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Role of Epithelial Interleukin-8 (IL-8) and Neutrophil IL-8 Receptor A in *Escherichia coli*-Induced Transuroepithelial Neutrophil Migration

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***Escherichia coli* stimulates neutrophil migration across human uroepithelial cell layers. This study investigated the role of the neutrophil chemokine interleukin-8 (IL-8) in this process. *E. coli* and IL-1α stimulated urinary tract epithelial layers to secrete IL-8 and induced transepithelial neutrophil migration. Anti-IL-8 antibody reduced neutrophil migration across epithelial cell layers, indicating a central role for this chemokine in the migration process. Furthermore, addition of recombinant IL-8 to unstimulated cell layers was sufficient to induce migration. The IL-8 dependence of neutrophil migration was maintained after removal of soluble IL-8 by washing of the cell layers. Flow cytometry analysis with fluorescein isothiocyanate-labelled IL-8 confirmed IL-8's ability to bind to the epithelial cell surface. Indirect immunofluorescence with confocal laser scanning microscopy showed that IL-8 associated with the epithelial cell layers. Prior incubation of neutrophils with antibodies to IL-8 receptor A (IL-8RA) reduced neutrophil migration. Anti-IL-8 RB antibody had no effect on neutrophil migration. These results demonstrate that IL-8 plays a key role in *E. coli*- or IL-1α-induced transuroepithelial migration and suggest that epithelial cell-produced IL-8 interacts with IL-8RA on the neutrophil surface.**

Neutrophils are recruited to mucosal sites in response to bacterial infection (14). In order to reach the urinary tract neutrophils must leave the blood vessels, cross the submucosa to the epithelial lining, and finally migrate across the epithelium into the lumen. The molecular mechanisms of bacterially induced neutrophil migration across epithelial layers have been studied in vitro (24). We recently showed that *Escherichia coli* stimulates neutrophil migration across urinary tract epithelial cell layers in vitro (4) and that epithelial intercellular adhesion molecule 1 (ICAM-1) and neutrophil Mac-1 are involved in this process. Antibodies to ICAM-1, CD11b, and CD18 but not CD11a blocked up to 85% of the *E. coli*-induced neutrophil migration. The chemotactic signal involved in this process has not been defined.

Interleukin-8 (IL-8) is present at mucosal surfaces during bacterial infection and has been proposed to play an important role as a neutrophil chemoattractant at these sites (3, 11, 12, 20, 29, 31, 32). Bacterial infections of the human urinary tract stimulate local production of IL-8 and a rapid influx of neutrophils into the urine. A role for IL-8 neutrophil recruitment was suggested by the strong correlation of urinary IL-8 levels with urinary neutrophil numbers in individual patients (3). Furthermore, anti-IL-8 antibody reduced the in vitro neutrophil chemotactic activity of urine obtained from patients with urinary tract infection (UTI) by 50% (20).

IL-8 has been implicated in induction of neutrophil influx to other mucosal sites during infection (23). IL-8 levels correlated with neutrophil numbers in bronchoalveolar lavage fluids from patients with chronic airway disease or *Pseudomonas aeruginosa* infection, and IL-8 accounted for most of the in vitro chemotactic activity in sputum samples obtained from these patients (29, 32).

Bacterial pathogens stimulate epithelial cells in vitro to secrete proinflammatory cytokines including IL-8, suggesting that epithelial cells are an important early source of this chemokine at the onset of infection (2, 3, 13, 15, 17, 19, 23, 34). The aim of the present study was to examine the role of epithelial IL-8 and neutrophil IL-8 receptors in *E. coli*-induced neutrophil migration across urinary tract epithelial layers in vitro.

MATERIALS AND METHODS

Reagents. Tryptic soy agar (TSA) and fetal calf serum were purchased from Difco (Detroit, Mich.). Human recombinant IL-1α and the blocking monoclonal mouse anti-human IL-8 antibody DM-7 were purchased from Genzyme (Boston, Mass.). Human recombinant IL-8, monoclonal mouse anti-human IL-8 (subclone 4G9/A5/A7), and polyclonal rabbit anti-human IL-8 antibody were kindly supplied by Sandoz Forschungsinstitut (Vienna, Austria). Polyclonal rabbit anti-rat antibody, monoclonal mouse immunoglobulin G (IgG) negative control antibody, fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG $F(ab')_2$ antibody, and FITC-streptavidin were purchased from Dakopatts AB (Stockholm, Sweden). FITC-labelled IL-8 was prepared as previously described (5), with modifications to the procedure reported by Offord et al. (28). Monoclonal anti-IL-8 receptor A (anti-IL-8RA) antibody (clone 9H1) (8) and monoclonal anti-IL-8RB antibody (clone 10H2) (9) were kindly provided by Genentech (San Francisco, Calif.). RPMI 1640 and gentamicin were from Life Technologies AB (Täby, Sweden). Polymorphprep was from Nycomed Pharma AS (Oslo, Norway). Transwell inserts (24.5-mm-diameter polycarbonate membrane with 3-μm-diameter pores) were from Costar (Cambridge, Mass.).

Bacteria. *E. coli* Hu734 is a *lac* mutant of the wild-type pyelonephritis strain GR12 (serotype O75:K5:H−, *hly*$^+$ ColV$^+$) (35). The strain was maintained on TSA plates. For experiments, Luria broth was inoculated with colonies from TSA plates and incubated overnight at 37°C. Under these conditions *E. coli* Hu734 expressed both type 1 and P fimbriae.

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TABLE 1. The role of IL-8 in *E. coli*- and IL-1α-induced transepithelial neutrophil migration

Antibody	% Neutrophil migration with[a]:		
	Medium	*E. coli*	IL-1α
None[b]	25.5 (3.9)	64.6 (5.2)	74.0 (5.5)
Polyclonal anti-IL-8	18.9 (1.2)	26.1 (3.2)	19.3 (3.0)
Control[c]	33.9 (2.7)	59.6 (3.2)	59.6 (3.9)
None[b]	22.7	71.2	82.4
Monoclonal anti-IL-8	6.7	18.7	16.1
Control[d]	22.7	61.5	68.2

[a] Values are means (SE) of seven separate experiments. Neutrophil migration ($t = 3$ h) across A-498 kidney epithelial cell layers prestimulated for 24 h with *E. coli* (10^8 bacteria/ml), IL-1α (1ng/ml), or medium was determined.

[b] The differences between the two sets of results with no antibody may be due to experimental variation in that these experiments were carried out on separate occasions.

[c] Rabbit anti-rat control antibody.

[d] Mouse IgG_1 irrelevant control antibody.

Cells. The A-498 kidney epithelial cell line (ATCC HTB44) was grown in cell culture medium (RPMI 1640 supplemented with 0.05 mg of gentamicin/ml, 2 mM glutamine, and 5% fetal calf serum) as previously described (15). Neutrophils were isolated from peripheral blood of healthy human volunteers (4). Briefly, heparinized blood was run on a Polymorphprep density gradient according to the manufacturer's instructions. Osmolarity was restored to the neutrophil suspension by the addition of an equal volume of 0.45% NaCl, and the remaining erythrocytes were removed by hypotonic lysis. The resulting cell suspension contained >97% neutrophils as determined by Wright's Giemsa stain and was 98% viable as determined by trypan blue exclusion.

Preparation of epithelial cell layers. Confluent A-498 epithelial cell layers were prepared on transwell inserts as previously described (4). Briefly, 1 ml of cells (~3×10^5 cells/ml) were seeded onto inverted transwell inserts and allowed to settle for 6 h at 37°C in a 5% CO_2 atmosphere. The remaining medium was then removed, and the transwell inserts were moved to cluster plates containing fresh medium. Plates were incubated at 37°C in a 5% CO_2 atmosphere until the cell layers were fully confluent (~14 days). When grown under these conditions, A-498 cells formed confluent nonpolarized layers on both sides of the transwell filter (4).

Neutrophil transepithelial migration assay. The neutrophil transmigration assay was performed as previously described (4). Briefly, 1.5 ml of fresh culture medium was added to the top well, and fresh medium or medium containing stimulant (*E. coli*, 10^8 bacteria/ml; IL-1α, 1 ng/ml) (2.7 ml) was added to the bottom well of transwell inserts. The cluster plates were then incubated at 37°C in a 5% CO_2 atmosphere for 24 h (a time previously shown to induce maximal neutrophil migration across A-498 cell layers [4]). Neutrophils (1.5×10^6 to 3×10^6) were added to the top well, samples (100 μl) were taken from the bottom well after 3 h, and neutrophil numbers were counted in a Bürker chamber. Migration was expressed as a percentage of the neutrophils added to the top well.

Antibodies to IL-8 were used to examine the role of IL-8 for bacterially induced neutrophil migration. After prestimulation, medium was removed from the top well and replaced with medium with or without antibody (polyclonal rabbit anti-human IL-8 antibody, monoclonal mouse anti-human IL-8 antibody [DM-7], polyclonal rabbit anti-rat control antibody, or monoclonal mouse IgG negative control antibody) (10 μg/ml) (4). At the same time antibody (10 μg/ml) was added to the bottom well medium.

To examine the role of IL-8 receptors in transepithelial neutrophil migration, neutrophils were incubated with anti-IL-8RA (clone 9H1) and anti-IL-8RB (clone 10H2) antibodies for 30 min at 37°C, washed twice in medium to remove excess antibody, and added to the top well of transwells as described above. Transmigration was quantitated as described above.

IL-8 analysis. IL-8 levels were quantitated by enzyme-linked immunosorbent assay (R&D, Barton Lane, United Kingdom) according to the manufacturer's instructions.

Confocal microscopy. Epithelial cell layers were grown to confluence on transwell inserts and stimulated with *E. coli* (10^8/ml) or IL-1α (1 ng/ml) for 24 h. Cell layers were washed in cell medium and fixed with 4% paraformaldehyde for 10 min. Paraformaldehyde was removed from the cell layers by two washes in phosphate-buffered saline (PBS). The polycarbonate filters were cut away from the transwell inserts, divided into two strips, and placed into 24-well plates (Falcon, Lincoln Park, N.J.) containing PBS with 0.3% bovine serum albumin (PBS-BSA) and primary antibody (monoclonal mouse anti-human IL-8 antibody or mouse IgG negative control antibody) (1 μg/ml) for 45 min at room temperature. Filter strips were washed four times in PBS, placed into new wells containing PBS-BSA and FITC-conjugated goat anti-mouse IgG $F(ab')_2$ antibody (1/10 dilution), and incubated for 45 min in the dark at room temperature. Staining of DNA was performed by incubating epithelial layers for 15 min with PBS containing Triton-X (0.1%) with propidium iodide (1 mg/ml) at 37°C in the dark. Finally, filters were air dried on glass slides and examined with an MRC 1024 confocal laser scanning microscope (Bio-Rad, London, England). To prevent UV light quenching, buffered glycerol (ACO, Stockholm, Sweden) containing 2% 1, 4-diazobicyclo-2.2.2 octane (Sigma, St. Louis, Mo.) was used as a mounting medium.

Flow cytometry. A-498 epithelial cells were grown to confluence in 25-ml culture flasks (Falcon) at 37°C in a 5% CO_2 atmosphere. Cells were detached from the flasks by incubation with 10 mM EDTA in RPMI 1640 for 20 min at 37°C in a 5% CO_2 atmosphere. A cell pellet was obtained by spinning the cell suspension at 1,000 rpm (RP centrifuge; Hettich Rotana, Malmö, Sweden) for 10 min. The resulting cell pellet was suspended in 100 μl of RPMI 1640 containing 0.3% BSA (RPMI-BSA) and FITC-IL-8 (5) (10 μg/ml) or FITC-streptavidin (10 μg/ml) and incubated on ice for 90 min. Cells were washed twice in RPMI-BSA and analyzed by flow cytometry in an EPICS Profile II (Coulter, Miami, Fla.). In each sample, 5,000 cells were counted.

Statistics. One-way analysis of variance was used to compare experimental groups.

RESULTS

***E. coli* and IL-1α stimulate neutrophil migration across uroepithelial cell layers.** Confluent A-498 epithelial cell layers on transwell polycarbonate filters were stimulated by addition of *E. coli* (10^8 bacteria/ml) or IL-1α (1 ng/ml) to the lower well. After a 24-h preincubation, medium in the upper well was replaced by medium containing neutrophils, and passage across the epithelial layer was measured after 3 h. Exposure of the cell layers to *E. coli* and IL-1α increased neutrophil migration from 26% in the control medium to 65 and 74%, respectively (Table 1).

***E. coli* and IL-1α induce epithelial IL-8 secretion.** The IL-8 secretion by A-498 epithelial cells exposed to *E. coli* or IL-1α was quantitated by enzyme-linked immunosorbent assay. IL-8 levels secreted by *E. coli*-stimulated epithelial layers after 24 h were 5 ng/ml (standard error [SE], 0.8) and 6.5 ng/ml (SE, 1.1) in the bottom and top wells, respectively. The levels of IL-8 in the upper and lower wells were higher in cells stimulated with IL-1α (>80 ng/ml) than in cells exposed to *E. coli* ($P < 0.01$) (Fig. 1).

Anti-IL-8 antibodies block *E. coli*- and IL-1α-induced transuroepithelial neutrophil migration. Addition of polyclonal anti-IL-8 antibody to the transwell medium reduced neutrophil migration across *E. coli*- and IL-1α-stimulated epithelial layers to background levels (Table 1). Similar results were obtained with monoclonal anti-IL-8 antibody DM-7 (Table 1). Thus, IL-8 produced by the stimulated cells appeared to be functional and responsible for the increased neutrophil migration across *E. coli*- and IL-1α-stimulated cell layers. In all experiments using control antibody it was noted that there was a

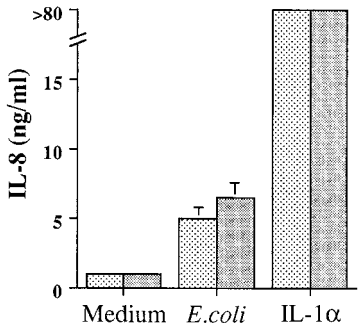

FIG. 1. IL-8 response of A-498 cells to stimulation with *E. coli* or IL-1α. Values are means (and SE) of three experiments. The background levels after 24 hours were 0.91 (SE, 0.17) and 0.98 ng/ml (SE, 0.09) in the bottom and top wells, respectively. ▥, top well; ▨, bottom well.

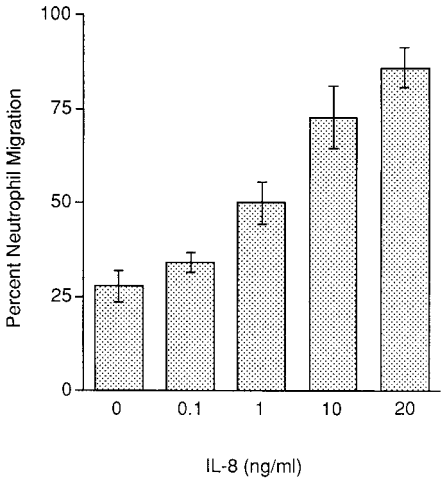

FIG. 2. Neutrophil migration (3 h) across confluent layers of the A-498 kidney epithelial cell line after addition of increasing concentrations of IL-8 to the bottom compartment. Values are means of four experiments. Error bars indicate SE.

slight reduction in neutrophil migration, which may have been due to nonspecific interference by the antibodies.

Recombinant IL-8 induces transuroepithelial neutrophil migration. The ability of IL-8 to support neutrophil migration across uroepithelial cell layers was further studied with recombinant IL-8. IL-8 (0.1 to 20 ng/ml) was added to the bottom wells of unstimulated cell layers, and neutrophils were added to the top wells. IL-8 induced a dose-dependent increase in neutrophil transmigration, with a maximum increase of 86% at 20 ng/ml (Fig. 2). The dose-related increase in migration was observed at IL-8 concentrations between 0.1 and 10 ng/ml with little additional migration at IL-8 concentrations above 10 ng/ml.

Cell-bound versus soluble IL-8. To examine the role of secreted versus cell-bound IL-8 in transepithelial neutrophil migration, cell layers prestimulated with *E. coli* or IL-1α for 24 h were removed from their cluster plates, washed three times in fresh medium, and transferred to new cluster plates containing fresh medium. Washing reduced the levels of soluble IL-8 in the top and bottom wells to below 0.03 ng/ml for *E. coli*-stimulated cell layers and to 0.16 and 0.07 ng/ml in the top and bottom wells, respectively, for IL-1α-stimulated cell layers. The removal of soluble IL-8 did not reduce neutrophil migration (Table 2), which was inhibited by monoclonal anti-IL-8 antibody (DM-7) from approximately 70 to 15% (Table 2). These results suggest that IL-8 was acting while bound to the epithelial cell surface.

TABLE 2. The role of cell-bound IL-8 in *E. coli*- and IL-1α-induced transepithelial neutrophil migration

Antibody (10 μg/ml)[b]	% Neutrophil migration with[a]:		
	Medium	*E. coli*	IL-1α
None	18.4 (3.4)	63.5 (3.5)	76.2 (1.7)
Monoclonal anti-IL-8	12.3 (1.0)	14.7 (3.4)	15.5 (1.5)
Control antibody	17.1 (2.6)	48.9 (3.6)	52.1 (5.2)

[a] Neutrophil migration (t = 3 h) across A-498 kidney epithelial cell layers prestimulated for 24 h with *E. coli* (10^8 bacteria/ml), IL-1α (1 ng/ml), or medium. Prestimulated cell layers were washed in PBS to remove unbound IL-8 and transferred to new cluster plates containing fresh medium. Results are the means (SE) of three separate experiments.

[b] Cell layers were exposed to medium, DM-7 monoclonal anti-IL-8, or irrelevant IgG control antibody.

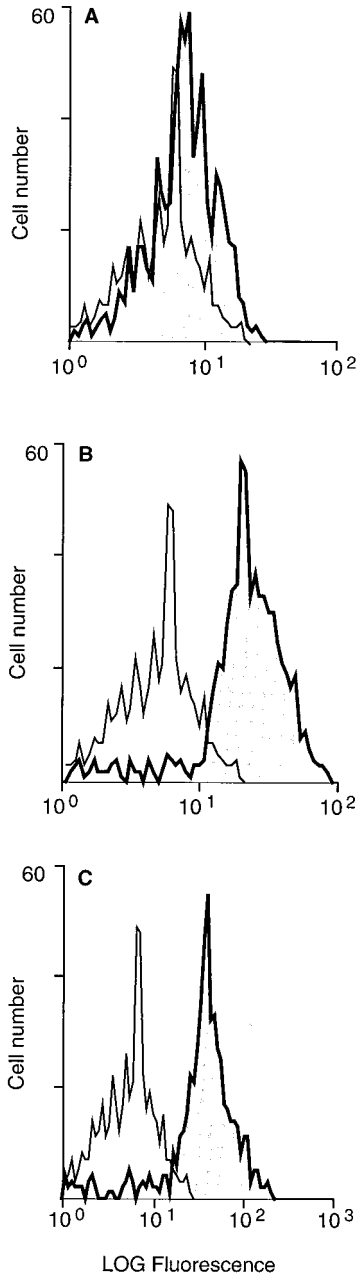

FIG. 3. FITC-IL-8 binding to A-498 epithelial cells as determined by flow cytometry analysis of unstimulated cells (A), IL-1α-stimulated cells (B), and *E. coli*-stimulated cells (C). Areas under the thin line denote the FITC-streptavidin background and areas under the thick line denote the binding of FITC-IL-8.

The ability of IL-8 to bind to urinary tract epithelial cells was examined by flow cytometry with FITC-labelled IL-8 (10 μg/ml). FITC-streptavidin was used as a control. Unstimulated epithelial cells bound more FITC-labelled IL-8 than FITC-streptavidin control (Fig. 3A). Prior stimulation of the epithelial cells with *E. coli* (10^8 bacteria/ml) or IL-1α (1 ng/ml) for 24 h increased the binding of FITC-labelled IL-8 to the epithelial cells (Fig. 3B and C).

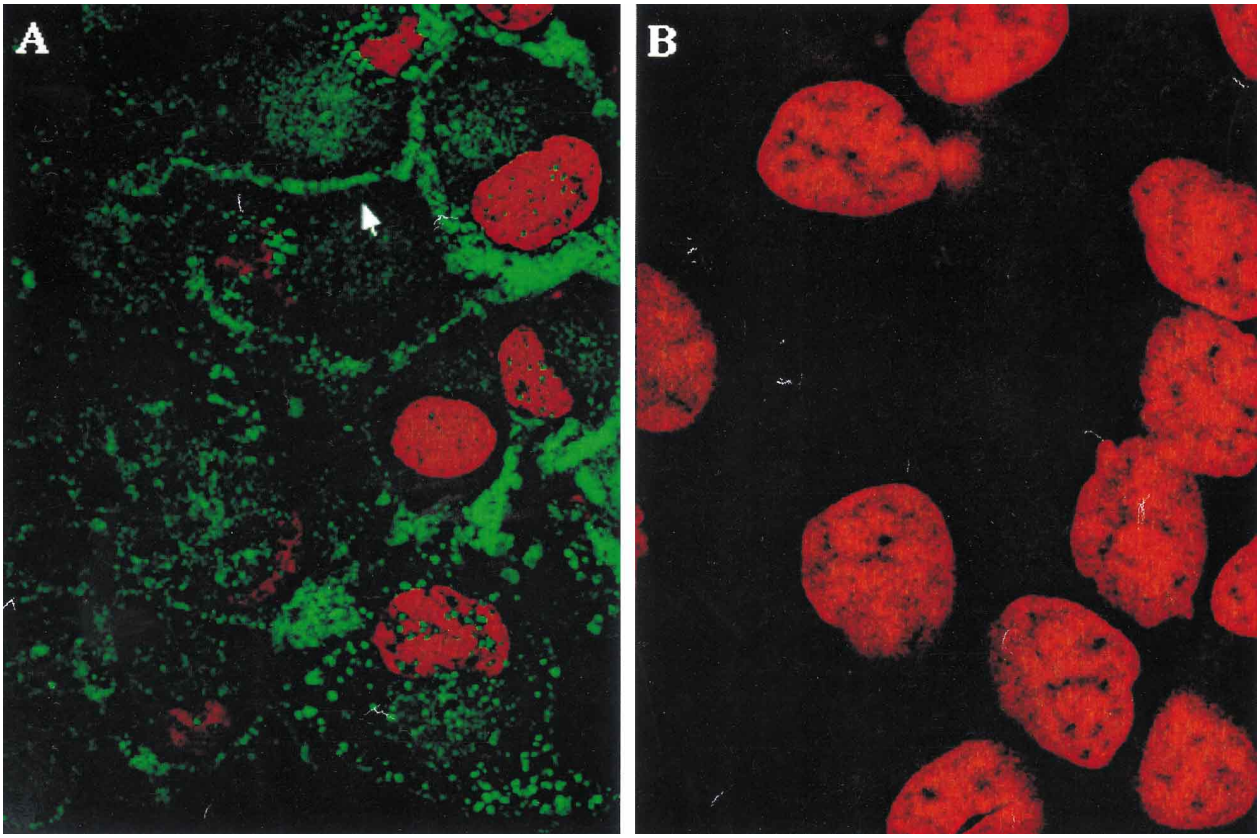

FIG. 4. Localization of IL-8 within A-498 epithelial cell layers. Confluent epithelial cell layers were stimulated for 24 h with IL-1α. (A) Staining with monoclonal anti-IL-8 antibody. (B) Staining with control antibody. Cell nuclei were stained with propidium iodide. The arrow depicts localization of IL-8 in the epithelial layer.

Attempts were made to localize IL-8 within epithelial cell layers stimulated with *E. coli* and IL-1α by using indirect immunofluorescence with monoclonal mouse anti-human IL-8 antibody. Figure 4 illustrates the presence of IL-8 in the epithelial cell layers. Additional granular staining was observed within the cells. There was no staining with the mouse IgG_1 control antibody.

Neutrophil receptors and neutrophil migration. The neutrophil IL-8 receptor involved in transepithelial migration was studied with monoclonal antibodies to IL-8RA and IL-8RB. Neutrophils were incubated for 30 min with antibodies to IL-8RA or IL-8RB, washed free of excess antibody, and added to *E. coli*- or IL-1α-stimulated cell layers. Anti-IL-8RA antibody reduced transepithelial neutrophil migration across *E. coli*- and IL-1α-stimulated epithelial layers from 74 to 41% and from 74 to 34%, respectively (Table 3). Incubation of the neutrophils with antibodies to IL-8RB did not inhibit migration.

DISCUSSION

IL-8 is a member of the α chemokine (C-X-C) family of cytokines whose members include NAP-2, PF-4, PBP, CTAP-111, ENA-78, *Gro*-α, *Gro*-β, and *Gro*-γ. It is produced by most cells, including lymphocytes, neutrophils, endothelial cells, fibroblasts, and epithelial cells (6). The primary target for IL-8 appears to be the neutrophil. In vitro, IL-8 induces neutrophil respiratory burst, granule release, and chemotaxis through numerous extracellular matrices including fibrin gels, matrigel (reconstituted basement membrane proteins), fibronectin, fibrinogen, and collagen IV (7, 22, 37). Furthermore, endogenous IL-8 secreted by activated endothelial layers supports transendothelial neutrophil migration (18).

This study examined the role of epithelial-derived IL-8 in *E. coli*- or IL-1α-induced neutrophil migration across confluent layers of the A-498 kidney cell line. Both agonists stimulated epithelial cell layers to secrete IL-8 during the 24-h period preceding the addition of neutrophils. Polyclonal and monoclonal IL-8 antibodies completely blocked *E. coli*- or IL-1α-induced neutrophil migration, suggesting that IL-8 was the major chemoattractant inducing transepithelial neutrophil migration. Further evidence for a role of IL-8 in transuroepithelial neutrophil migration was obtained with recombinant protein. Recombinant IL-8 induced neutrophil migration across unstimulated cell layers at concentrations similar to those secreted by stimulated epithelial layers. IL-8-dependent

TABLE 3. Role of neutrophil IL-8RA and IL-8RB in migration across *E. coli*- and IL-1α-stimulated urinary tract epithelial layers

Antibody (10 μg/ml)[b]	% Neutrophil migration with[a]:		
	Medium	*E. coli*	IL-1α
Monoclonal anti IL-8 RA	11.6 (0.7)	41.5 (4.4)	33.9 (5.4)
Monoclonal anti-IL-8 RB	16.9 (1.8)	66.9 (2.9)	80.2 (6.0)
Medium control	18.8 (1.8)	74.3 (2.8)	73.7 (8.2)

[a] Neutrophil migration (3 h) across A-498 kidney epithelial cell layers prestimulated for 24 h with *E. coli* (10^8 bacteria/ml), IL-1α (1 ng/ml), or medium. Results are the means (SE) of three separate experiments.

[b] Neutrophils were incubated for 30 min with each antibody or with medium prior to addition to the A-498 cell layers.

neutrophil migration occurred across *E. coli*- and IL-1α-stimulated epithelial layers after removal of soluble IL-8 and was blocked with monoclonal anti-IL-8 antibody, suggesting that soluble IL-8 was not involved in the migration process. Indirect immunofluorescence staining of IL-8 layers showed IL-8 to be present throughout the cell layer and to be associated with the epithelial cell surface. Flow cytometry analysis with FITC-IL-8 showed that IL-8 could bind to the epithelial cell surface and that stimulation with *E. coli* and IL-1α increased FITC-IL-8 binding, indicating upregulation of receptor expression (1). Interestingly, McCormack and coworkers recently demonstrated that *Salmonella typhimurium* stimulated intestinal T84 epithelial cells to imprint a chemotactic gradient onto a subepithelial matrix in vitro. Neutrophil transmatrix migration was blocked with anti-IL-8 antibody (25). However, IL-8 was not involved in neutrophil migration across T84 epithelial layers (24). The present study shows the ability of IL-8 to bind directly to urinary tract epithelial cells. This suggests that urinary tract epithelial cells are able to present IL-8 to migrating neutrophils.

Two high-affinity neutrophil IL-8 receptors, IL-8RA and IL-8RB, have been cloned and extensively characterized (16, 27). These receptors are members of the seven transmembrane G protein-linked receptor family with 77% amino acid sequence homology (16, 27). While IL-8RA appears to be a specific receptor for IL-8, IL-8RB binds additional members of the α chemokine family (NAP-2, *gro*, and MGSA) with high affinity (21, 26). We used monoclonal antibodies to IL-8RA (8) and IL-8RB (9) to examine the relative roles of these receptors in transepithelial neutrophil migration. The results show that IL-8RA is involved in *E. coli*- and IL-1α-induced neutrophil migration across urinary tract epithelial layers. Similar results were obtained in studies examining the presence of IL-8RA and IL-8RB on neutrophils in IL-8-induced migration across polycarbonate membranes (8, 9). Monoclonal antibody to IL-8RA blocked the majority of IL-8-induced neutrophil migration across polycarbonate filters, while only a minor portion of chemotaxis was blocked with antibodies to IL-8RB. In contrast, neutrophil migration in response to *gro*-α was blocked with antibody to IL-8RB but not with antibody to IL-8RA. The lack of effect of IL-8RB antibody in the present study suggested that other chemokines which bind with high affinity to IL-8RB and induce neutrophil migration via this receptor are not involved in neutrophil migration in our model.

The nature of the role of the epithelial IL-8 receptor(s) in neutrophil migration in UTI remains to be determined. Preliminary data has shown that the A-498 kidney and J82 bladder epithelial cell lines constitutively express IL-8RA and IL-8RB mRNA and that more IL-8RA than IL-8RB protein is detected on the epithelial surface following stimulation with IL-1α or *E. coli* (1). However, antibodies to IL-8RA and IL-8RB were unable to block FITC-IL-8 binding to the epithelial surface, suggesting that these receptors are not involved in IL-8 presentation to neutrophils (13a). Proteoglycans represent a second class of chemokine receptors that present chemokines at the endothelial surface to neutrophils in the circulation (33, 36) and enhance the activity of IL-8 for neutrophils (38). The role of proteoglycans in the present model is under further investigation.

The infection-related recruitment of neutrophils into the urine is often called pyuria and has been used to diagnose UTI. We propose the following molecular model of *E. coli*-induced neutrophil migration during UTI. *E. coli* first stimulates mucosal cells to secrete cytokines including IL-8 (3, 4). The degree of IL-8 secretion is influenced by the virulence of the infecting strain (3). A chemotactic gradient is formed, and neutrophils migrate from the vasculature to the subepithelial tissue. This gradient is likely to involve IL-8 since staining of human urinary tract mucosa has shown IL-8 to be present during inflammation (13b). The results of the present study suggest that mucosal IL-8 is able to bind to urinary tract epithelial cells and is recognized by IL-8 receptors on neutrophils. As previously shown in this model, neutrophil Mac-1 and epithelial ICAM-1 expression are involved in migration across the urinary tract epithelium in vitro (4). A recently reported molecule, CD47, has been implicated in neutrophil migration across intestinal epithelial cell layers in vitro but its significance in the urinary tract has not yet been investigated (10, 30).

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