Effects of *Escherichia coli* and *E. coli* Lipopolysaccharides on the Function of Human Ureteral Epithelial Cells Cultured in Serum-Free Medium

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*Escherichia coli* is the microorganism most commonly isolated from human urinary tract infections (6). The ability to adhere to and colonize the urogenital mucosa is an important virulence attribute of uropathogenic strains of *E. coli* (43). A great deal is known about the molecular mechanisms of attachment of *E. coli* to epithelial cells from the urinary tract (1, 25, 42). Earlier studies have shown that the attachment of *E. coli* to epithelial cells in the urinary tract enhances the ability of the toxins produced by these bacteria (e.g., lipopolysaccharides [LPS]) to trigger mucosal inflammation (25). The injection of adherent *E. coli* strains into the mouse urinary tract resulted in a mucosal and systemic inflammatory response, and inflammatory cells were recruited to the infection site (37). The cellular mechanisms triggered by the attachment of *E. coli* to epithelial cells in the lower urinary tract or by the production of LPS by these bacteria, leading to a fast local inflammatory response in vivo, have not been completely defined.

Studies in pre-B lymphocytes have demonstrated that treatment with LPS causes an increase in the intracellular Na⁺ content as a result of the activation of an amiloride-sensitive Na⁺/H⁺ exchanger. Stimulation of the expression of a cell surface immunoglobulin follows (32–35). We have postulated that LPS may have a similar effect on Na⁺ content in uroepithelial (UT) cells and that this increase or changes in ionic gradients coupled to it (e.g., pH) may serve as a signal to stimulate gene expression in UT cells. Of special interest was the intercellular adhesion molecule-1 (ICAM-1), a member of the immunoglobulin superfamily, which is a specific ligand of lymphocyte function-associated antigen-1 (LFA-1) (7, 9, 10). By increasing the avidity of this interaction (28), ICAM-1 may play an important role in the interaction of UT cells with lymphocytes during the inflammatory response. ICAM-1 is widely represented on various tissues, including bone marrow–derived cells (2, 3), vascular endothelium (9, 20), fibroblasts, and some epithelial cells (8), e.g., intestinal (23), renal tubular (22), thymic epithelial (40), and retinal pigment epithelial (15) cells. Constitutive expression varies with the cells examined, but activation studies on humans with interleukin-1, gamma interferon, and tumor necrosis factor alpha indicate that these cytokines can induce ICAM-1 expression considerably (30). ICAM-1 has been shown to be important in both afferent recognition and efferent cytotoxic immune responses (3, 27). Increased levels of ICAM-1 in response to bacterial toxins, although a necessary event in the inflammatory response, could contribute to cytotoxic injury following infection in the lower urinary tract.

The in vivo studies carried out so far do not allow complete separation of systemic host-related mechanisms of LPS action from local effects at the mucosal epithelium. The molecular basis of the local host-pathogen interactions could not be studied previously with cultured epithelial cells because differentiated and polarized UT cells were not available until recently (14, 31). In the present study, epithelial cell cultures isolated from the human ureter (UT cells) (14, 46) served as a model system with which to explore the mechanisms of action of *E. coli* and *E. coli* LPS in UT cells.
MATERIALS AND METHODS

Abbreviations. DIDS, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid; LPS-1, LPS from E. coli O55:B5; LPS-5, LPS from E. coli O127:B8; PBS, phosphate-buffered saline; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; MES, 4-morpholinepropanesulfonic acid.

Cell culture. Cells were isolated from human ureters which were obtained from Tissue Procurement, University Hospital, University of Alabama at Birmingham, within 1 to 2 h after surgical removal. Pieces of normal ureter are routinely available as a byproduct of kidney transplantation surgery. The procedures for preparing primary cultures and subcultures of the UT cells were generally those described previously (14, 31, 46). Briefly, primary UT cultures were initiated from explants of ureteral uroepithelium. Cultures were grown on substrates of reconstituted type I tail collagen in Ham's F12 medium (GIBCO) supplemented with 1 μg of hydrocortisone (Merek, Sharpe and Dohme) per ml, 5 μg of transferrin (Sigma) per ml, 10 μg of insulin (SQubb) per ml, 0.1 mM nonessential amino acids (Microbiological Associates, Walkersville, Md.), 2 mM L-glutamine (GIBCO), and 2.7 mg of dextrose (Amend Drug and Chemical Co., Irvington, N.J.) per ml. This medium, designated F12*, was supplemented with 1% fetal bovine serum (Sterile Systems, Inc., Logan, Utah) for primary UT cultures. The culture medium was changed three times per week. UT cells cultured by these techniques have a generation time of approximately 25 h and spontaneously senesce after 35 to 50 population doublings.

For the seeding of secondary cultures of UT cells, explants were removed from 7- to 9-day-old growing (almost confluent) primary cultures of UT cells. The cells were dispermic with 0.1% EDTA (Sigma) and collected as previously described (31). The number of viable cells was determined by direct examination with a hemacytometer after the cells were stained for 5 min with 0.1% trypsin blue (GIBCO). The dispersed cells were centrifuged at 4°C for 5 min at 200 × g, the cell pellet was gently resuspended in 10 ml of a balanced salt solution (39) containing 5% fetal bovine serum (GIBCO), and the centrifugation step was repeated. The cell pellet was resuspended in 1 ml of complete serum-free F12*, and an aliquot was removed to a cell-counting chamber for routine determination of total cell number. Routinely, approximately 10⁶ cells were seeded directly onto plastic in 35-mm petri dishes in complete serum-free F12* containing 0.25% bovine pituitary extract and 10 μg of insulin per ml. For immunohistochemical studies, cells were seeded on glass cover slips in an identical growth medium. Cells were maintained at 37°C in a humidified environment with 5% CO₂ and re-fed fresh complete growth medium every other day.

For further passage of proliferating secondary cultures, almost-confluent monolayer cultures were enzymatically dissociated by incubation for 6 to 8 min at 0 to 4°C in a balanced salt solution containing 0.25% trypsin; 10% fetal bovine serum was then added to the cell suspension, and the cells were harvested by low-speed centrifugation as described previously (46). The cell pellet was resuspended in 1 ml of complete, serum-free F12* containing 0.5% bovine pituitary extract and 10 μg of insulin per ml. The next steps of the procedure were identical to those described in the previous paragraph.

Radioactive labeling of E. coli. A derivative of E. coli ATCC 11303 Luria strain B was isolated by Suling and collaborators at the Southern Research Institute, Birmingham, Ala., and was kindly provided for these studies. A modification of the procedure described by Cohen et al. (5) was used to label E. coli with ³⁵SO₄²⁻. Briefly, cultures of E. coli were grown in Luria-Bertani broth containing (in grams per liter) tryptone, 10; yeast extract, 5; and NaCl, 10, at pH 7.5 and 37°C from about 10⁶ to about 10⁷ cells per ml. At that time, 0.1 ml of culture was transferred to 10 ml of sulfur-free minimal medium containing (in grams per liter): KH₂PO₄, 2; K₂HPO₄, 9.2; sodium citrate, 0.5; MgCl₂, 6H₂O, 0.17; NH₄Cl, 0.81; and dextrose, 1.0, plus ³⁵SO₄²⁻ (1 μCi/ml). The cultures were incubated at 37°C for various times. ³⁵SO₄²⁻-labeled cultures were washed twice at 4°C with HEPES-Hanks, which contains (millimolar): NaCl, 137; KCl, 5; CaCl₂, 1.3; MgSO₄, 7H₂O, 0.8; Na₂HPO₄, 0.35; KH₂PO₄, 2.6; and HEPES, 10, titrated with NaOH to the desired pH. The labeled E. coli were resuspended in the same buffer at 37°C and used to infect UT cells. An aliquot of labeled E. coli was used to determine the number of cells, and ³⁵S was counted in another aliquot by liquid scintillation spectrometry. The viability of E. coli used in all experiments was determined on aliquots which were seeded overnight on tryptic soy agar. Viability was expressed as growing colonies as a percentage of the cells seeded and was found to be 70 to 80% in the experiments presented in this article.

Attachment of E. coli to UT cells in vitro. To quantitate the attachment of E. coli to UT cells, UT cells were incubated for various times at 37°C with E. coli labeled with ³⁵SO₄²⁻ by the method described above. The composition of the incubation medium is given in detail in the Results. After the incubation, the medium containing unattached bacteria was removed, the UT cells were washed four times in an ice-cold isosmotic medium, and the cells were extracted in 0.1 N NaOH. The radioactivity in a sample of the extract was counted by liquid scintillation spectrometry.

Immunohistochemical studies. Cells were prepared for immunohistochemical studies as described previously (47). Briefly, cells grown on glass coverslips were washed with PBS and fixed with 3.5% formaldehyde (20 min). After free aldehyde groups were quenched with NH₄Cl in PBS (15 min), the cells were washed once with PBS for 15 min, permeabilized with 0.1% Triton X-100 in PBS for 10 min, and then washed three times for 15 min each in PBS. Permeabilization was necessary to determine the spatial distribution of species of interest, not only at the cell surface, but also in intracellular pools. Non-specific binding was evaluated by incubating the sample with 1% bovine serum albumin for 30 min at 37°C. The cells were then incubated with primary antibody for 45 min at 37°C, washed three times with PBS for 15 min each, and then incubated for 45 min at 37°C with secondary antibody (affinity-purified fluorescein-conjugated goat anti-mouse immunoglobulin G [IgG]; Cappel). Finally, the cells were mounted in a mounting medium containing (wt/vol) 20% polyvinyl alcohol (Sigma type II), 30% glycerol, 100 mg of chlorobutanol, 5% Tris-Cl (1 M; pH 9), and 0.0005% phenol red. To detect nonspecific staining, control samples were treated with secondary antibody only, and no staining was observed in these cells (results not shown).

Monoclonal antibody against ICAM-1 was purchased from AMAC, Inc., Westbrook, Maine. Actin was labeled with phalloidin coupled to rhodamine (Molecular Probes, Eugene, Ore.).

Transmission electron microscopy. UT cells were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, embedded in Epon, sectioned, and poststained in uranyl acetate for observation in a Hitachi HU 600 transmission electron microscope.

Transport studies. Uptake was measured as described

Vol. 61, 1993
EFFECTS OF LPS ON UROEPITHELIAL CELLS

3305

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previously (11, 13, 14). Before the assay, the cells were washed three times in an isosmotic medium. Generally, uptake was initiated by adding 1 ml of medium containing 32NaCl or 35SO42-. The composition of the medium varied from experiment to experiment and is given in detail with the results. After incubation for various times, uptake was stopped by removing the labeled medium and rapidly washing the cells at 4°C four times with 3 ml of medium, generally containing 100 mM sucrose, 100 mM NaNO3, 4 mM magnesium glutonate, and 10 mM HEPES titrated with Tris base to the desired pH. Finally, the cells in each dish were extracted with 1 ml of 0.1 N NaOH for 30 min at room temperature, and the radioactivity in a 0.5-ml sample of the extract was measured by liquid scintillation counting. Results are expressed as nanomoles taken up per milligram of cellular protein.

**Assays.** Protein concentrations were determined by the method of Lowry et al. (26).

**Statistical analysis.** All transport experiments and assays were performed with two to four replicate samples on two to seven cell preparations. Results are means ± standard deviation (σn – 1).

**RESULTS**

**Adhesion of E. coli to human UT cells in vitro and effect on function.** Transmission electron microscopy studies showed close interaction of the fuzzy-coated E. coli with the microvilli on the apical side of the cells (Fig. 1) (14), demonstrating unequivocally that E. coli adhered to UT cells.

Since previous studies had suggested that sulfate transport in mammalian cells may have a role in regulation of the intracellular pool of inorganic sulfate available for macromolecular sulfation (12, 13), I was interested in testing the possibility that adherence of E. coli to UT cells may affect sulfate transport in these epithelial cells. Earlier studies with UT cells (14) had shown that a DIDS-sensitive component of sulfate uptake, i.e., the carrier-mediated component of sulfate transport, was very sensitive to changes in extracellular pH (pHo). The pHo profile of this transport system indicated that maximum uptake occurred at about pHo 6, whereas half of this activity was observed at pH 7.4.

To measure the effect of E. coli under conditions in which sulfate transport was maximal, it was important to ascertain that E. coli adherence was not affected by lowering pHo. Therefore, the adhesion of E. coli to monolayers of UT cells in media of pH 7.4 and 6 was measured first. Preliminary time course studies (results not shown) had indicated that after 18 h of incubation, E. coli labeling had reached a steady state, the average amount of radioactive label per E. coli cell being 0.042 ± 0.003 cpm of 35S per bacterium, about 10-fold more label per E. coli cell than in previous studies (5). The number of labeled E. coli associated with the UT cultures was determined as described in Materials and Methods, and it was found to be proportional to the number of E. coli in the incubation suspension.

To estimate the adhesion of E. coli to monolayers of UT cells under conditions of varying pHo, confluent monolayers of UT cells were incubated for 1 h at 37°C in HEPES-Hanks medium at pH 7.4 or 6, containing 107 E. coli labeled with 35SO42-, for 18 h. The number of labeled E. coli associated with the UT culture in HEPES-Hanks medium was similar, at pH 7.4 and 6 (252 ± 0.5 and 288 ± 14 kcpm bound per monolayer, respectively), indicating that lowering the extracellular pH did not markedly affect the ability of E. coli to adhere.

To test the possibility that E. coli affects sulfate transport in UT cells, confluent UT cultures were first incubated for 1 h at 37°C in HEPES-Hanks at pH 6, containing 1 mM
Na<sub>2</sub>SO<sub>4</sub> and 10<sup>7</sup> unlabeled <i>E. coli</i> per ml. Control confluent UT cells were preincubated in the same medium but without bacteria. After the preincubation, nonadhering bacteria were removed, and the UT monolayer was gently washed four times with HEPES-Hanks, pH 6. Sulfate uptake into UT cells was then measured for 1 min in a medium identical to the preincubation medium but with 35SO<sub>4</sub><sup>2-</sup>, in the presence or absence of 0.1 mM DIDS, a sulfate transport inhibitor (14). Incubation with <i>E. coli</i> inhibited DIDS-sensitive sulfate uptake to half of that in untreated cells (<i>P < 0.01</i>) but had no effect on the DIDS-insensitive component of sulfate uptake (Fig. 2).

It was possible that the effect of <i>E. coli</i> on UT cells is mediated by LPS, macromolecular substances bound to the surface of these bacteria. To test the possibility that LPS affect the function of UT cells in vitro, I carried out the studies described below.

**Effect of LPS on UT cell function.** LPS-5, at concentrations as low as 5 μg/ml, inhibited DIDS-sensitive (<i>P < 0.01</i>) but not DIDS-insensitive sulfate uptake in UT cells (Fig. 3). The conditions under which the experiments aimed at testing the effect of <i>E. coli</i> (Fig. 2) and LPS (Fig. 3) were carried out were different. However, it should be noted that the percent inhibition by LPS (i.e., DIDS-sensitive sulfate uptake in the presence of LPS [25 μg/ml] as a percentage of DIDS-sensitive uptake in the absence of the toxin) and the percent inhibition of DIDS-sensitive sulfate uptake observed in <i>E. coli</i>-infected UT cells are of the same order of magnitude. I interpret this to suggest that the inhibitory effect of <i>E. coli</i> on sulfate transport may be mediated by bacterial toxins.

Studies with pre-B lymphocytes have demonstrated that treatment with LPS caused an increase in the cellular Na<sup>+</sup> content (34). I postulated that LPS may have a similar effect in UT cells. To test this possibility, two types of UT cells were used: sparse, rapidly proliferating cells and almost-confluent quiescent monolayers. The cells were first equilibrated with <sup>22</sup>NaCl for 30 min at 37°C in 5% CO<sub>2</sub>-95% O<sub>2</sub> in F12* medium. After the equilibration, LPS-1 (25 μg/ml) was added, and incubation was continued for the times indicated in Fig. 4. Control cells were incubated in an identical medium containing the same amount of ethanol as that added with the LPS solution. A transient increase in the cellular Na<sup>+</sup> content was observed in rapidly proliferating cells, and this increase was 21, 29, 38, and 52% higher after 1, 5, 10, and 30 min of incubation, respectively, in the presence of LPS-1 than after incubation in its absence (Fig. 4). This
FIG. 5. Effect of LPS-5 on the spatial distribution of ICAM-1 in UT cells. Log-phase cells (A and B) or quiescent, confluent cell monolayers (C and D) were incubated in the last 4 days of their growth with (B and D) or without (A and C) 25 μg of LPS-5 per ml. The cells were then fixed and probed with an antibody against ICAM-1 as described in Materials and Methods. Arrowheads, ICAM-1 clusters on the surface of UT cells.

LPS-stimulatable “overshoot” in the Na⁺ content observed in rapidly proliferating cells dissipated after 60 min. An identical trend was observed in the presence of LPS-5 (results not shown).

Similar studies with pre-B lymphocytes had shown that following treatment with LPS, intracellular pH and cellular Na⁺ content are increased by activation of an amiloride-sensitive Na⁺/H⁺ exchanger. Moreover, LPS stimulated the expression of mature immunoglobulin on the cell surface (33, 35). Immunohistochemical studies (Fig. 5) were carried out to test the possibility that LPS may affect the cell surface expression of ICAM-1, a glycoprotein belonging to the immunoglobulin superfamily (41).

Subconfluent, rapidly proliferating cells (Fig. 5A and B) or confluent, quiescent cells (Fig. 5C and D) were incubated in the last 4 days of growth with or without LPS-5 (25 μg/ml). The cells were then fixed and prepared for immunohistochemistry as described in detail in Materials and Methods. Two types of staining were observed: (i) clusters of stained material at the circumference of the cells (arrowheads in Fig. 5A and B), indicating the presence of ICAM-1-containing structures at the surface of rapidly proliferating UT cells but not of quiescent cells (Fig. 5C and D) and (ii) diffuse intracellular staining as a result of interaction of the anti-ICAM-1 antibody with the interior of the permeabilized cells.

To quantify the amount of staining, I focused on the clusters of ICAM-1-containing material at the cell surface of rapidly proliferating cells, since these cell surface structures were the most relevant for a possible role of the UT cell ICAM-1 in cell-cell interactions. To ascertain that the clusters observed are not an artifact of the permeabilization process, rapidly proliferating, nonpermeabilized, LPS-treated and untreated cells were also probed with the antibody against ICAM-1. A pattern of clustering similar to that in the respective permeabilized samples was observed (results not shown). One hundred randomly chosen cells from untreated cultures and cultures treated with LPS-5 (25
µg/ml) were examined, and the number of cells displaying specific numbers of clusters of ICAM-1-containing material on the cell surface was counted. The results shown in Fig. 6 are the number of cells expressing the respective number of ICAM-1-containing clusters as a percentage of the total number of cells examined. Most of the cultured UT cells did not express any cell surface clusters of ICAM-1-containing material. However, there were significantly fewer nonexpressing UT cells in the LPS-treated cell sample (71.7% ± 2.9%) than in the untreated sample (85.7% ± 2.1%).

The untreated, rapidly proliferating cell population that expressed cell surface ICAM-1 clusters displayed a Gaussian distribution with respect to the number of clusters per cell (Fig. 6), with a mean of three clusters per cell. The percentage of cells expressing three, four, or five clusters per cell was 2.2 times higher in cells treated with LPS-5 than in untreated cells. A significant number of LPS-treated cells expressed 8 to 10 clusters per cell, whereas no untreated cells did (Fig. 6).

It was possible that the spatial redistribution of ICAM-1 on the UT cell surface was the result of cytoskeletal reorganization in the presence of the toxin. To test this possibility, LPS-treated cells were stained with rhodamine-conjugated phalloidin (Fig. 7). Whereas control cells displayed a fine pattern of actin (Fig. 7A), thick bundles of actin were observed in cells treated with LPS (Fig. 7B).

**DISCUSSION**

Gram-negative infection with *E. coli* induces an inflammatory response which is caused, in part, by the LPS component of the outer membrane (25). Effector mechanisms that mediate host resistance to *E. coli* infection have been studied in an ascending in vivo urinary tract infection model. In contrast to models of systemic infection, this model mimicked natural infection in that bacteria had to overcome the mucosal barrier before reaching the tissues and activating host defenses (19). In vivo studies carried out with this model and normal mice, as well as with mice containing a mutation which renders them nonresponsive to LPS, have suggested that the capacity of the animal to react with LPS is necessary for the induction of local inflammatory response as well as for the clearance of bacteria following infection. For example, LPS responder mice reacted to infection by a significant increase in the number of urinary leukocytes, whereas LPS nonresponders did not. These studies supported the role of LPS in the recruitment of leukocytes to the urinary tract as a first, early line of defense, in contrast to a later antibody- or T-cell-mediated response (37). By their very nature, these in vivo studies could not completely distinguish systemic mechanisms of LPS action from local effects at the mucosal epithelium. In the present study, epithelial cells isolated from the human ureter (14, 46) served as a model system with which to explore the mechanisms of *E. coli* and *E. coli* LPS action in cell culture.

As shown previously in in vitro studies with cultured intestinal epithelial cells (29), unequivocal proof for *E. coli* adherence to UT cells was obtained by transmission electron microscopy studies (Fig. 1), in which *E. coli* were shown to adhere to microvilli on the apical side of the cells (14). The main objective of the present study was to test the possibility that *E. coli* and the LPS they produce affect the function of UT cells. The epithelium of the lower urinary tract is lined by a mucous layer of acidic sulfated glycoconjugates (45) that serve as a defense barrier. Since previous studies with human lung fibroblasts had shown that DIDS-sensitive sulfate uptake at the cellular plasma membrane, i.e., sulfate uptake via a carrier-mediated system (11), was a limiting factor for glycoconjugate sulfation (12), the possibility that *E. coli* affects this transport system in UT cells was tested. Incubation of UT cells with *E. coli*, which possesses the ability to adhere to UT (Fig. 1), inhibited DIDS-sensitive sulfate uptake to half of that in the untreated UT cells (Fig. 2).

The possibility existed that the *E. coli* effect on sulfate uptake in UT cells was mediated by LPS, macromolecules bound to the surface of these gram-negative bacteria. This possibility was supported by the fact that incubation of UT cells with LPS inhibited DIDS-sensitive sulfate uptake (Fig. 3), and the maximal inhibition observed in the presence of LPS was similar to that caused by whole *E. coli* (Fig. 2).

In conclusion, *E. coli* and LPS inhibited carrier-mediated sulfate uptake in UT cells, suggesting that the intracellular pool of sulfate available for sulfation may be lower in infected cells and may lead to the production of undersulfated glycoconjugates in UT cells. Further studies will be necessary to test the latter possibility in these epithelial cells.

Studies with pre-B lymphocytes have demonstrated that treatment with LPS causes an increase in the cellular Na⁺ content as a result of the activation of an Na⁺/H⁺ exchanger. Stimulation of the expression of a cell surface immunoglobulin follows (32-35). I postulated that LPS may have a similar effect on Na⁺ content in UT cells and that this increase (or changes in ionic gradients coupled to it, e.g., pH and Ca²⁺) may serve as a signal that affects the expression of a member of the immunoglobulin superfamily, ICAM-1, on the surface of UT cells. ICAM-1 is expressed in a variety of cells (2, 3, 9, 15, 20, 22, 23, 40) and has been shown to regulate LFA-1-mediated leukocyte adhesion to target cells when rapid and effective yet controlled responses to immune or inflammatory stimuli are required (7, 10).

Treatment of rapidly proliferating UT cells with LPS resulted in a transient increase in the cellular Na⁺ content, which is consistent with the possibility that Na⁺/H⁺ ex-
FIG. 7. Effect of LPS-5 on actin organization in UT cells. Cells were incubated in the last 4 days of their growth without (A) or with (B) 25 μg of LPS-5 per ml. The cells were then fixed and probed with rhodamine-phalloidin as described in Materials and Methods.
change was being activated in UT cells. LPS had no effect on the Na⁺ content in quiescent cells (Fig. 4), LPS are amphiphilic, i.e., they consist of a hydrophilic polysaccharide and a hydrophobic lipid A domain (17). I speculate that, via its polysaccharide domain, LPS may interact with rapidly proliferating epithelial cells, e.g., in a wound, in a process that mimics their interaction with extracellular matrix glycoproteins. It may not be coincidental that, as in the case of LPS (34) (Fig. 4), adhesion of anchorage-dependent cells to fibronectin via integrin α5β1 (21) or to fibrinogen, collagens, laminin, and vitronectin (36) also leads to an increase in Na⁺ content and intracellular pH by activating an Na⁺/H⁺ exchanger. Physiological alterations of cytoplasmic pH after adhesion have been shown to correlate directly with the effect of fibronectin on DNA synthesis (21). It is possible that indirect activation of an Na⁺/H⁺ exchanger by LPS may mimic this signalling pathway, producing a differentiation-modulating effect in rapidly proliferating UT cells. This has been shown to be the case in studies in which LPS induced the expression of an immunoglobulin in IgM⁺ pre-B lymphocytes and caused them to differentiate to a more mature IgM⁺ lymphocyte (35).

To test the possibility that LPS affects the expression of ICAM-1, UT cell treated with LPS for 4 days were probed for ICAM-1. Diffuse staining with an anti-ICAM-1 antibody could be detected in the perinuclear region of quiescent, permeabilized UT cultures (Fig. 5C), and treatment with LPS had no visible effect (Fig. 5D). In contrast to the quiescent cells, some of the ICAM-1 had redistributed from the intracellular pool to clusters on the cell surface in a few cells of a rapidly proliferating untrated culture (Fig. 5A), possibly focal adhesions. Treatment with LPS stimulated this process of clustering to a significant extent (Fig. 5B and 6). A similar effect has been reported recently in the presence of proinflammatory cytokines, which caused a significant increase in the expression of ICAM-1 on the surface of human airway epithelial cells (44).

The fact that LPS affected the spatial distribution of actin in UT cells (Fig. 7) suggested that actin may be involved in LPS-stimulated clustering of ICAM-1 on the surface of UT cells. This possibility is consistent with recent studies demonstrating that ICAM-1 associates with actin-containing cytoskeleton and α-actinin (4), a cytoskeletal protein localized at focal adhesions. In conclusion, my studies with UT cells suggest that under normal circumstances, ICAM-1 may not be expressed on the surface of UT cells lining the intact mucosa in vivo. However, under conditions of injury to the epithelial layer, when cells proliferate rapidly, ICAM-1 clusters on the surface of the urotheleum, and this clustering is stimulated in the presence of LPS, allowing a more effective binding of leukocytes to the epithelial cells. The stimulation of ICAM-1 expression by LPS in UT cells may be a mechanism underlying earlier findings in vivo indicating a role for LPS in the recruitment of leukocytes to the urinary tract as a first, early line of defense following urinary tract infection (37). Alternatively, since induction of ICAM-1 on monocytes and potentially other cell types has been implicated in enhanced antigen-presenting capacity (38), the expression of ICAM-1 on the surface of UT cells exposed to LPS may be involved in autoimmune reactions following infection in the lower urinary tract.

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