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Stable Expression of Lipooligosaccharide Antigens during Attachment, Internalization, and Intracellular Processing of *Neisseria gonorrhoeae* in Infected Epithelial Cells

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Immunoelectron microscopy enables the detection and localization of bacterial antigens during in vitro infection (J. F. L. Weel and J. P. M. van Putten, Microb. Pathog. 4:213–222, 1988). In this study, we have used this method to get information on the role of lipooligosaccharides (LOS) in the pathogenesis of neisserial infections at the mucosal level. Ultrathin cryosections of Chang conjunctiva epithelial cells infected with *Neisseria gonorrhoeae* (3 to 18 h) were incubated with LOS-specific monoclonal antibodies and gold-labeled protein A and viewed in the electron microscope. Our results demonstrate that the probed LOS determinants are stably expressed during the adherence, internalization, and intracellular processing of the bacteria. There was no indication of an adaptation of the gonococcal LOS expression to the host cell environment or of a degradation of the probed epitopes. The gold particles, representing LOS molecules, were predominantly located at the bacterial membranes, but sometimes the host cell plasma membrane was labeled as well, suggesting that LOS or LOS-containing membrane fragments interacted with the eucaryotic cells. This was confirmed when purified LOS was added to the cells. Two hours after LOS exposure, gold particles were observed at the plasma membrane of a subpopulation of the cells. After 18 h of LOS exposure, gold particles were also found in large vacuoles inside the cells, suggesting that LOS molecules were internalized by the cells. The function of observed LOS binding and endocytosis in the pathogenesis of neisserial infections remains to be defined.

Every strain of *Neisseria* is able to produce several glycolipids with M_rs of 3,200 to 7,100; these glycolipids are termed lipooligosaccharides (LOS) (15). The LOS repertoire is a relatively stable characteristic of a strain, but within the population of bacteria there may be a high frequency of interconversion of the LOS that is predominantly expressed (16). The degree to which the different LOS molecules are produced appears to be regulated at a single cell level, and every cell probably has the ability to produce the entire LOS repertoire of the strain (16). The factors that regulate LOS expression remain to be defined, but external factors such as selective pressure (pyocin sensitivity [4, 12] and immunological pressure) and growth conditions (2, 6, 13) influence LOS expression. The ability of a strain to make different LOS molecules contributes to the antigenic heterogeneity of neisserial LOS (10).

LOS plays an important role in immunity against *Neisseria* infections. LOS determinants confer resistance to killing of the bacteria by human serum (17, 20) but may also serve as a target for bactericidal (3) and chemotaxis-inducing (7) antibodies. The significance of LOS and its variability in the pathogenesis of *Neisseria* infections at the level of the mucous membrane is much less evident. Purified LOS of *Neisseria meningitidis* and *N. gonorrhoeae* damage the ciliary activity in the human fallopian tube organ culture model, but this toxic effect was not found in the nasopharyngeal infection model (19). In fact, the role of LOS as a virulence determinant at the primary site of neisserial infections (nasopharynx, urethra, cervix, and conjunctiva) is still obscure.

In the present study, we systematically investigated the role of LOS in the first stages of neisserial infection by determining the stability and the topological distribution of LOS molecules during the attachment, invasion, and intracellular processing of *N. gonorrhoeae* in Chang conjunctiva epithelial cells. The LOS was detected by using LOS-specific monoclonal antibodies (MAbs) and postembedding immunoelectron microscopy, a method that has previously been shown to be very useful in the detection of gonococcal antigens in eucaryotic cells (25). Our results indicate a remarkable stability of the probed LOS epitopes during the attachment, internalization, and (morphological) degradation of the gonococci and also that LOS does interact with and is endocytosed by a subset of the infected epithelial cells.

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MATERIALS AND METHODS

Bacterial strain. *N. gonorrhoeae* 830563 (auxotype Pro$^-$, serotype pIA), isolated from a patient with a disseminated gonococcal infection, was grown for 16 h on Gc medium base (Difco Laboratories, Detroit, Mich.) containing 1% Vitox (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) at 37°C in a humidified atmosphere of 5% CO_2 in air. The colonies were selected for piliation and opacity with the help of an inverted microscope.

Cell culture. Chang conjunctiva epithelial cells (Flow Laboratories, Irvine, Ayrshire, United Kingdom) were seeded at a density of $3 \times 10^3/cm^2$ and grown in 25-cm^2 culture flasks (Nunclon Delta; Kamstrup, Roskilde, Denmark) in Dulbecco modified Eagle medium supplemented with 5% fetal bovine serum (tissue culture medium) in a CO_2 incubator. Upon reaching confluence (day 5), the cells were used in the infection experiments.

MAb production. BALB/c mice were immunized at days 1, 8, and 15 with lithium acetate-extracted outer membrane fractions (20 μg of protein) prepared from iron-starved

gonococci. On day 18, spleen cells and NS-1 myeloma cells were fused by the method described by Tam et al. (21) and allowed to grow for 10 days. Then, the antibody-producing hybridomas were selected by enzyme-linked immunosorbent assay, using microdilution plates coated with outer membrane fractions (50 μg of protein per plate) or purified LOS (150 μg per plate), and cloned. The LOS specificity of the MAbs was confirmed by gel immunoradioassay (14).

The inter- and intrastrain variabilities in expression of the LOS epitopes recognized by the MAbs were investigated by enzyme-linked immunosorbent assay, immunolight microscopy (J. P. M. van Putten et al, submitted for publication), and immunoelectron microscopy. In enzyme-linked immunosorbent assay, MAb 7B1E recognized LOS of 4 (including strain 830563) of 14 gonococcal isolates. MAb 4B7F recognized a LOS epitope that was expressed in detectable amounts by 8 (including strain 830563) of the 14 isolates. MAb 4D7F reacted with LOS of two of eight isolates and not with the predominantly expressed LOS molecule of strain 830563. These data indicate interstrain variability in the expression of the probed epitopes.

Immunomicroscopy of whole microorganisms of a single strain revealed that all three MAbs recognized epitopes that showed intrastrain variation in expression. The intrastrain variability in gonococcus strain 830563, the strain used in this study, is described in Results.

Infection of the monolayer. *N. gonorrhoeae* 830563 (pili$^+$, pII$^+$) was suspended in 1 ml of phosphate-buffered saline (PBS; 140 mM NaCl, 2.5 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 1 mM $MgCl_2$, pH 7.4) to a concentration of 10^8 CFU/ml. From this suspension, 0.5 ml was added to 5 ml of tissue culture medium. This suspension was used as an inoculum to infect the Chang cells (25-cm^2 flasks) with a bacteria/epithelial cell ratio of ca. 15:1.

After 3 h of incubation at 37°C, the unattached bacteria were removed by rinsing the cells several times with 5 ml of medium, and fresh medium was added to enable further infection of the cells. At various intervals postinfection, the medium was discarded and the cells were washed three times with PBS and fixated in PBS containing 0.1% glutaraldehyde (Merck AG, Darmstadt, Federal Republic of Germany) and 2% paraformaldehyde (PFA) (Merck) (30 min, 20°C), followed by an incubation (3 to 6 h, 20°C) in PBS–2% paraformaldehyde. After fixation, the cells were scraped with a rubber policeman in PBS–2% paraformaldehyde and embedded in gelatin (2% [wt/vol], final concentration; Sigma Chemical Co., St. Louis, Mo.). The cells were pelleted in an Eppendorf centrifuge (10,000 × *g*, 30 s, 20°C) and stored in PBS–2% paraformaldehyde at 4°C until further processing for immunoelectron microscopy.

Addition of purified LOS to cells. Gonococcal LOS from strain 830563 was purified by the hot phenol-water extraction method of Westphal and Jann (26). The LOS was further purified by several centrifugations at 100,000 × *g* and lyophilized. Silver-stained sodium dodecyl sulfate-polyacrylamide gels of the purified material (10 μg) indicated that the LOS was essentially free of protein.

Before addition to the cells, the purified LOS was dissolved in tissue culture medium and sonicated in a B15 Sonifier (Branson Sonic Power Co., Danbury, Conn.) for 10 min just prior to use. A concentration of 10 μg of LOS per ml was used in the experiments. The further processing of the cells was similar to that of the infected monolayers.

Ultramicrotomy and immunolabeling. The fixated specimens were frozen and sectioned with an ultracryomicrotome as described previously (25). Then, the sections were subjected to immunolabeling to mark the LOS antigens. First, the grids were incubated with anti-LOS antibodies in a dilution of 1/1,000 in PBS for 30 min at 20°C. After four 5-min washes with PBS, the sections were incubated (30 min, 20°C) with gold-conjugated protein A (5 or 10 nm), prepared by the method of Slot and Geuze (18), and diluted in PBS plus 1% gelatin to an optical density at 520 nm of 0.06. After four 5-min washes with PBS and two 5-min washes with distilled water the sections were adsorption stained as described by Tokayasu (23). When double-labeling experiments were performed, after the first immuno-labeling step (10-nm gold particles) the grids were incubated with unlabeled protein A (10 μg/ml; 30 min, 20°C), washed with PBS, and exposed to a different LOS antibody (30 min, 20°C). These antibodies were marked with 5-nm gold-conjugated protein A. All sections were viewed in a Philips EM 201, 300, or 420 electron microscope at 60 kV.

RESULTS

Postembedding immunolabeling of gonococcal LOS and intrastrain variability in LOS expression. The applicability of postembedding immunoelectron microscopy to detection of gonococcal LOS antigens was investigated by incubating cryosections (70 nm) of the bacteria (strain 830563) with LOS-specific MAbs and subsequently with gold-conjugated protein A. Immunolabeling of the sections with MAb 7B1E resulted in extensive gold labeling of the gonococcal outer membrane as well as of membrane blebs (Fig. 1A). Unlabeled bacteria were found sporadically (Fig. 1B). When MAb 4B7F was used to probe the cells, a different pattern of labeling was observed in that about 10 to 15% of the bacteria were found to be free of gold particles. These bacteria still expressed the 7B1E epitope (Fig. 1C). These results indicate that the 4B7F epitope is different from and much more variably expressed than the 7B1E epitope. When the sections were incubated with 4D7F, a MAb that did not react with strain 830563 in gel immunoradioassay and enzyme-linked immunosorbent assay, no immunolabeling was obtained (Fig. 1D).

The observed intrastrain variability in the expression of the probed epitopes was found in both gonococci grown on agar plates and bacteria grown in tissue culture medium in the presence of epithelial cells. Importantly, similar results were obtained when the bacteria were probed prior to the sectioning procedure (preembedding labeling) (data not shown). This finding suggests that immunolabeling of the LOS determinants obtained in the cryosections reflects that of whole microorganisms.

Taken together, our results demonstrate that specific labeling of LOS epitopes can be obtained by the procedure used and that the MAbs described are useful in detecting variability in the expression of LOS determinants after sectioning of the specimen.

Detection of LOS antigens in infected epithelial cells. The localization and processing of LOS in the various stages of gonococcal infection were investigated in the Chang conjunctiva epithelial cell infection model (25). Confluent Chang cells were infected with *N. gonorrhoeae* 830563 (pili$^+$, PII$^+$), previously shown to be invasive in these cells (25). After various periods, the infected cells were fixated, cryosectioned, and subjected to immunolabeling with LOS-specific MAb 7B1E.

Electron microscopic observation of 3-h-infected cells revealed numerous extracellular bacteria that were either directly or indirectly (by clumping together) attached to the

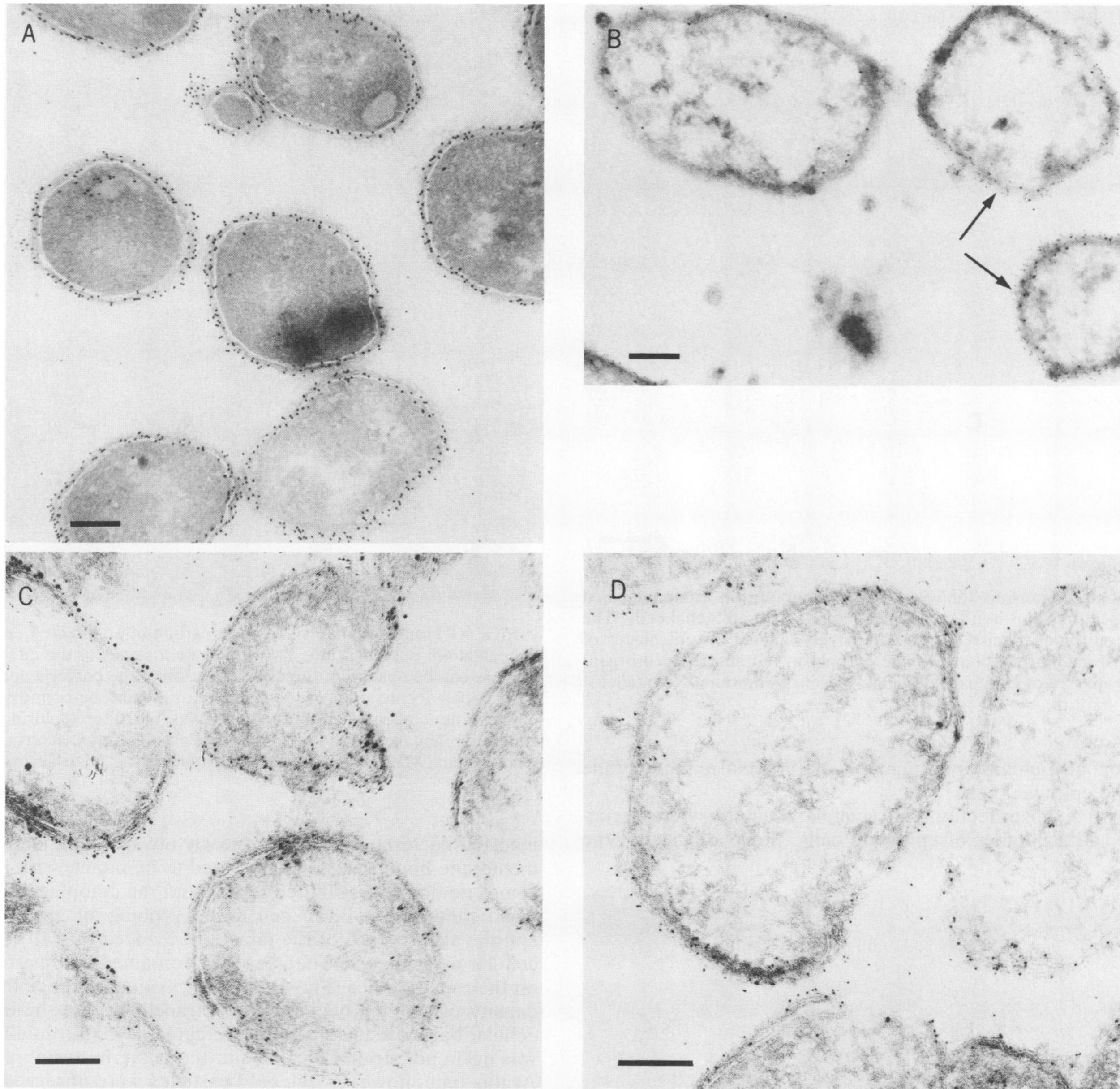

FIG. 1. Electron micrographs of gonococci. (A) Transmission electron micrograph of cryosectioned gonococci incubated with MAb 7B1E and protein A-gold. All bacterial membranes, but not the cytosol, are loaded with gold particles. The immunolabel is randomly distributed on the bacterial cell surface. (B) Transmission electron micrograph of three gonococci incubated with MAb directed against protein I (10 nm) and with MAb 7B1E (5 nm). Only two of the gonococci (arrows) reacted with the LOS-specific MAb 7B1E (small gold particles), while all three bacteria were labeled for protein I, illustrating that the 7B1E epitope is not uniformly expressed by the whole population of bacteria. (C) Transmission electron micrograph of gonococci incubated with MAb 4B7F (10 nm) and subsequently with MAb 7B1E (5 nm). The 4B7F epitope (large gold particles) appears to be absent in a subpopulation of the bacteria. These bacteria do express the 7B1E epitope (small gold particles). (D) Transmission electron micrograph of gonococci incubated with MAb 4D7F (10 nm) and subsequently with MAb 7B1E (5 nm). Only LOS epitopes recognized by MAb 7B1E (small gold particles) could be detected on the bacterial membranes. Bars, 0.25 μm (A and B) and 0.2 μm (C and D).

plasma membrane of the epithelial cells. All adherent bacteria were found to contain gold particles on their membranes, including those parts of the gonococcal membrane that were in contact with the host cell membrane and with neighboring bacteria (Fig. 2). The density of the labeling of the adherent bacteria was relatively constant and comparable to that of the nonadherent bacteria (not shown), suggesting that adherence of the bacteria did not alter the expression of the probed LOS epitope. Interestingly, the immunolabeling was not always restricted to the bacterial membranes. In a limited number of epithelial cells, the gold probe appeared to react with microvilli and plasma membranes of the host cells (Fig. 3). This gold labeling of the host cell membranes was further investigated by subjecting uninfected Chang epithelial cells to immunolabeling. In these experiments, no labeling of the epithelial membranes was obtained (data not shown), suggesting that the observed labeling of the infected epithelial cells was due to an interaction of LOS-containing

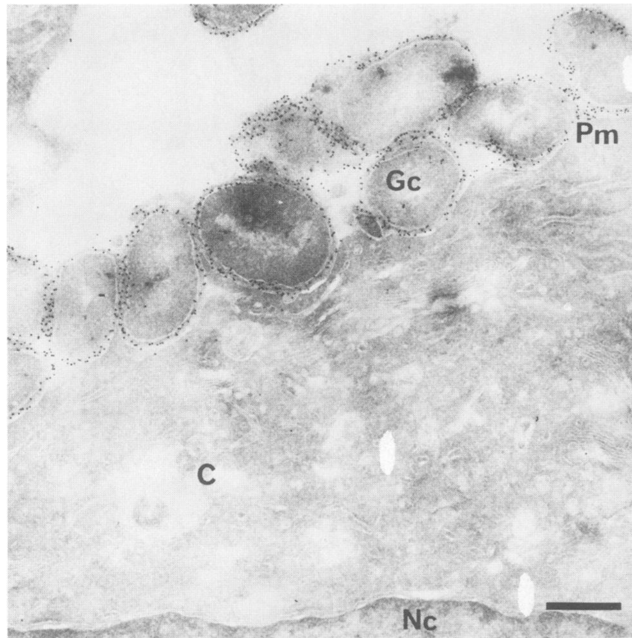

FIG. 2. Transmission electron micrograph of immunolabeled cryosections of 3-h-infected Chang conjunctiva epithelial cells. The adherent gonococci (Gc) are extensively labeled also at places of intense membrane contact. The cytoplasm (C) and the cell organelles are free of gold particles. Pm, Plasma membrane; Nc, nucleus. Bar, 0.5 μm.

gonococcal membrane fragments and the plasma membrane of the epithelial cells.

At 6 h postinfection, intracellular bacteria were recognized in a number of epithelial cells. Morphologically, the ingested bacteria, which were nearly always contained in a membrane-bound vacuole, appeared to be intact, with ribosomes randomly distributed throughout the cytoplasm and a well-defined thre -layer cell wall. Probing of the 7B1E epitope at this stage of the infection revealed that all extracellular as well as ingested bacteria contained gold particles on their membranes (Fig. 4). There was no difference in the density of labeling between the extracellular and the intracellular bacteria. In some of the infected cells, the gold label was again not strictly localized at the bacterial membranes. At this time, however, the gold particles were observed not only at the epithelial cell surface, but also at the membranes of endocytic vacuoles. Sometimes these vacuoles seemed to contain no gonococci (data not shown). Other cell organelles, the nucleus, and the cytoplasm were always found to be free of gold particles.

At 12 h postinfection, nearly all of the Chang epithelial cells contained numerous vacuoles, many of which were filled with gonococci. The morphology of the ingested bacteria was less homogeneous than in the early infection period. Besides morphologically well-preserved gonococci, bacteria were seen that showed extreme blebbing of their membranes or a condensation of their cytoplasm, resulting in an increased electron density in the electron microscope. Immunolabeling of the 12-h-infected cells with MAb 7B1E resulted in extensive gold labeling of the extracellular bacteria as well as of the morphologically well-preserved and the apparently disintegrating intracellular bacteria (Fig. 5). In the intact bacteria, the gold particles were localized at the bacterial membranes and sometimes at the membrane of the gonococcus-containing vacuole. With morphologically disin-

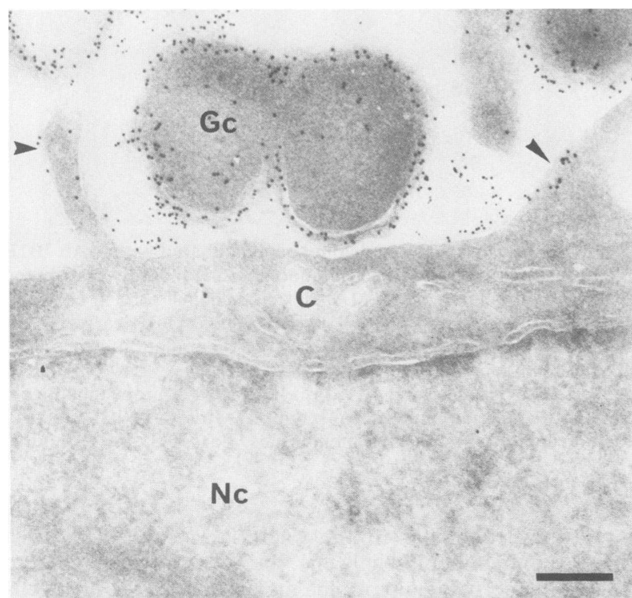

FIG. 3. Transmission electron micrograph of bacteria attached to an epithelial cell (3 h). The attached gonococcus (Gc) is surrounded by microvilli. The gold particles are located not only at the bacterial membrane but also at the microvilli and other parts of the plasma membrane (arrows). Nc, nucleus; C, cytoplasm. Bar, 0.25 μm.

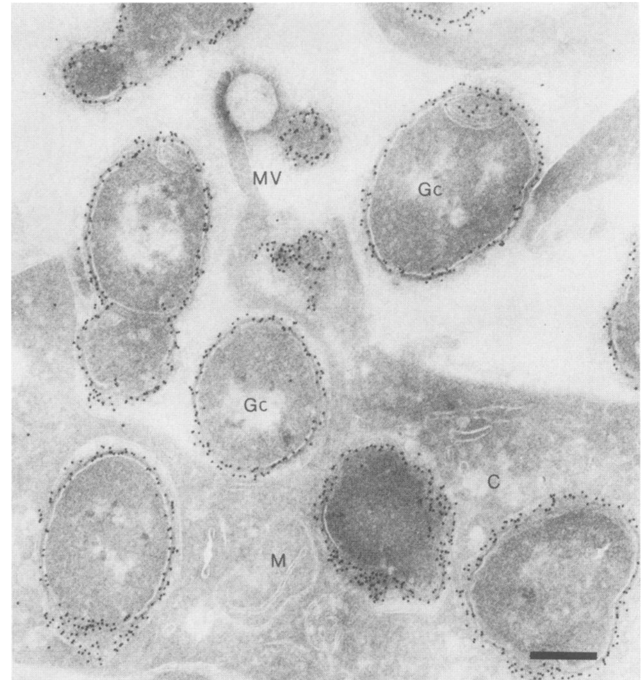

FIG. 4. Transmission electron micrograph of an infected epithelial cell at 6 h postinfection. Two subsequent stages of the infection process can be observed: the internalization of the bacteria and the intracellular localization of the bacteria in membrane-bound vacuoles. Immunolabeling of the bacteria was not altered during the adherence, uptake, and intracellular processing of the bacteria. Gc, Gonococcus; MV, microvilli; M, mitochondrion; C, cytoplasm. Bar, 0.25 μm.

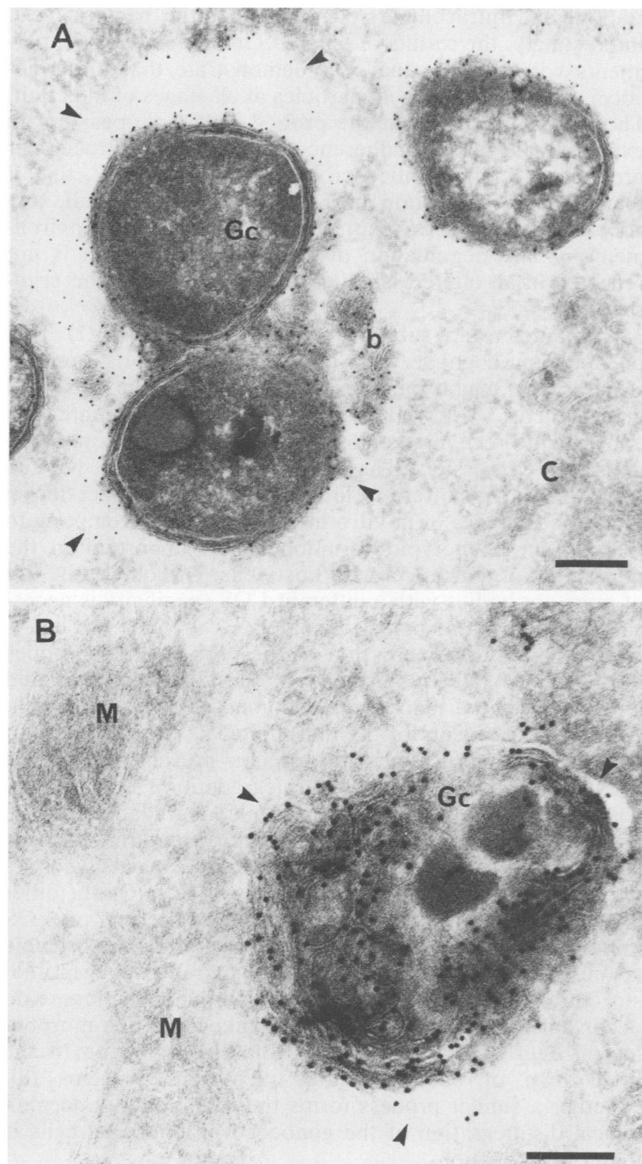

FIG. 5. Transmission electron micrographs of intracellular gonococci (Gc) at various stages of morphological disintegration (12 h postinfection). At the early stage of disintegration, the bacteria showed extreme blebbing (b) of the outer membrane and a condensation of the cytosol (A). Later, the cytosol disappeared, leaving membrane remnants behind (B). The labeling of the LOS antigens did not seem to be affected by the degradation process. Gold particles were found at the bacterial membranes as well as at the vacuole membrane (arrows). M, Mitochondrion; C, cytoplasm. Bar, 0.2 μm.

tegrated bacteria, the immunolabel was found both on the bacterium and scattered throughout the vacuole. Other cellular compartments were always found to be free of gold particles, with the exception of some small vesicles that contained heavily gold-labeled amorphous material. Because of the specificity of the labeling for gonococcal LOS, this material probably represents remnants of degraded bacteria or internalized blebs.

Interestingly, when the much more variable 4B7F epitope was probed, the pattern of labeling was basically comparable to that of 7B1E. (i) The labeling of the attached and internalized bacteria was not different with respect to density and distribution of the gold particles. (ii) The percentage of bacteria that expressed the 4B7F remained constant at the various stages of the infectious process. (iii) The 4B7F LOS determinant could be localized at both the microvilli and other parts of the plasma membrane.

Interaction of purified LOS and epithelial cells. The presence of gold particles (i.e., LOS molecules) at the host cell plasma membrane and even in endocytic vacuoles that apparently did not contain gonococci suggests that LOS (or LOS-containing blebs) can be bound and internalized by Chang conjunctiva epithelial cells. This was further investigated by exposing the epithelial cells to purified LOS (10 μg/ml) of the invasive strain 830563 for 2 and 18 h prior to subjecting the cells to the postembedding immunolabeling procedure.

Electron microscopy of the 2-h LOS-exposed cells revealed that a subset of the cells contained gold particles. Sometimes these particles were bound to microvilli, but more often they were located at other parts of the plasma membrane (Fig. 6A). The sparse cytoplasmic vacuoles (as well as other cell organelles) in these cells were nearly always free of gold spheres. The apparent presence of LOS molecules on the cell surface was not accompanied by morphological alterations of the cells.

Exposure of the cells to LOS for 18 h resulted in a considerable loss of microvilli and a strong vacuolization of the cells. Many of the cytoplasmic vacuoles were filled with large membranous structures. Immunolabeling of these cells demonstrated that some of the remaining microvilli and some of the cytoplasmic vacuoles contained gold particles (Fig. 6B). The labeling of the vacuoles appeared to be restricted to the membranous structures, which therefore probably represent LOS aggregates. The presence of LOS in the cells was not accompanied by a decrease in the number of viable cells (as judged by trypan blue exclusion) or by a loss of the integrity of the monolayer.

DISCUSSION

Use of postembedding immunoelectron microscopy in studies on the pathogenesis of neisserial infections allows the detection of bacterial antigens during the attachment, internalization, and intracellular processing of the bacteria in infected host cells (25). In this study, we have successfully applied this method to provide information with respect to the topological distribution and the stability in expression of gonococcal LOS during in vitro infection.

Before attempting LOS detection in infected cells, it was important to examine (i) the preservation of LOS antigens throughout the fixation and cryosectioning of the specimen, (ii) the specificity of the immunolabeling, and (iii) the homogeneity in the expression of the probed LOS epitopes in the inoculum. Our finding of an extensive gold labeling of gonococcal membranes and membrane blebs with MAbs 7B1E and 4B7F but not with the antibody with a different LOS specificity (4D7F) demonstrates that the probed LOS epitopes remained intact during the handling of the specimen and that specific labeling of LOS molecules was obtained. This means that the previously observed damage of gonococcal antigenicity with the use of glutaraldehyde as a fixative (25) was not due to loss of the probed LOS determinants. The observation that with MAb 7B1E nearly all of the cryosectioned bacteria were found to contain gold particles but with MAb 4B7F only a subpopulation was able to

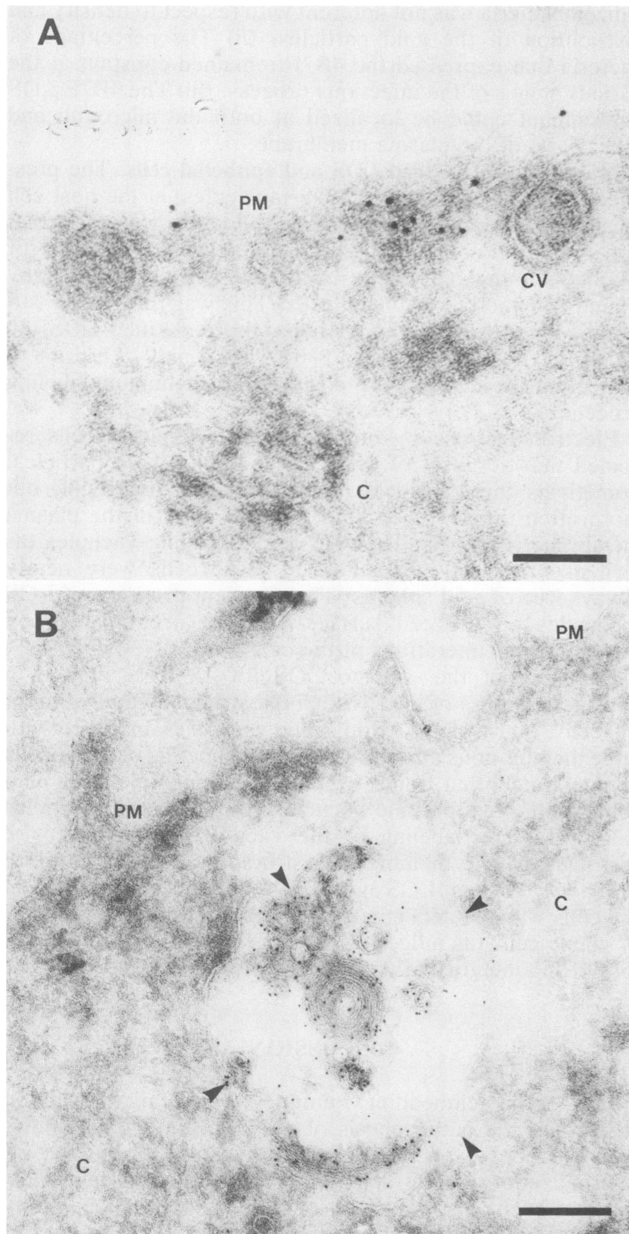

FIG. 6. Transmission electron micrographs of epithelial cells that have been exposed to purified LOS for 2 to 18 h. After 2 h of LOS exposure, spots of immunolabel were seen located at the plasma membrane (PM) of the cells (A). Note the location of the gold particles next to two coated vesicles (CV). After 18 h of exposure (B), numerous gold particles were found in large membrane-bound vacuoles (arrows) inside the cells, suggesting the uptake of LOS molecules. C, Cytoplasm. Bar, 0.2 μm.

do so indicates that there is intrastrain variability in expression of the probed LOS epitopes and that the postembedding immunolabeling technique used is suitable to detect alterations (phenotypic variation and degradation) in these determinants that might occur during the various stages of the infectious process.

In the infection experiments, we used the Chang conjunctiva cell line as a model for gonococcal infection (24). This cell system previously has been shown to reflect human mucosal epithelium with respect to the adherence, internalization, and intracellular localization of the bacteria (25). Interestingly, the results of our LOS immunolabeling experiments with the infected cells demonstrate that the gonococci are loaded with gold particles at all stages of infection. These data indicate that the probed LOS epitopes remain expressed during the adherence, uptake, and intracellular processing of the gonococci or, more specifically, that if there is any interaction of LOS with the host cell, any adaptation of the LOS expression to the host cell environment, or any degradation of LOS determinants, it is not reflected in an altered labeling of the epitopes in the cryosections.

LOS antigens are subject to phenotypic variation (2). This phenomenon might serve as an evasion of the host immune response or might function in adapting to the host cell environment. MAbs 7B1E and 4B7F used in the infection experiments recognize epitopes that show intrastrain variability in expression. The observation that there was no alteration in the pattern of labeling of the gonococci during the various stages of in vitro infection makes it tempting to reason that phenotypic variation is not important in the interaction of gonococci with host cells. Of course, the use of more antibodies with a different LOS specificity is necessary to address this point adequately.

The stability in expression of the probed LOS epitopes during in vitro infection is illustrated best by the intense gold labeling of the (partially) degraded gonococci inside the cell. Although the reason for the morphological disintegration of some of the intracellular bacteria is presently unknown (perhaps autolysis or lysosomal degradation), this discrepancy of morphological and antigenic degradation of the gonococci suggests that at least some LOS determinants are resistant to breakdown by endogenous gonococcal glycolytic enzymes (1) or lysosomal glucosidases or both. On the other hand, the extreme blebbing and the scattering of LOS immunolabel throughout the gonococcus-containing vacuole at the early stage of gonococcal disintegration suggest that structural alterations in the gonococcal outer membrane do occur during in vitro infection. In leukocytes, the morphological degradation of gonococci has been related to the breakdown of bacterial outer membrane proteins (8). Whether a similar process forms the basis for the morphological disintegration of the gonococci in epithelial cells is under investigation.

An unexpected and intriguing observation in our studies was that the gold particles were not strictly localized at the bacterial membranes. In the early infection, we viewed in the electron microscope not only the expected intense labeling of attached gonococci, but also some spots of gold particles on microvilli and other parts of the host cell plasma membrane. Recently, Mandrell et al. demonstrated immunological cross-reactivity between LOS antigenic determinants and glycoconjugates on eucaryotic plasma membranes (11). The indication that the gold labeling we viewed indeed represented antigenic determinants of gonococcal LOS and not cross-reacting host cell components was obtained from the observation that immunolabeling of uninfected cells was negative. This premise holds on the assumption that the presence of the bacteria did not induce exposure of previously nonexposed cross-reactive host cell components.

The presence of LOS molecules at the host cell membranes might result from adhesin-containing blebs or from membrane fragments of adherent gonococci that were disrupted from the plasma membrane during the handling of the specimen. Alternatively, it is possible that the labeling originates from a direct interaction of LOS molecules and

the plasma membrane of the host cells. Evidence to support this hypothesis was obtained when the cells were exposed to hot phenol-water-extracted LOS: labeling of both the microvilli and the plasma membrane was obtained, but only in a subset of the exposed epithelial cells. The latter is of interest since LOS might give rise, because of its partially hydrophobic nature, to nonspecific lipid-lipid interactions. A specific interaction of gonococcal LOS and host cells previously has been found with respect to the toxic effect of LOS on ciliated epithelial cells (19). This toxicity shows a remarkable host and tissue specificity and is related to the LOS binding capacity of the various host cells (5). The reason for the observed selective binding of LOS to Chang conjunctiva cells is unclear, but might also be related to an uneven distribution of LOS receptors among the cells in the monolayer.

Prolonged exposure of Chang conjunctiva cells to purified LOS resulted in the internalization of membrane-bound LOS molecules. Although the specificity of this endocytosis, also found to occur in the fallopian tube organ culture model (5), is inconspicuous, it might contribute to the course of the infection. The endocytosis was accompanied by a decrease in the number of microvilli and an increase in the vacuolization of the cells, suggesting interference with host cell functions. These effects, however, did not result in a reduction of the number of viable cells or a detachment of the epithelial cells, as have been observed with infected epithelial cells in vivo (9) and in the human cornea organ culture model (22).

A role for LOS in the interaction of gonococci with mucosal cells at the primary site of infection (urethra, cervix, or conjunctiva) is unknown. The present data indicate that LOS determinants are stably expressed throughout the first steps of the infectious process and raise the possibility that LOS is involved in the early interaction of the bacteria with the host cells by binding to the host cell membrane and perhaps by interfering with eucaryotic cell functioning.

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