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Localization of Gonococcal Lipopolysaccharide and Its Relationship to Toxic Damage in Human Fallopian Tube Mucosa

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An experimental model using human fallopian tubes in organ culture was used to study the localization of purified gonococcal lipopolysaccharide (LPS). LPS was visualized by light microscopy with immunoperoxidase staining. Immediately after addition to fallopian tube organ cultures, gonococcal LPS aggregated on the tips of cilia. By 1 to 2 h after exposure, LPS could be seen distributed throughout the cytoplasm of ciliated and nonciliated cells in structures resembling vesicles. By 12 h, there were sloughed, ciliated cells present in the fallopian tube lumen, which had positive LPS stain on their surfaces as well as in their cytoplasm. By 24 h, LPS was distributed throughout the cytoplasm. Control experiments with rabbit oviduct organ cultures showed that LPS failed to attach, enter, or damage mucosal cells. These studies illustrate the initial localization of LPS on human mucosal cells and its uptake into the cells, which are coincident with toxicity for ciliated epithelial cells.

The mechanisms by which gonococci damage host cells in general, and human fallopian tube mucosa in particular, remain unresolved. Recent studies have implicated two cell surface components of *Neisseria gonorrhoeae* which can damage human fallopian tube mucosa (6–8, 15, 16). Both gonococcal lipopolysaccharide (LPS) and peptidoglycan fragments, which are shed during active growth of gonococci, were capable of producing damage to fallopian tube mucosa (7, 16). Damage by these toxins resulted in loss of ciliary activity and sloughing of ciliated epithelial cells in a manner similar to that observed during active gonococcal infection.

Previous studies (6–8, 15) have indicated that gonococcal LPS is a major contributor to the toxic damage seen in gonococcus-infected human fallopian tube organ cultures (FTOC). However, direct evidence of LPS contacting, attaching to, and entering fallopian tube mucosal cells has been lacking. The studies presented here demonstrate that purified gonococcal LPS can attach to, enter, and damage the cell.

MATERIALS AND METHODS

Microorganisms. Transparent colony type 1 organisms of *N. gonorrhoeae* 2686 were used to prepare LPS (14). Stock cultures were maintained in defibrinated sheep blood at −70°C. The organisms were cultivated on a medium consisting of gonococcal agar base (Difco Laboratories, Detroit, Mich.) plus 2% (vol/vol) IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md.).

Preparation of gonococcal LPS. Gonococcal LPS was isolated and purified by the method of Johnson and Perry (10) as modified by Gregg et al. (7). The protein content of purified gonococcal LPS was quantitated either by the method of Lowry et al. (13) or by amino acid analysis (7). Amino acid analysis was also used to detect diaminopimelic acid as an indication of peptidoglycan contamination in the LPS preparation (16). LPS was tested for its biological activity by the *Limulus* amoebocyte lysate assay as previously described (7). The lysate, *Escherichia coli* LPS standard, and methodology were supplied by Associates of Cape Cod, Woods Hole, Mass.

Human FTOC. Organ cultures were prepared from human fallopian tubes as previously described (14). In brief, fallopian tubes were removed from nonpregnant, premenopausal women during the course of hysterectomy for surgical indications. These tubes were freed of adventitial tissues, opened longitudinally, and cut into square pieces (3 by 4 mm). Three pieces were placed, mucosal side up, in plastic tissue culture dishes (35 by 10 mm; Becton Dickinson Labware, Oxnard, Calif.) and covered with 2 ml of 0.05 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid-buffered Eagle minimal essential medium containing Earle salts and L-glutamine (HEPES-MEM). This medium was supplemented with colistin sodium methanesulfonate (Warner Chilcott, Morris Plain, N.J.) in a final concentration of 3 μg/ml and with vancomycin hydrochloride (Eli Lilly & Co., Indianapolis, Ind.) in a final concentration of 5 μg/ml. Twenty-four hours after organ cultures were established, the medium was removed, and the tissue was rinsed three times with HEPES-MEM without antibiotics. The latter medium (without antibiotics) was used for organ cultures thereafter. The cultures were then exposed to LPS or unexposed according to experimental design.

Rabbit oviduct organ cultures. Oviducts were obtained aspetically from rabbits weighing approximately 3 kg as previously described (25). Rabbit oviduct organ cultures were prepared and maintained identically to human FTOC (14).

Addition of LPS to FTOC or oviduct organ cultures. Purified, lyophilized LPS was suspended in HEPES-MEM and sonicated in an ultrasonic cleaner (Branson Sonic Power Co., Danbury, Conn.) for 15 min just prior to use. A concentration of 100 μg of LPS per ml was used in all experiments. Organ cultures were shaken gently for the first 4 h of the 24-h incubation period. At the termination of the experiment, each organ culture was checked for bacterial and fungal contamination by inoculating the culture medium on blood and chocolate agar.

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Quantitation of mucosal damage. Damage to fallopian tube or oviduct mucosa was assessed by measurement of the percentage of the periphery of the mucosa with ciliary activity at 24 h after exposure to LPS as previously described (14).

Preparation of antisera. Antibody to live, whole gonococcal cells was prepared by immunizing rabbits with three intravenous injections of 10^8 CFU/ml at 10-day intervals. The sera were tested for antibody to LPS 10 days after the final immunization by passive hemagglutination (26). Rabbits with positive titers to LPS were exsanguinated, and the sera were separated and stored at −20°C until used.

Antibody to purified LPS was prepared by intravenous injections of LPS and polymyxin B in graded doses at 10-day intervals. An initial dose of 10 μg of LPS was given intravenously and followed at 10-day intervals with the following mixtures containing increasing concentrations of reagents: 100 μg of LPS–2,000 μg of polymyxin B; 250 μg of LPS–5,000 μg of polymyxin B; and 500 μg of LPS–10,000 μg of polymyxin B. The LPS-polymyxin B mixture was incubated at ambient temperature for 30 min prior to injection and then filter (0.45 μm [pore size]; Millipore Corp., Bedford, Mass) sterilized. Polymyxin B was used to reduce the toxicity of LPS for rabbits (20). Rabbits were tested for antibody and exsanguinated 10 days after the last immunization.

Quantitation of antibody directed against LPS in each antiserum was determined by an enzyme-linked immunosorbent assay in which purified gonococcal LPS was used as the antigen (1). Antisera prepared against live gonococci had a titer of 51,200, while anti-LPS antisera had a titer of 6,400.

Immunocytochemical examination of tissues. Organ culture tissues were removed and fixed in formal sublimate (22). Tissues were paraffin embedded, sectioned at 3 μm, attached to gelatin-coated microscope slides, and air-dried. Tissue sections were deparaffinized in 2% iodine in xylene, dehydrated in graded alcohols, and placed in 0.05 M Tris-buffered saline (pH 7.6; TBS) for 5 min. The tissue was then stained by a modification of the peroxidase-antiperoxidase (PAP) method of Curran and Greogry (5). Excess TBS was removed from the tissue, and the sections were incubated in TBS containing 2% goat serum for 30 min in a humid chamber. The 2% goat serum was removed, a 1:1,500 dilution of rabbit anti-gonococcus LPS serum was applied, and the sections were incubated overnight at 4°C in a humid chamber. Following overnight incubation, the tissue was washed three times in TBS, and goat anti-rabbit serum was incubated with the tissue for 30 min. Tissues were washed three times in TBS and incubated in a 1:50 dilution of rabbit PAP (Miles Laboratories, Elkhart, Ind.) in TBS containing 1% goat serum for 30 min. The tissue was rinsed three times in TBS and placed in 0.05 M Tris hydrochloride (pH 7.6) for 10 min. The tissue slides were then placed in a humid chamber, and 0.05% 3,3′-diaminobenzidine tetrahydrochloride (Litton Bionetics, Kensington, Md.) was incubated with the tissue for 8 min. Following incubation, the tissues were washed for 5 min in TBS. The tissues were then placed in distilled water and counterstained with hematoxylin (Fisher Scientific Co., Pittsburg, Pa.) for 3 min. The tissues were dehydrated in graded alcohol followed by xylene and mounted in a synthetic mounting medium. The slides were then examined by light micorscopy. Controls consisted of (i) saline, (ii) commercial normal rabbit serum, and (iii) preimmune serum. Sections of gonococcus-infected tissue were processed with whole gonococcal antiserum as a positive control.

RESULTS

Purity testing and biological activity of gonococcal LPS. The purified LPS contained <1% protein. In addition, amino acid analysis failed to detect the presence of the peptidoglycan marker, diaminopimelic acid, which indicated that the LPS was free of detectable peptidoglycan contamination. The biological activity of the LPS preparation was confirmed by its ability to gel *Limulus* amoebocyte lysate in concentrations of 3.0 to 6.0 pg/ml.

Toxic effects of gonococcal LPS on human fallopian tube mucosa. Gonococcal LPS in concentrations of 100 μg/ml produced extensive damage to fallopian tube mucosa by 24 h. The percentage of the periphery of the mucosa with ciliary activity was measured at 0 and 24 h in both LPS-exposed and nonexposed tissues. The ciliary activity of LPS-exposed tissue was reduced by >80%, indicating that LPS had greatly damaged the ciliated epithelium. This was consistent with results reported in previous studies with purified LPS, gonococcus-infected mucosal fallopian tube tissues, and filter-sterilized toxic supernatants from gonococcus-infected organ cultures (15). Although in previous studies LPS in concentrations as low as 0.015 μg/ml damaged fallopian tube mucosa, a concentration of 100 μg/ml was used in the present study to provide adequate visualization of the cellular localization of the LPS.

Immunocytochemical localization of LPS in fallopian tube mucosa. The appearance of normal human fallopian tube mucosa is illustrated in Fig. 1A. This is a representative section of control tissue unexposed to LPS, which was removed for fixation at 0 h. This tissue was reacted with anti-whole gonococcal cell antiserum and stained with PAP. There was no endogenous reactive material in normal human fallopian tube mucosa, and there was no nonspecific tissue reaction with the stain.

In contrast, Fig. 1B demonstrates the appearance of mucosa exposed to LPS and immediately fixed at 0 h. Small, irregular aggregates of stained material were seen associated primarily with the tips of ciliated cells. Sections of this same tissue treated with normal rabbit serum were not reactive with PAP. Tissue harvests at 30 min after exposure to LPS had increased amounts of stained material attached to the tips of cilia but otherwise appeared similar to the 0-h specimens.

After 1 h of exposure to LPS, dramatic changes in mucosal staining became evident (Fig. 2A). Darkly stained material was still present on the surface of the mucosa. However, this occasionally seemed to represent a stained cell rather than the previous irregular, extracellular aggregates. Mucosal damage was evident, because stained, ciliated cells were seen sloughed from the epithelium. One such stained, sloughed, cilicated cell can be seen in the upper right of Fig. 2A. A striking feature at that time was the appearance of stained, circular bodies resembling vesicles, which were distributed throughout the mucosal cells. These vesiclelike structures appeared in two morphological forms; one was a tight, circular form with a heavily stained border, and the other was a larger, vacuolelike form with stained material in the interior.

After 2 to 12 h, increased numbers of sloughed cells were seen (Fig. 2B and C). Many of these cells were heavily stained throughout the cell body. Stained cilia on sloughed cells were commonly noted, and the stained circular bodies (vesiclelike structures) throughout the mucosal cell cytoplasm were a prominent feature at those times. By 2 h, the stained, vesiclelike structures were evident in both ciliated and nonciliated epithelial cells.

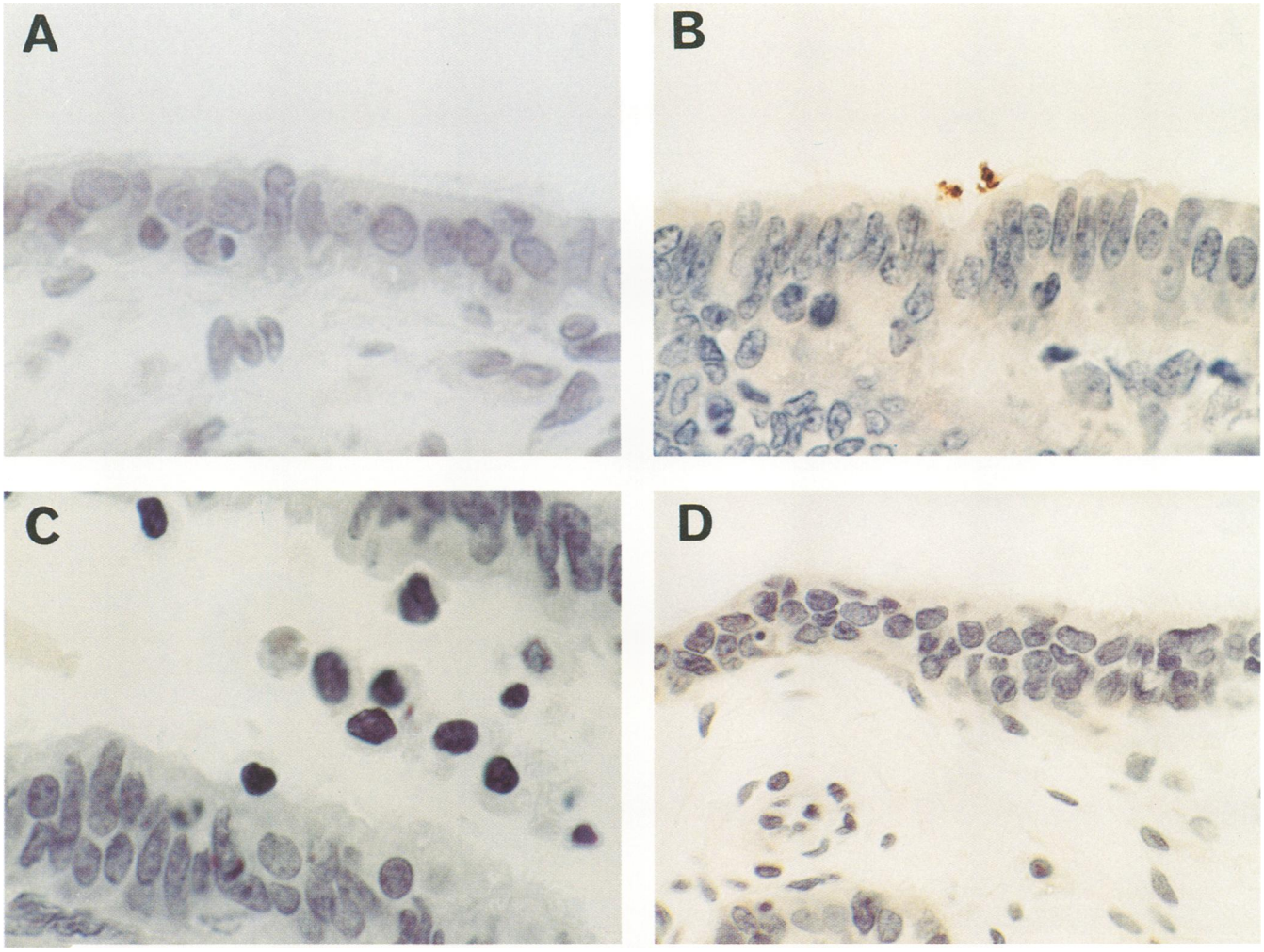

FIG. 1. (A) Control FTOC tissue without exposure to LPS at 0 h but reacted with LPS antibody and PAP. (B) LPS-exposed FTOC at 0 h, reacted with LPS antibody and PAP. Note the aggregates of LPS on the tips of the cilia. (C) LPS-exposed FTOC tissue 12 h after exposure, reacted with normal rabbit serum and PAP. (D) Control FTOC without exposure to LPS at 24 h, reacted with LPS antibody and PAP. Magnification, ×875.

By 12 h, progressive damage to the mucosa had occurred (Fig. 2C). There were numerous, heavily stained, degenerating, sloughed cells. Few ciliated cells remained in the mucosa, which appeared severely damaged. The majority of the stained, circular bodies had disappeared; only very rarely could one be found.

At 24 h (Fig. 2D), all vestiges of the circular bodies had disappeared, and only a few ciliated cells remained attached to the mucosa. Fragmented, stained, sloughed cells were evident in the lumen. At this period, for the first time, uniform, light staining appeared throughout the cytoplasm of the remaining mucosal cells. There were also some crystalline aggregates of LPS which appeared on the borders of mucosal cells and in association with sloughed cells.

Throughout the experiment and at the 12- and 24-h terminations, control organ cultures which were not exposed to LPS (Fig. 1C and D) maintained good ciliary activity, and numerous ciliated cells remained in the mucosal surface. When reacted with antigonococcal antiserum, no brown-staining cells, aggregates, or fragments could be detected.

Control experiments with rabbit oviduct organ cultures. Control experiments with rabbit oviduct organ cultures were performed to determine whether gonococcal LPS attached to or entered genital epithelial cells of a species whose genital mucosa was resistant to toxic damage by gonococcal LPS. The percentage of the periphery of the mucosa with ciliary activity was measured at 0 and 24 h in both LPS-exposed and nonexposed tissues. Gonococcal LPS in concentrations of 100 μg/ml failed to produce any detectable damage to rabbit oviduct mucosa by 24 h. The ciliary activity of LPS-exposed tissue was retained at 100% of zero-time values and was indistinguishable from the ciliary activity of unexposed oviduct organ culture. This was consistent with results reported in previous studies.

Immunocytochemical studies of LPS-exposed oviduct tissue failed to demonstrate any localization, attachment, or uptake of LPS (data not shown). The vesiclelike structures observed in human FTOC tissue were absent. Occasional stained aggregates were seen in the lumen but were not attached to, nor within, any ciliated or nonciliated cells at any of the time intervals studied (0, 2, 6, and 24 h). In addition, there were no sloughed, ciliated cells observed in

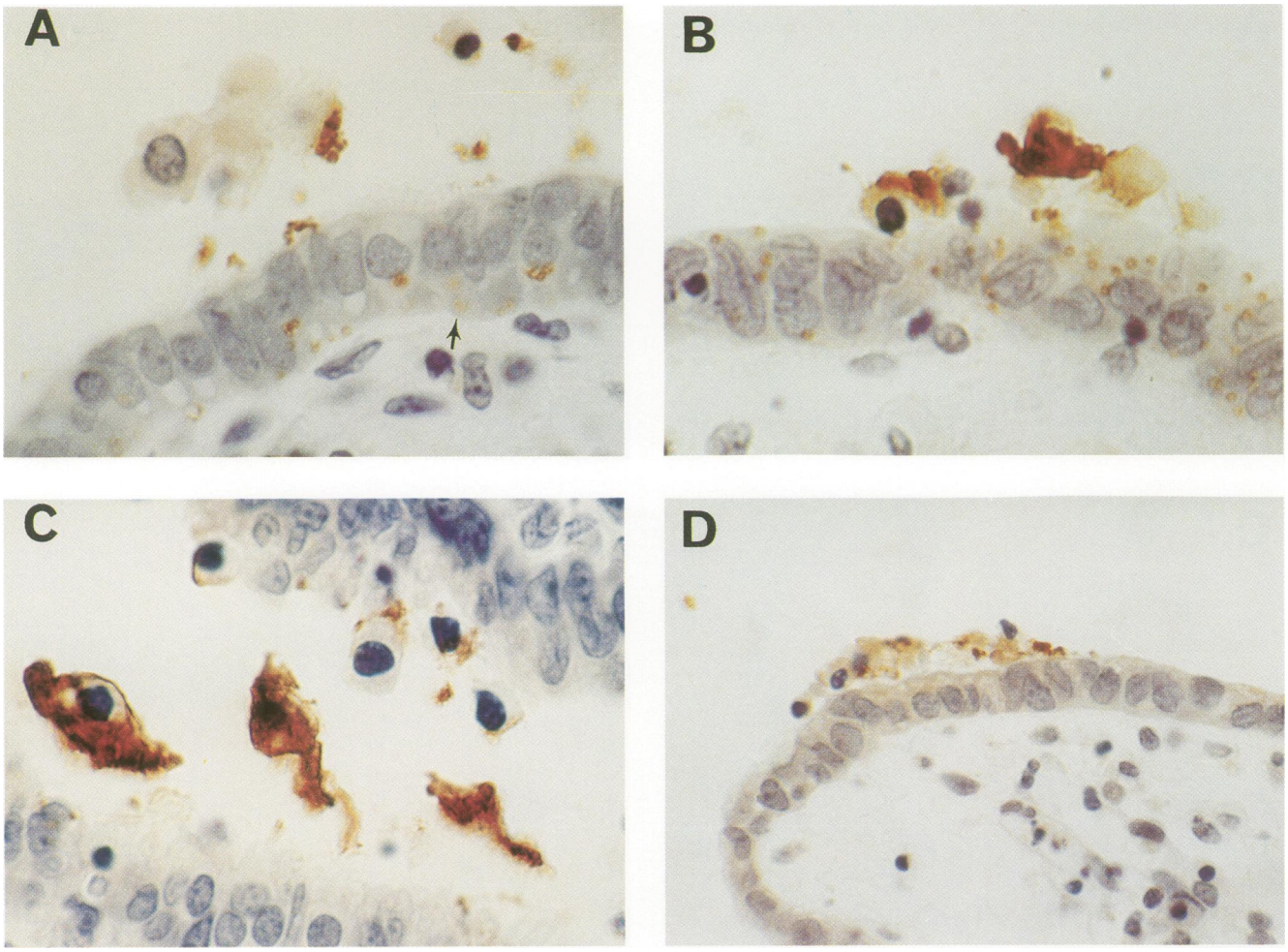

FIG. 2. (A) LPS-exposed FTOC at 1 h, reacted with LPS antibody and PAP. There is wide distribution of vesiclelike structures throughout the cell; there are two types of vesicular structure, one with a tight band of intense staining and a second with more diffuse staining (arrow). (B) LPS-exposed FTOC at 2 h, reacted with LPS antibody and PAP. There is wide distribution of vesiclelike structures throughout the cells and heavily stained, sloughed cells adjacent to the intact mucosal surface. (C) LPS-exposed FTOC at 12 h, reacted with LPS antibody and PAP. There are heavily stained, sloughed cells in the lumen and a decline in the number of vesiclelike structures. (D) LPS-exposed tissue at 24 h, reacted with LPS antibody and PAP. Note the general reactivity throughout the cytoplasm and aggregates of LPS with associated sloughed cells. Magnification, ×875.

LPS-exposed tissues, and there were no detectable differences in the numbers of ciliated cells observed in the epithelia of LPS-exposed or nonexposed tissues.

DISCUSSION

There have been few reports describing direct toxic activity of gonococcal products on host tissue. Gonococcal LPS has been reported to be lethal for mice (24) and chicken embryos (19). Melly et al. (15) and Gregg et al. (7) reported the toxic activity of gonococcal LPS on human fallopian tube mucosa. They demonstrated that LPS was responsible for most of the toxicity of supernatant fluids from organ cultures infected with gonococci and that this toxicity could be substantially reduced by absorption of the supernatant with *Limulus* amoebocyte lysate. The toxicity of gonococcal LPS was manifested in fallopian tubes by loss of ciliary activity and by sloughing of ciliated epithelial cells from the mucosa. The mechanisms by which LPS produces this damage are unclear.

To establish the cellular localization of LPS in mucosal epithelial tissue, we attempted to detect the interaction of gonococcal LPS with human fallopian tube mucosa by using a probe consisting of polyclonal antibody to LPS and PAP staining. This technique allowed us to visualize the initial attachment of the LPS aggregates to the tips of ciliated cells. At 1 h, not only was LPS present on the mucosal surface, but evidence of damage to the mucosa was becoming apparent. At this time, stained ciliated cells were beginning to slough from the mucosa into the lumen. In addition, LPS-containing, vesiclelike, circular structures appeared, which were distributed throughout the cytoplasm of both ciliated and nonciliated cells. Thus, it appears that the entrance of LPS into the cells might be correlated with the first morphological evidence of mucosal damage. Both the number of sloughed, ciliated cells and vesiclelike structures within mucosal cells increased significantly by 2 h.

Figures 1B and 2A and B suggest that LPS molecules may enter the epithelial cell in a manner similar to that of

receptor-mediated endocytosis of other toxins (18). It appears that LPS attached initially to the tips of cilia on ciliated cells (presumably through an LPS receptor). In addition, it is also entirely possible that small particles of LPS which are beyond the limits of resolution by light microscopy might have attached to nonciliated cells or to the membranes of ciliated cells as well as to the tips of cilia. From our observations, it seems that LPS aggregates on the tips of cilia subsequently migrated to the epithelial cell membrane, where they became incorporated into vesiclelike structures. These structures became distributed throughout the cytoplasm of both ciliated and nonciliated cells. LPS then appeared to be released from a structure which lacked the defined organization of the initial vesiclelike body and became evenly distributed throughout the cytoplasm. Although it is not possible to see electron-dense clathrin coat areas in our micrographs, the stained, circular bodies in the light micrographs were similar to structures reported by others (17, 21).

By 6 h after exposure to LPS, heavily stained, sloughed, ciliated cells were present in the lumen. Stained LPS occurred on cell surfaces specifically on the cilia of these cells and within the cytoplasm.

Several other mammalian cells have been shown to interact with LPS. This is exemplified by reports that bacterial endotoxin binds to erythrocytes in vivo (2, 3, 23) and that a direct relation exists between the affinity of murine erythrocytes for LPS and the lethal effects of endotoxin for several mouse strains (9). Erythrocyte membranes contain a specific receptor to which LPS binds. This receptor has been isolated and is a water-soluble, heat-labile lipoglycoprotein with a molecular weight of about 250,000. This receptor contains a high amount of *N*-acetylneuraminic acid and small amounts of glucose, mannose, and fucose (11).

Lipopolysaccharides have also been reported to bind to leukocytes, platelets, and lymphocytes (4), as well as to monocytes and macrophages (12). Thus, there is evidence to indicate the presence of LPS receptors on a variety of mammalian cells. Our experiments with rabbit oviduct mucosa indicated that gonococcal LPS did not attach to nor damage mucosal epithelial cells. Neither ciliated nor nonciliated rabbit epithelial cells took up LPS, indicating the lack of LPS receptors in rabbit tissue. Despite many years of intense study, the exact mechanism by which endotoxin induces toxic manifestations remains unclear. Indeed, in our observations, we have seen the toxic effects of purified LPS, which resulted in sloughing of ciliated epithelial cells while adjacent nonciliated cells, which also had ingested LPS, did not slough. Why these differences between cellular types exist remains unexplained.

In summary, we have demonstrated the initial localization of gonococcal LPS on cilia of human genital mucosal cells, its uptake into cells, and its toxicity for ciliated epithelial cells. However, the exact mechanisms of biological interaction with mucosal cells and the processing of gonococcal LPS remain elusive.

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LITERATURE CITED

1. **Apicella, M. A., and N. C. Galiardi.** 1979. Antigenic heterogeneity of the non-serogroup antigen structure of *Neisseria gonorrhoeae* lipopolysaccharides. Infect. Immun. **26**:870–874.
2. **Boyden, S. V.** 1953. Fixation of bacterial products by erythrocytes in vivo and by leucocytes. Nature (London) **171**:402–403.
3. **Buxton, A.** 1959. The in vivo sensitization of avian erythrocytes with *Salmonella gallinarum* polysaccharide. Immunology **2**:203–210.
4. **Cooper, K. E.** 1971. Some physiological and clinical aspects of pyrogens, p. 5–21. *In* G. E. W. Wolstenholme and J. Birch (ed.), Symposium on pyrogens and fever. Churchill Livingstone, Inc., New York.
5. **Curran, R. C., and J. Gregory.** 1978. Demonstration of immunoglobulin in cryostat and paraffin sections of human tonsil by immunofluorescence and immunoperoxidase techniques. J. Clin. Pathol. **31**:974–983.
6. **Gregg, C. R., A. P. Johnson, D. Taylor-Robinson, M. A. Melly, and Z. A. McGee.** 1981. Host species-specific damage to oviduct mucosa by *Neisseria gonorrhoeae* lipopolysaccharide. Infect. Immun. **34**:1056–1058.
7. **Gregg, C. R., M. A. Melly, G. G. Hellerqvist, J. G. Coniglio, and Z. A. McGee.** 1981. Toxic activity of purified lipopolysaccharide of *Neisseria gonorrhoeae* for human fallopian tube mucosa. J. Infect. Dis. **143**:432–439.
8. **Gregg, C. R., M. A. Melly, and Z. A. McGee.** 1980. Gonococcal lipopolysaccharide: a toxin for human fallopian tube mucosa. Am. J. Obstet. Gynecol. **138**:981–984.
9. **Hill, G. J., and D. W. Weiss.** 1964. Relationships between susceptibility of mice to heat-killed salmonella and endotoxin and the affinity of their red blood cells for killed organisms, p. 422–427. *In* M. Landy and W. Braun (ed.), Bacterial endotoxins. Rutgers University, New Brunswick, N.J.
10. **Johnson, K. G., and M. B. Perry.** 1976. Improved techniques for the preparation of bacterial lipopolysaccharides. Can. J. Microbiol. **22**:29–34.
11. **Kabir, S., D. L. Rosenstreich, and S. E. Mergenhagen.** 1978. Mode of attachment to cell membranes, p. 70–87. *In* J. Jeljaszewicz and T. Wadstrom (ed.), Bacterial toxins and cell membranes. Academic Press, Inc., New York.
12. **Larsen, N. E., and R. Sullivan.** 1984. Interaction between endotoxin and human monocytes: characteristics of the binding of ^{3}H-labeled lipopolysaccharide and ^{51}Cr-labeled lipid A before and after the induction of endotoxin tolerance. Proc. Natl. Acad. Sci. USA **81**:3491–3495.
13. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
14. **McGee, Z. A., A. P. Johnson, and D. Taylor-Robinson.** 1976. Human fallopian tubes in organ culture: preparation, maintenance, and quantitation of damage by pathogenic microorganisms. Infect. Immun. **13**:608–618.
15. **Melly, M. A., C. R. Gregg, and Z. A. McGee.** 1981. Studies of toxicity of *Neisseria gonorrhoeae* for human fallopian tube mucosa. J. Infect. Dis. **143**:423–431.
16. **Melly, M. A., Z. A. McGee, and R. S. Rosenthal.** 1984. Ability of monomeric peptidoglycan fragments from *Neisseria gonorrhoeae* to damage human fallopian-tube mucosa. J. Infect. Dis. **149**:378–386.
17. **Morris, R. E.** 1985. Receptor-mediated endocytosis is required for expression of *Pseudomonas* and diphtheria toxin activity, p. 91–95. *In* Loretta Leive (ed.), Microbiology—1985. American Society for Microbiology, Washington, D.C.
18. **Pastan, I., and M. C. Willingham.** 1983. Receptor-mediated endocytosis: coated pits, receptosomes and the golgi. Trends Biochem. Sci. **8**:250–255.
19. **Peacock, W. L., Jr., and J. D. Schmale.** 1969. Toxic constituents of *Neisseria gonorrhoeae*. Nature (London) **221**:760–761.
20. **Rifkind, D.** 1967. Prevention by polymyxin B of endotoxin lethality in mice. J. Bacteriol. **93**:1463–1464.
21. **Saelinger, C. B.** 1985. Route of toxin or virus entry as determinant of virulence, p. 83–84. *In* Loretta Leive (ed.), Microbiol-

ogy—1985. American Society for Microbiology, Washington, D.C.
22. **Sowter, C., and Z. A. McGee.** 1976. Evaluation of a new technique for the demonstration of gonococci and other microorganisms in host cells. J. Clin. Pathol. **29:**433–437.
23. **Springer, G. F., and R. E. Horton.** 1964. Erythrocyte sensitization by blood group-specific bacterial antigens. J. Gen. Physiol. **47:**1229–1250.
24. **Tauber, H., and W. Garson.** 1959. Isolation of lipopolysaccharide endotoxin. J. Biol. Chem. **234:**1391–1393.
25. **Taylor-Robinson, D., S. Whytock, C. J. Green, and F. E. Carney, Jr.** 1974. Effect of *Neisseria gonorrhoeae* on human and rabbit oviducts. Br. J. Vener. Dis. **50:**279–288.
26. **Vos, J. G., J. Buys, J. G. Hanstede, and A. M. Hagenaars.** 1979. Comparison of enzyme-linked immunosorbent assay and passive hemagglutination method for quantification of antibodies to lipopolysaccharide and tetanus toxoid in rats. Infect. Immun. **24:**798–803.