal colonization (e.g., vaginal, ectocervical, urethral, oropharyngeal, and tonsillar sites) have also been used. Although potentially of more relevance, these isolated cells have similar disadvantages to those described for buccal cells. Human erythrocytes of various blood groups (ABO-Rh) are also a favorite cell type for attachment assays. Sterility, availability, and uniformity of size are advantages. Hemagglutination assays are most commonly used for attachment studies. Recently a hemadsorption assay on nitrocellulose disks (16) also has been used to more precisely define the phenotypes of meningococci which bind to erythrocytes. Caution must be exercised when observations made with erythrocytes are generalized to interactions with mucosal cells. For example, Lambden et al. (31) noted increased binding of protein II (PII)-containing gonococci to buccal cells but not to erythrocytes. Gonococci that did not express PII exhibited increased binding to erythrocytes.

Primary epithelial cell lines (e.g., uterine, endometrial, conjunctival, and ectocervical cells) overcome many of the disadvantages of isolated cells but are often difficult to establish and maintain. Cell lines, often of tumor origin, are less fragile and have been extensively used. HeLa cells (a human cervical carcinoma cell line), HEp-2 cells (a human laryngeal carcinoma cell line), human endometrial carcinoma cell lines, and Chang conjunctival epithelial cells have been the most widely used (see above) (2, 4, 21, 52, 66). These cell lines have been used for cytotoxicity and invasion studies as well as attachment assays.

Infection of organ cultures has provided an experimental means of studying the interactions of gonococci and meningococci with intact mucosal surfaces (10, 26, 36, 38, 53, 70). Human fallopian tube organ cultures (FTOC) are used because they are a site of natural infection (gonococcal salpingitis). Infections in this model produce changes similar to those noted in pathologic specimens obtained during salpingitis. Human nasopharyngeal tissue in organ culture (NPOC) has been used to study *N. meningitidis* (53). The specificity of the events observed in these models and their correlation with events observed during natural infection are major advantages. Difficulty in obtaining these tissues, variability among tissue from different individuals, and absence of important components of the inflammatory response (e.g., leukocytes, circulating antibody, and complement) are disadvantages. In addition, they require antibiotics for sterilization and have limited viability. Nevertheless, human organ culture assays have provided valuable clues about mechanisms of gonococcal and meningococcal cytotoxicity, attachment, and invasion.

PATHOGENESIS

Contact of gonococci or meningococci with human mucosal surfaces (e.g., urogenital tract for *N. gonorrhoeae*, nasopharynx for *N. meningitidis*) initiates a series of events resulting in attachment of the organisms to epithelial cells and multiplication (colonization) at the mucosal surface. These events may also lead to internalization of gonococci or meningococci by epithelial cells and possibly transport of the organisms across the normally protective mucosal barrier. Clinically this process may result in asymptomatic gonococcal or meningococcal carriage, or in signs of inflammation, indicating injury to host cells. For example, most individuals who harbor meningococci or gonococci in the nasopharynx are asymptomatic. Overt pharyngitis resulting from infection by either organism is unusual. In contrast, infection of the human urethra or human fallopian tube by gonococci often results in marked inflammation and tissue damage. Table 1 is a summary of pathogenic events noted following gonococcal or meningococcal infection of isolated human cell, cell culture, and organ culture assays.

Mucosal Cytotoxicity

Many of the clinical signs of gonococcal or meningococcal infection are due to migration of leukocytes and activation of complement at the site of infection. However, there is increasing evidence that gonococci and meningococci exert direct cyctotoxic effects that may potentiate the inflammatory response. In contrast, commensal *Neisseria* strains do not normally cause overt cytotoxicity. By using the cell and organ culture experimental models described above, the mechanisms by which *N. gonorrhoeae* and *N. meningitidis* may directly induce cytopathic changes at mucosal surfaces have been partially determined.

Virji et al. studied the cytotoxic effect of gonococci on Chang epithelial cells (66–68). Variants of *N. gonorrhoeae* P9 that expressed pili or PII were cytotoxic, whereas gonococci that did not express these components were not. Pili and PII also increased the attachment of the gonococci to this cell line. These data suggest that close, direct contact facilitated by attachment ligands may be important in local cytotoxicity.

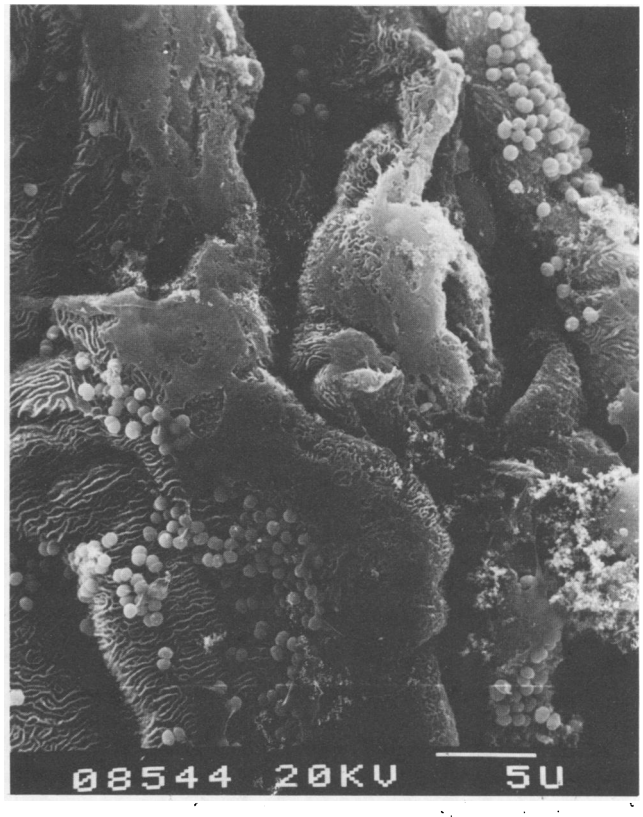

FIG. 1. Scanning electron micrograph showing attachment of piliated meningococci to human buccal epithelial cells. Meningococci attach to microplicae of these cells, usually in areas free of mucus.

Damage to ciliary activity was noted early in the course of

TABLE 1. Events noted with gonococcal or meningococcal infection of human cell, cell culture, and organ culture assays

Cell or organ type and infection	Attachment[a]	Cytotoxicity[a]	Invasion[a]
N. gonorrhoeae			
Buccal epithelial cells	$P^+ > P^-$, $PII^+ > PII^-$	N	N
Cervical epithelial cells	$P^+ > P^-$, $PII^+ > PII^-$	N	N
Erythrocytes	$P^+ > P^-$, $PII^- > PII^+$	N(?)	N
HeLa cells	$PII^+ > PII^-$	N	$PII^+ = PII^-$, $P^+ = P^-$
Chang conjunctival cells	$PII^+ > PII^-$	Y	Y
HEC-I-B cells	Y	Y	$P^+ = P^-$
HEp-2 cells	Y	N(?)	Y
FTOC	$P^+ > P^-$, $PII^+ > PII^-$(?)	$P^+ > P^-$	$P^+ = P^-$
N. meningitidis			
Buccal epithelial cells	$P^+ > P^-$	N	N
Erythrocytes	$P^+ > P^-$	N(?)	N
NPOC	$P^+ > P^-$	Y	Y
Commensal *Neisseria* spp.			
Buccal epithelial cells	Y	N	N
Chang conjunctival cells (*N. mucosa*)	Y		N
HEC-I-B cells (*N. lactamica*)	Y	N	N
HeLa cells (*N. sicca*)	Y	N	N
NPOC (*N. subflava*)		N	
NPOC (*N. lactamica*)	Y		Y

[a] Abbreviations: Y, observed; N, not observed; P^+, piliated; P^-, nonpiliated; PII^+, expressing II; PII^-, not expressing PII.

FIG. 2. Scanning electron micrograph showing attachment of meningococci to nonciliated columnar epithelial cells but not ciliated cells of human nasopharyngeal organ cultures. Note the general loss of short microvilli on nonciliated cells to which meningococci attach. They are replaced by a few elongated microvilli (arrows) interacting with attaching meningococci.

human FTOC infection with gonococci and human NPOC infection with meningococci (55). Loss of ciliary activity was accompanied by sloughing of ciliated cells. Unlike *Bordetella pertussis*, which damages ciliated cells after direct attachment (15), the damage to the ciliated cells was not associated with the attachment of gonococci or meningococci to these cells (Fig. 2) or to the presence of organisms within ciliated cells. Infection with the commensal species *N. subflava* did not result in significant damage to human FTOC or NPOC ciliary activity. Lipopolysaccharide (LPS) appears to be a major toxin of gonococci for the ciliated cells of human FTOC (18, 19, 36, 41). Gonococcal peptidoglycan fragments also damage FTOC ciliary activity (42). Both piliated and nonpiliated gonococci and meningococci damage FTOC and NPOC ciliary activity (36, 39, 57), but piliated organisms damage ciliary activity more rapidly than nonpiliated organisms do. Ciliated cells of the FTOC were damaged by <10 μg of purified gonococcal or meningococcal LPS per ml (19). By 1 to 2 h after exposure to gonococcal LPS, vesicles containing LPS were distributed throughout the cytoplasm of ciliated cells (9). Polymyxin B neutralized LPS-induced damage, suggesting that the lipid A portion of LPS was the toxic moiety. In contrast, purified gonococcal and meningococcal LPS at 100 μg/ml did not damage NPOC from humans (57) or FTOC from rabbits, pigs, and cows (17). These studies indicate that *N. gonorrhoeae* and possibly *N. meningitidis* damage ciliated epithelial cells indirectly by release of LPS, peptidoglycan monomers, and possibly other toxins from the organisms and suggest that there are differences in the susceptibility of ciliated cells to these toxins. The selectivity of the LPS toxicity for humans and for specific human mucosal surfaces may be responsible in part for the host specificity of infections and the variability in severity of human mucosal infections due to gonococci or meningococci.

Pili are not necessary for damage to ciliated cells (39, 55, 57) but facilitate the attachment of meningococci and gonococci to nonciliated epithelial cells of both mucosal surfaces (39, 56). The increased attachment associated with pili may allow more effective delivery of toxic factors to adjacent ciliated epithelial cells. Other outer membrane proteins of gonococci or meningococci did not appear to be required for damage to ciliated cells (55) but detailed studies to address this question have not been performed. Immunoglobulin A1 protease activity was also not critical for epithelial cell damage seen in gonococcal infection of FTOC (7, 8). Similar results have been noted when immunoglobulin A1 protease activity-deficient mutants of *Haemophilus influenzae* were used to infect NPOC (13). However, in view of recent studies defining other products of the immunoglobulin A1 protease gene (J. Pohlner and T. Meyer, personal communication) which may affect host cells and the finding of other substrates cleaved by this protease, these studies must be viewed cautiously.

Attachment

Although nonspecific factors (e.g., surface charge, pH, Derjaguin-Landau-Verwey-Overbeek [DLVO] theory, ionic bridging, and hydrophobic interactions) may be important (72), attachment of gonococci and meningococci to human cells is selective. In the FTOC and NPOC models (7, 10, 39, 53), gonococci and meningococci attach only to microvilli of nonciliated columnar epithelial cells. Attachment to ciliated cells is not observed. Similarly, attachment of piliated meningococci differs markedly among epithelial cells from different sites (54). In contrast, nonpiliated meningococci attach equally but in small numbers to all cell types. These data suggest that gonococcal and meningococcal attachment is mediated by specific ligands that selectively recognize receptors on certain types of human cells. The number and distribution of receptor sites for these ligands may in part determine sites of mucosal colonization and infection. As reviewed by Brooks (5), the host factors important in attachment of gonococci are poorly understood. The characteristics of the receptors for gonococci or meningococci and the influence of hormonal factors are currently areas of intense investigative interest.

Studies with human cells, cell lines and organ cultures have implicated three gonococcal and meningococcal surface components in attachment: (i) pili, (ii) heat-modifiable outer membrane proteins (e.g., gonococcal protein II), and (iii) the major porin proteins. However, it is critical to note that different mechanisms are probably operative in the attachment of gonococci and meningococci to different kinds of cells.

Pili are hairlike surface appendages which radiate several thousand nanometers from the surfaces of meningococci and gonococci. Numerous studies (1, 4, 6, 60, 64–67) with a variety of cells and organ cultures have implicated pili as major attachment ligands of meningococci and gonococci. Experiments with human volunteers have confirmed these findings (29, 63). Owing to their radiation from the cell

surface, these organelles probably make the initial contact with host cells. There is experimental evidence that supports this concept (70).

No clear differences in the degree of attachment were reported when different antigenic types of gonococcal pili were used in assays of attachment to buccal epithelial cells or to erythrocytes (50). In contrast, Trust et al. (65) noted that although all piliated meningococci attached to buccal cells, only certain strains bound to erythrocytes. In studies with antisera raised to purified pili, attachment and virulence for Chang conjunctival epithelial cells were reduced only in the presence of homologous antisera to pili (67). Heterologous antisera to pili were largely ineffective in reducing the attachment and cytotoxicity to these cells. Similar results are reported for attachment of gonococci to buccal epithelial cells (64) and rhesus monkey kidney cells (1). Schoolnik et al. (49) noted that a ^{125}I-labeled gonococcal pilin fragment, TC-2, bound to endocervical cells from healthy women but not to buccal or HeLa cells. Rothbard et al. (48) found that antibodies to synthetic peptides 69-84 and 41-50 inhibited a heterologous gonococcal strain from binding to the human endometrial carcinoma cell line ENCA-4. However, these antibodies were not effective in blocking attachment of meningococci to buccal epithelial cells, even though the epitopes were present on pilins of these strains (58). Thus, pili are important in the attachment of meningococci and gonococci to a variety of human cells, and they appear to mediate attachment through several different mechanisms.

For gonococci, PII also appears to be an important attachment ligand, at least to certain kinds of human cells. Lambden et al. (31) found that gonococci that expressed PII (regardless of the molecular weight of the PII) attached in greater numbers to buccal epithelial cells. Elkins et al. (11) noted that gonococci expressing PII showed increased adherence to primary cultures of uterine and ectocervical cells. Bessen and Gotschlich studied PII binding to HeLa cells (2) and noted that the receptor had a protein configuration (3). Lammel et al. (32) noted that a monoclonal antibody directed at a gonococcal PII decreased the adherence of gonococci expressing the PII to HEC-1-B cells. Similar results were reported by Sugasawara et al. for HeLa cells (59). PIIs are also important in binding to human polymorphonuclear leukocytes (11). In contrast, James et al. (25) and Draper et al. (10) found that transparent gonococci which lacked PIIs appeared to have increased attachment to FTOC and cervical explant tissue. Lambden et al. (31) also noted that in contrast to buccal cells, gonococci lacking PIIs demonstrated the greatest binding to erythrocytes. Gonococci lacking PIIs are often recovered from the fallopian tubes of women with salpingitis. These data indicate that PII may be important in attachment to certain kinds of human cells and a disadvantage in attachment to others. The question remains, however, an open one. Recently, Lammel et al. (C. J. Lammel, N. P. Dekken, and G. F. Brooks, personal communication) studied the ability of four PII-expressing gonococcal clones and a PII-negative clone to attach to human fallopian tube tissue. Differences in attachment and damage to FTOC mucosal cells occurred with different PII-expressing clones ($PII^+ > PII^-$).

Meningococci express heat-modifiable surface proteins (class V proteins) that are similar biochemically to PII. The role of these proteins in meningococcal attachment is much less clear. We noted (56) that meningococci that formed opaque colonies exhibited increased attachment to buccal epithelial cells, but we could not establish a relationship between colony phenotype and the expression of class V proteins. Others have observed (M. Hagman, P. Olan, and D. Danielsson, personal communication) that meningococci isolated from urogenital specimens and containing class V proteins showed an increased attachment to human vaginal cells but not to buccal epithelial cells.

As discussed in the next section, the major porin proteins of gonococci and meningococci have been shown to insert into eucaryotic membranes. These proteins may further enhance gonococcal attachment and initiate invasion.

Mucosal Invasion

Invasion of mucosal surfaces by *N. gonorrhoeae* has been noted histopathologically since the late 1800s. Harkness (22) reviewed data showing that in patients with acute gonorrhea, gonococci had penetrated the mucosal surface and were multiplying in the subepithelial space by day 3 of infection. However, it is unclear whether squamous cells of the cervix are truly invaded by gonococci (12). Intracellular gonococci are observed in histopathological specimens within nonciliated columnar epithelial cells of the urethra, cervix, and fallopian tubes (71, 72). Human fallopian tube organ cultures have been a major experimental model in the study of this event (38, 40, 70). Recently, a similar invasion event has been noted in human nasopharyngeal organ cultures infected with *N. meningitidis* (53). Shaw et al. (51, 52) developed an assay to study gonococcal invasion by using HEC-1-B human endometrial cells; Bessen and Gotschlich (2) and Richardson and Sadoff (46) studied this event in HeLa or human amnionic cells from primary cultures. Chang conjunctival epithelial cells (23, 67) and HEp-2 cells (4) have also been used to study gonococcal invasion, as have a variety of other mammalian cell cultures (4, 43, 69).

Several investigative groups have used the human fallopian tube model to study gonococcal mucosal invasion (40, 70). Approximately 1% of attached gonococci invade by 8 h after infection in this model. Attachment of gonococci to nonciliated mucosal cells is the first step in a process of internalization of gonococci by these cells. Similar to the relationship of cytotoxicity and attachment, attachment and invasion are closely associated. Virji et al. found increased cytotoxicity and invasion of Chang conjunctival cells by gonococci that exhibited the greatest attachment (67, 68).

After attachment of gonococci to nonciliated epithelial cells of the human fallopian tube, the microvilli of these cells surround gonococci and draw them to the surface of the mucosal cell. Later in the course of infection, the membranes of some of the nonciliated cells seem to retract and pinch off a membrane-bound vacuole containing gonococci. Similar membrane-bound vacuoles containing gonococci are noted following gonococcal infection of tissue culture cells (4, 43, 69). This process is quite similar to the phagocytosis of bacteria by professional phagocytes, but destruction of gonococci after entry into phagocytic vacuoles is not observed. Gonococci are rapidly transported in these vacuoles across the epithelial cell. In the FTOC model there is an orderly parting of the basement membrane, with release of gonococci into the subepithelial space.

In the NPOC model, similar events are observed following meningococcal infection (53). However, after meningococci enter via phagocytic vacuoles, they remain in an apical location within the epithelial cell (Fig. 3). Meningococci can be seen in the subepithelial space, but their exact route of access remains unclear. In contrast, when *H. influenzae* is used to infect the NPOC model, the route of mucosal invasion is primarily between epithelial cells (13).

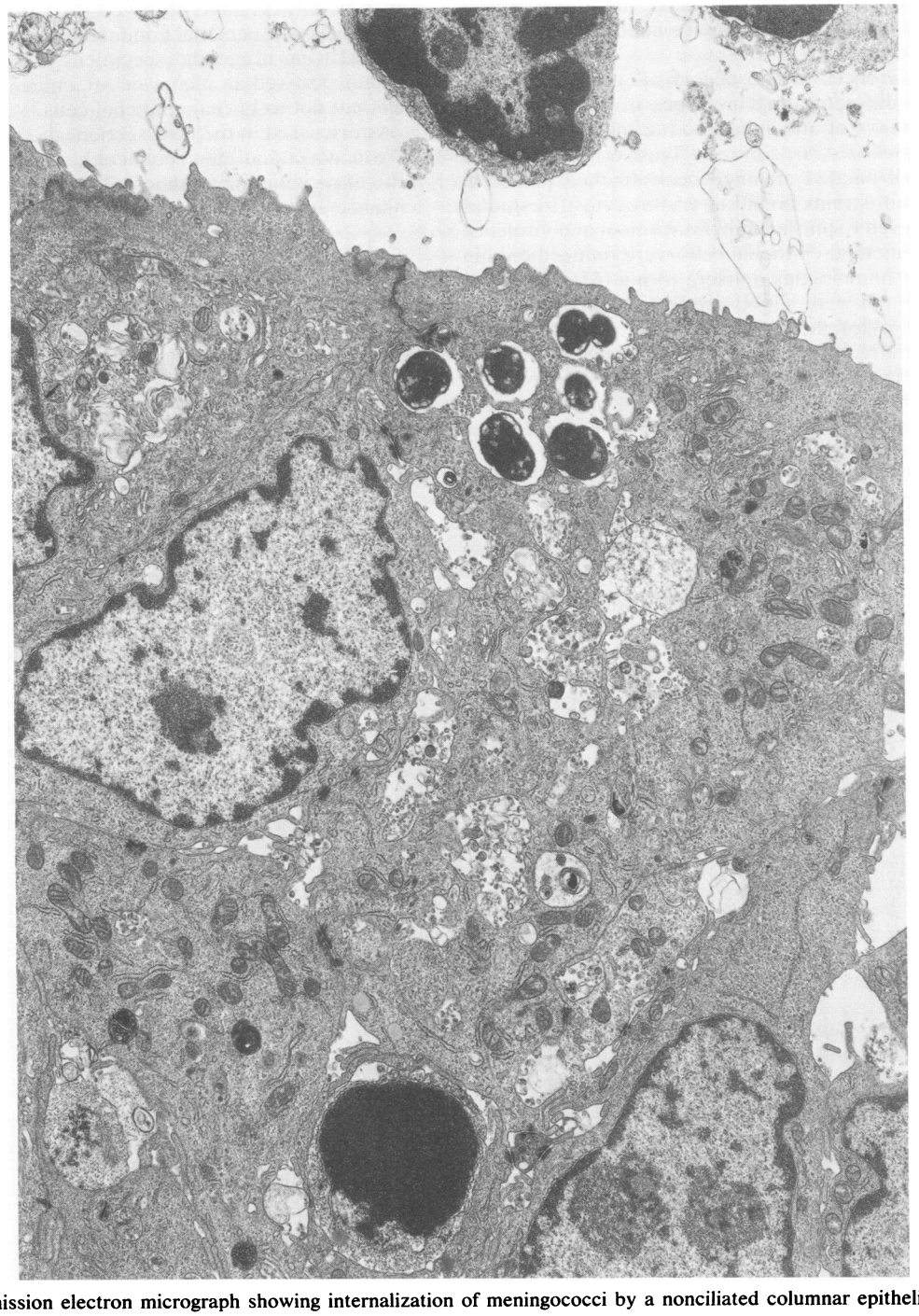

FIG. 3. Transmission electron micrograph showing internalization of meningococci by a nonciliated columnar epithelial cell of human nasopharyngeal organ cultures.

Using the endometrial cell line HEC-1-B, Shaw and Falkow (51) noted that after gonococcal attachment, the organisms were embraced by microvilli and entered these cells in membrane-bound vacuoles. However, 8 h after infection, gonococci were found in the cytoplasm free of membrane-bound vacuoles. At 12 h, hundreds of internalized gonococci were noted free in the cytoplasm. Lysis of the invaded cells was also noted. In contrast, *N. lactamica* was adherent but not invasive in this model and *N. sicca* was not invasive for HeLa cells (2).

Outer membrane blebs are also internalized by epithelial cells (9). LPS antigens on ingested meningococci and gonococci or on blebs may remain unaltered after internalization (J. F. L. Weel, S. Gigengack, C. T. P. Hopman, Y. Pannekoele, and J. P. M. van Putten, personal communication), in contrast to the processing that may occur after ingestion by polymorphonuclear leukocytes.

The molecular events that lead to internalization of gonococci and meningococci are also being actively studied. Engulfment requires viable organisms (46). Engulfment was inhibited when cell microtuble- or microfilament-dependent movement was disrupted by cytochalasin B or demecolcine.

Gonococci and meningococci appear to enter epithelial cells by a process called parasite-directed endocytosis (37). The process involves a mechanism similar to classical phagocytosis (microfilament dependent, blocked by cytochalasin) but appears to be dependent on microbial factors for initiation and occurs with host cells that are not normally phagocytic. Pili are not required for entry. James (24) noted the adherence of gonococci expressing PIIs (opaque colonies) to human embryonic fibroblast cell cultures, with the subsequent formation of microcolonies. Transparent variants (which presumably lacked PIIs), produced at the periphery, were observed to translocate across the tissue cultures by twitching mobility and were more invasive. Other studies have found no differences in the invasiveness of attaching PII^+ or PII^- gonococci (2).

The major porin proteins (gonococcal proteins I [PIA and PIB] and meningococcal class II and III proteins) have been proposed as candidates for the gonococcal and meningococcal invasin. The porin proteins have been shown to insert into lipid bilayers (34). Layh et al. (33) found that gonococcal PIA inserted into mammalian cells identically to its orientation in the gonococcal membrane. Heckels et al. (23) noted that monoclonal antibodies to gonococcal PIA and PIB blocked cytotoxicity and invasion of Chang conjunctival epithelial cells.

Events after Bloodstream Invasion

Few studies with human cells, cell cultures, or organ cultures have addressed events (e.g., endothelial cytotoxicity, penetration of the blood-brain barrier) that occur after bloodstream invasion by gonococci or meningococci. Pathologic specimens suggested that events similar to those noted at mucosal surfaces occurred after dissemination. For example, gonococci have been seen inside A cells of the synovial membrane (14), and gonococcal peptidoglycan monomers have been implicated in cell damage at synovial membranes (42, 47). Wispelwey et al. (B. Wispelwey, A. J. Hesse, E. J. Hansen, and W. H. Scheld, Clin. Res. 35:495A, 1987), using a model of isolated cerebral capillaries from the rat brain, showed that the LPS of *H. influenzae* altered the permeability of the blood-brain barrier. Whether meningococcal, but not gonococcal, LPS produces similar results would be of great interest.

SUMMARY

Human cells, cell cultures, and organ cultures have been extremely useful for studying the events that occur when gonococci and meningococci encounter human mucosal surfaces. The specificity and selectivity of these events for human cells are striking and correlate with the adaptation of these pathogens for survival on human mucous membranes. To colonize these sites, meningococci and gonococci have developed mechanisms to damage local host defenses such as the mucociliary blanket, to attach to epithelial cells, and to invade these cells. Attachment to epithelial cells mediated by pili, and to some types of cells mediated by PIIs, serves to anchor the organism close to sources of nutrition and allows multiplication. Intracellular invasion, possibly initiated by the major porin protein, may provide additional nutritional support and protection from host defenses. Mucosal invasion may also result in access of gonococci and meningococci to the bloodstream, leading to dissemination.

ACKNOWLEDGMENTS

I thank Juanita Coleman, Sheryl Hall, and Patricia Spellman for invaluable help in preparation of the manuscript.

Current studies from my laboratory were supported by the Medical Research Service of the Veterans Administration and by a grant from the Children's Research Center of Emory University.

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