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Epithelial Cells Secrete the Chemokine Interleukin-8 in Response to Bacterial Entry

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Bacterial invasion of mucosal surfaces results in a rapid influx of polymorphonuclear leukocytes. The chemotactic stimulus responsible for this response is not known. Since epithelial cells are among the first cells entered by many enteric pathogens, we investigated the ability of epithelial cells to provide an early signal for the mucosal inflammatory response through the release of chemotactic cytokines. As shown herein, the chemokine interleukin-8 (IL-8), a potent chemoattractant and activator of polymorphonuclear leukocytes, was secreted by intestinal and cervical epithelial cells in response to bacterial entry. Moreover, a variety of different bacteria, including those that remain inside phagosomal vacuoles, e.g., *Salmonella* spp., and those that enter the cytoplasm, e.g., *Listeria monocytogenes*, stimulated this response. Increased IL-8 mRNA levels could be detected within 90 min after infection. Neither bacterial lipopolysaccharide nor noninvasive bacteria, including *Escherichia coli* and *Enterococcus faecium*, induced an IL-8 response. Moreover, tumor necrosis factor alpha, which is known to be expressed by some epithelial cells, was not detected in the culture supernatants after bacterial entry, and addition of anti-tumor necrosis factor alpha antibodies had no effect on the IL-8 response following bacterial entry. These data suggest the novel concept that epithelial cells serve as an early signaling system to host immune and inflammatory cells in the underlying mucosa following bacterial entry.

Mucosal surfaces are lined by epithelial cells that form a barrier between potentially pathogenic microorganisms and host tissues. Penetration of this layer by invasive bacteria leads initially to an acute inflammatory response, a hallmark of which is the local accumulation of polymorphonuclear leukocytes. The mechanisms which initiate this response are not well understood. We hypothesized that intestinal epithelial cells, the first host cells to come in contact with enteric pathogens, secrete a chemotactic mediator in response to bacterial entry.

The chemokines are a family of small polypeptides which have chemoattractant properties for inflammatory cells (22). The best-studied member of this group is interleukin-8 (IL-8), which is secreted by several cell types, including monocytes and macrophages, fibroblasts, endothelial cells, and keratinocytes (2, 10, 19, 22). The most important function of IL-8 is the attraction and activation of polymorphonuclear leukocytes. Other functions of IL-8 have been demonstrated, including chemotaxis of basophils (22) and a role in angiogenesis (14). Moreover, intradermal injection of IL-8 initiates an acute inflammatory response, which is characterized by the local accumulation of polymorphonuclear leukocytes (5, 16).

Recently, evidence has been presented that epithelial cells have the capacity to express and secrete several cytokines constitutively or after stimulation with the proinflammatory cytokines tumor necrosis factor alpha (TNF-α) or IL-1 (6, 15, 21, 25, 29, 30, 32). The importance of these findings to host defense is currently unclear. In the present study, we have examined the possibility that production of cytokines by host epithelial cells may be an important primary event in host defense by analyzing a model system in which epithelial cells are entered by pathogenic bacteria. We report herein that enteric and cervical epithelial cells upregulate steady-state levels of IL-8 mRNA and secrete IL-8 in response to bacterial entry. These findings suggest that epithelial cells are in integral component of an early signaling system important for the activation of immune and inflammatory cells in the underlying mucosa following bacterial entry.

MATERIALS AND METHODS

Cell lines. Human T_{84} colonic epithelial cells (20) were a gift from K. Dharmsathaphorn and were used between passage 16 and 35. Human HeLa cervical epithelial cells (ATCC CCL 2) and human WI-38 lung fibroblasts (ATCC CCL 75) were obtained from the American Type Culture Collection, Rockville, Md. Cells were grown in 50% Dulbecco's modified Eagle's medium (DME)–50% Ham's F12 medium, supplemented with 2 mM glutamine and 5% newborn calf serum (for T_{84} cells) or 10% fetal calf serum (for HeLa and WI-38 cells), at 37°C in a water-saturated atmosphere of 95% air and 5% CO_2.

To obtain polarized monolayers, T_{84} cells were seeded in Transwell cultures (6.5-mm-diameter polycarbonate microporous membranes with 3.0-μm pore size; Costar, Cambridge, Mass.) at 3×10^5 per well in a total volume of 1.2 ml (0.2 ml in the top reservoir; 1.0 ml in the bottom reservoir) and cultured for 7 days before infection. To assess monolayer integrity, 1×10^5 to 2×10^5 cpm of [^{3}H]mannitol (specific activity, 15 to 30 Ci/mmol) was added to the top reservoir, and radioactivities in the top and bottom reservoirs were separately determined after 3 h in culture.

Bacteria and cytokines. The following bacteria and cytokines were used in these studies: *Salmonella dublin* (3), *Yersinia enterocolitica* 08, *Shigella dysenteriae* (clinical isolate identified by the California State Health Department, Berkeley), *Escherichia coli* DH5α, *Listeria monocytogenes* 4b (ATCC 19115), *Enterococcus faecium* (ATCC 35667),

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TABLE 1. Bacterium-induced IL-8 secretion by human epithelial cells and fibroblasts[a]

Additions to cultures		IL-8 secreted (pg/ml)[b]		
Bacteria or cytokines[c]	Cell invasion	Epithelial cells		WI-38 lung fibroblasts
		T_{84} (colon)	HeLa (cervix)	
S. dublin	+	913 ± 47 (5)	2,922 ± 361 (13)	3,363 ± 641 (2)
L. monocytogenes	+	1,661 ± 228 (6)	6,065 ± 1,561 (10)	1,601 ± 142 (2)
Y. enterocolitica	+	238 ± 61 (4)	2,090 ± 530 (3)	4,553 (1)
S. dysenteriae	+	226 ± 35 (5)	3,647 ± 494 (3)	5,090 (1)
E. coli DH5α	−	<50 (5)	540 ± 104 (10)	515 ± 183 (4)
E. faecium	−	<50 (4)	<50 (1)	378 ± 5 (4)
LPS		<50 (6)	268 ± 72 (4)	331 ± 78 (5)
TNF-α		1,259 ± 164 (8)	4,704 ± 1,119 (10)	26,862 ± 4,836 (6)
None		<50 (8)	139 ± 29 (16)	265 ± 45 (6)

[a] Cultures of human epithelial cells or fibroblasts were infected with various bacteria as described in Materials and Methods, and after 4 h in culture, the concentration of IL-8 was determined in the supernatants.
[b] Results are means ± standard errors of the means of the values obtained from the number of determinations given in parentheses.
[c] LPS and TNF-α were used at 100 ng/ml.

Salmonella typhimurium (ATCC 14028), *E. coli* serotype O29:NM (ATCC 43892), *E. coli* serotype O157 (ATCC 43894), recombinant human TNF-α (Genentech, South San Francisco, Calif.), recombinant human IL-1α (Immunex Corporation, Seattle, Wash.), and bacterial lipopolysaccharide (LPS) from *E. coli* serotype O111 (Sigma Chemical Co., St. Louis, Mo.).

Infection protocol. Cells were seeded into 24-well Costar tissue culture plates at 0.5×10^5 to 3×10^5 per well in a 1-ml volume and allowed to adhere for 24 h. Salmonellae were grown at 37°C in Trypticase soy broth to late-log phase at which time they are maximally invasive (17). Yersiniae were grown in Trypticase soy broth at 22°C overnight, and listeriae and shigellae were grown in Trypticase soy broth at 37°C overnight. Cell cultures were infected with bacteria that had been washed three times and resuspended in 50% DME–50% Ham's F12 medium at a bacterium/cell ratio between 5:1 and 100:1 and further incubated for 2 h to allow bacterial entry to occur. After removal of the extracellular bacteria, the cultures were incubated for 4 h in the presence of 50 μg of gentamicin per ml to kill the remaining extracellular bacteria. Preliminary experiments had shown that this concentration of gentamicin reduces the number of extracellular bacteria from all strains used in these studies more than 10^5-fold within 2 h. Subsequently, the gentamicin was removed and the cells were lysed with 0.1% sodium dodecyl sulfate (SDS) in isotonic saline (for gram-negative bacteria) or distilled water (for gram-positive bacteria), and the number of released viable bacteria was determined. This treatment did not affect bacterial viability if bacteria were plated for enumeration within 1 h after lysis. Parallel cultures were stimulated with TNF-α, IL-1α, or LPS for 4 h before the IL-8 concentration was measured. In some experiments, polyclonal goat anti-human TNF-α antibodies (R & D Systems, Minneapolis, Minn.) were added to the cultures at 10 μg/ml during the 2-h infection period and during the subsequent 4-h culture period in the presence of gentamicin.

ELISA for IL-8 and TNF-α. The amount of IL-8 and TNF-α secreted into the supernatants was determined by an enzyme-linked immunosorbent assay (ELISA) using optimal concentrations of polyclonal goat anti-human IL-8 antibodies and goat anti-human TNF-α antibodies (R & D Systems), respectively, as capturing antibodies, polyclonal rabbit anti-human IL-8 antibodies and rabbit anti-human TNF-α antibodies (Endogen, Boston, Mass.), respectively, as detecting antibodies, and alkaline phosphatase-labeled monoclonal mouse anti-rabbit immunoglobulin G (Sigma) as a second-step antibody. Bound alkaline phosphatase was visualized with the substrate *p*-nitrophenylphosphate (Sigma). The sensitivities of the ELISA for IL-8 and TNF-α were 50 and 20 pg/ml, respectively. The bioactivity of IL-8 secreted by the employed cell lines was confirmed by measuring calcium mobilization in human neutrophils (36).

RNA extraction and Northern (RNA) blot analysis. RNA was extracted by using acid guanidinium thiocyanate-phenol-chloroform as described previously (4). Forty micrograms of total RNA was size fractionated on a formaldehyde-agarose gel, blotted onto nitrocellulose, and probed with a ^{32}P-labeled cDNA fragment of human IL-8 (18) and, after the blots were stripped, with a ^{32}P-labeled cDNA fragment of human glyceraldehyde-3-phosphate dehydrogenase (ATCC 57091). Hybridizations were performed at 42°C for 16 h with a solution of 50% formamide, 10% dextran sulfate, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 50 mM Na_2PO_4 (pH 7.0), 5× Denhardt's solution, 0.1% SDS, 250 μg of salmon sperm DNA per ml, and 5 μg of polyuridylic acid per ml. After hybridization, nonspecifically bound radioactivity was removed by washing the blots twice in 0.1× SSC–0.1% SDS at 60°C for 20 min each, after which the blots were exposed to X-ray film at −70°C using an intensifying screen.

RESULTS

Epithelial cells secrete IL-8 after exposure to invasive bacteria. As a source of human epithelial cells, we employed two cell lines, T_{84} colonic epithelial cells (20) and HeLa cervical epithelial cells. Monolayers of T_{84} or HeLa cells were infected with bacteria, and after 4 h of culture, IL-8 secretion was determined. Infection of T_{84} and HeLa monolayers with the gram-negative invasive bacteria *S. dublin*, *Y. enterocolitica*, and *S. dysenteriae* and the gram-positive invasive bacterium *L. monocytogenes* stimulated increased IL-8 secretion (Table 1). Of note, the number of intracellular *S. dublin*, *L. monocytogenes*, *Y. enterocolitica*, and *S. dysenteriae* bacteria recovered from T_{84} and HeLa cells were comparable in these experiments (data not shown). In contrast, unstimulated cells secreted little, if any, IL-8, and the addition to the monolayers of noninvasive bacteria, i.e., *E. coli* DH5α and *E. faecium*, had no significant effect on

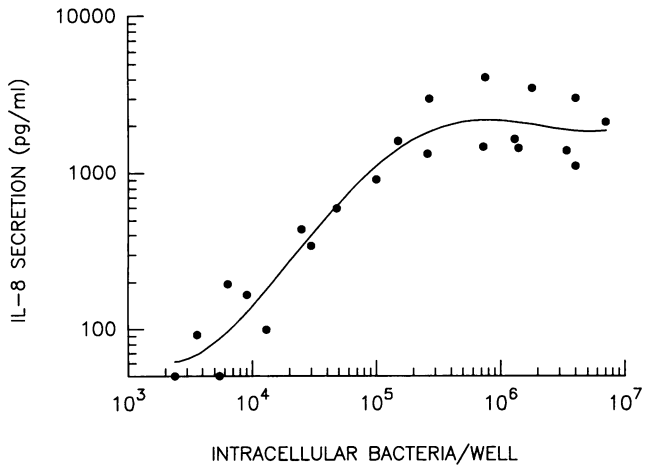

FIG. 1. Relationship of the number of intracellular *S. dublin* bacteria and IL-8 secretion in HeLa epithelial cells. Cultures of HeLa epithelial cells in 24-well plates (1 ml per well) were infected with various doses of *S. dublin* and incubated for 2 h to allow bacterial invasion to occur. After removal of the extracellular bacteria, the cultures were further incubated in the presence of gentamicin for an additional 4 h. At the end of the culture period, the concentration of IL-8 in the supernatant was determined, and the number of viable intracellular bacteria was determined after the HeLa cells were lysed. Values represent individual measurements from three independent experiments. Comparable results were obtained in three additional experiments using T_{84} cells.

IL-8 secretion, even when these bacteria were allowed to grow in the culture medium for the 4-h incubation period. Similar results were obtained after infection of WI-38 normal human lung fibroblasts (Table 1), showing that IL-8 secretion in response to bacterial entry is not limited to epithelial cells. Bacterial LPS did not stimulate IL-8 secretion by these cell lines, while TNF-α stimulated IL-8 secretion in all three cell lines (Table 1). As shown in Fig. 1 for HeLa cells infected with *S. dublin*, there was a direct relationship between the number of intracellular bacteria and the amount of IL-8 secreted into the supernatants. Comparable relationships between numbers of intracellular bacteria and level of IL-8 secretion were found for *S. dublin* LD842 (a virulence plasmid-cured strain) (3), *S. typhimurium*, *Y. enterocolitica*, *S. dysenteriae*, and *L. monocytogenes* 4b when T_{84} and HeLa cells were used (data not shown).

Bacterial entry is required for increased IL-8 secretion. To determine if cell entry was required for bacterium-induced IL-8 secretion, we employed an *S. dublin invA* mutant (SB133) that is isogenic with the parental *S. dublin* (8). The mutant attaches to epithelial cells but is far less efficient than the parental strain in entering epithelial cells (8). As shown in Table 2, the *invA* mutant of *S. dublin* entered HeLa epithelial cells 50-fold less efficiently than the parental *S. dublin* strain and did not induce IL-8 secretion, while the wild-type invasive strain stimulated IL-8 secretion efficiently. When cell entry by wild-type *S. dublin* was prevented by preincubating the monolayers with cytochalasin D, an agent that inhibits actin polymerization and bacterial entry (7), both bacterial entry and IL-8 secretion were markedly decreased (Table 2). We also compared IL-8 secretion after incubation of HeLa cells with an enteroinvasive *E. coli* (serotype O29:NM) with that after incubation with a noninvasive, enterohemorrhagic *E. coli* (serotype O157). HeLa cells in-

TABLE 2. Entry is required for bacterium-induced IL-8 secretion by epithelial cells[a]

Expt	Additions to cultures		No. of bacteria/well			IL-8 secreted (pg/ml)
	Bacteria	Other	Inoculum	Extracellular[b]	Intracellular[c]	
1	*S. dublin* (wild type)	None	1.5×10^8	4.2×10^8	2.2×10^6	1,434
	S. dublin (*invA*)	None	4.3×10^7	7.5×10^8	5.2×10^4	<50
	S. dublin (wild type)	Cytochalasin D[d]	1.5×10^8	4.0×10^8	2.0×10^5	229
	None	TNF-α[e]				1,657
	None	None				<50
2	*S. dublin* (wild type)	None	1.5×10^8		3.4×10^6	1,387
	S. dublin (*invA*)	None	4.3×10^7		5.0×10^3	57
	None	None				<50
3	*E. coli* O29:NM	None	2.4×10^5	2.1×10^8	3.1×10^5	1,155
	E. coli O157	None	2.8×10^5	3.5×10^8	3.5×10^4	<50
	None	IL-1α[f]				1,328
	None	None				<50
4	*E. coli* O29:NM	None	2.4×10^7		8.7×10^5	2,788
	E. coli O157	None	1.6×10^7		1.3×10^4	354
	None	TNF-α[e]				5,187
	None	None				188

[a] Subconfluent monolayers of HeLa epithelial cells in 24-well tissue culture plates were infected with *S. dublin*, an *invA* mutant of *S. dublin* (8), or *E. coli* at a bacterium/cell ratio of 1,000:1 and incubated for 4 h without antibiotics. The viability of HeLa cells was maintained despite the presence of more than 5×10^8 bacteria per well. At the end of the culture period, culture supernatants were removed and filtered, and the IL-8 concentration was determined by ELISA. Control experiments had shown that the IL-8 is stable in the presence of high numbers of the bacteria used in these experiments.

[b] At the end of the 4-h culture period, supernatants containing noninvaded (e.g., extracellular) bacteria were removed from each well, and the number of extracellular bacteria per well was determined.

[c] The cultures were washed after the 4-h culture period and further incubated in the presence of 50 μg of gentamicin per ml for an additional 30 min. Subsequently, the cells were lysed, and the number of viable intracellular bacteria was determined.

[d] Cultures were preincubated for 45 min with 2.5 μg of cytochalasin D per ml before infection. Cytochalasin D was present throughout the 4-h culture period. The addition of cytochalasin D to TNF-α-stimulated cells did not decrease the amount of IL-8 secreted.

[e] TNF-α was used at 100 ng/ml.

[f] IL-1α was used at 1 ng/ml.

TABLE 3. Bacterial infection and IL-8 secretion by polarized T_{84} monolayers[a]

Expt	Addition to culture	No. of bacteria/well[b]			IL-8 secreted (pg/well)[c]		
		Inoculum (apical)	Basolateral (2 h after infection)	Intracellular	Apical	Basolateral	Total
1	*S. dublin*	1.0×10^7	5.5×10^3	ND[d]	877	3,980	4,857
	L. monocytogenes	2.0×10^7	1.0×10^1	ND	897	6,738	7,635
	S. dysenteriae	2.0×10^6	0	ND	102	1,999	2,101
	None				74	753	827
2	*S. dublin*	4.8×10^6	1.3×10^3	8.5×10^4	295	1,203	1,498
	S. dublin invA	5.4×10^6	0	5.1×10^3	61	441	502
	E. coli DH5α	2.8×10^6	0	1.0×10^2	16	253	269
	None				13	256	269

[a] T_{84} cells were grown as confluent polarized monolayers on microporous membranes (Transwells; Costar). The formation of tight junctions between the cells was confirmed functionally by the lack of permeability to [^{3}H]mannitol added to the apical culture reservoir; less than 1% of the added [^{3}H]mannitol appeared in the basolateral reservoir after 3 h in control cultures. Bacteria were added to the apical reservoir, and the cultures were incubated for 2 h. Subsequently, the cultures were rinsed three times and further incubated in the presence of 50 μg of gentamicin per ml.

[b] The number of bacteria which had passed through the monolayers (e.g., bacteria in the basolateral reservoir) was determined 2 h after infection. The number of viable intracellular bacteria was determined 4 h after gentamicin addition (e.g., 6 h after infection) after the cells were lysed.

[c] The IL-8 concentration in the apical and basolateral media was measured at the end of the culture period (16 h in experiment 1; 4 h in experiment 2) and multiplied by the respective culture volume (0.2 ml for the apical reservoir; 1 ml for the basolateral reservoir).

[d] ND, not done.

cubated with the enteroinvasive strain of *E. coli* had 10- to 50-fold higher numbers of intracellular bacteria than those incubated with the noninvasive *E. coli* O157 (Table 2). Concomitantly, the invasive *E. coli* strain stimulated IL-8 secretion, while the noninvasive strain did not. These results indicate that entry is required for bacterium-induced IL-8 secretion by epithelial cells.

Bacterial entry at the apical surface of epithelial cells stimulates IL-8 secretion from the basolateral surface. Epithelial cells that line mucosal surfaces are polarized. At mucosal surfaces, initial bacterial entry occurs at the apical surface. To assess IL-8 secretion in response to entry of bacteria via the apical surface, T_{84} cells were grown as confluent polarized monolayers on microporous membranes (Transwells; Costar). Apical infection of the T_{84} monolayers with *S. dublin*, *L. monocytogenes*, and *S. dysenteriae* caused a substantial increase of total IL-8 secretion relative to that of uninfected control cultures (Table 3). More than 80% of the secreted IL-8 was recovered in the lower reservoir in these experiments, indicating that IL-8 was preferentially secreted at the basolateral surface. The monolayers remained intact during the 3-h incubation even though 0.03% of the inoculated *S. dublin* passed through the cells into the lower chamber. Additionally, we found that the *S. dublin invA* mutant entered the polarized T_{84} cells with less than 10% of the efficiency of the wild-type *S. dublin* and, in parallel, induced only a small increase in IL-8 secretion (Table 3).

Increased IL-8 secretion is paralleled by increased mRNA expression for IL-8. Increased secretion of IL-8 after bacterial entry was due to increased IL-8 synthesis, as shown in Fig. 2 for HeLa cells infected with *S. dublin*. Control cells did not express detectable levels of IL-8 mRNA by Northern blot analysis. IL-8 mRNA was detected within 90 min of infection with *S. dublin* or stimulation with the cytokine TNF-α. Maximal levels of IL-8 mRNA were seen by 3 h after infection. Despite continued stimulation and viability of the monolayers, IL-8 mRNA levels decreased after 5 h in both groups. Similar results were obtained when T_{84} cells were infected with *S. dublin* (data not shown).

Increased IL-8 secretion following bacterial entry is not mediated by secreted TNF-α. Epithelial cells from various organs, including intestine (6), kidney (13), ovary (31), and lungs (31), are known to express mRNA for TNF-α or secrete TNF-α, and, as shown above, TNF-α increased IL-8 secretion by T_{84} and HeLa epithelial cells. Thus, we asked if secretion of TNF-α is important for the IL-8 response of these cells after bacterial entry. In cultures of T_{84} cells that were infected with *S. dublin* or *L. monocytogenes*, TNF-α could not be detected (less than 20 pg/ml), whereas IL-8 was secreted at high levels (*S. dublin* infected, 3,905 pg/ml; *L. monocytogenes* infected, 6,510 pg/ml; unstimulated, 62 pg/ml). Similarly, no TNF-α was found in cultures of HeLa cells infected with *S. dublin* or *L. monocytogenes*, while IL-8 secretion was easily detectable (*S. dublin* infected, 41.3 ng/ml; *L. monocytogenes* infected, 32.2 ng/ml; unstimulated, 5.6 ng/ml). Furthermore, the addition of anti-TNF-α antibod-

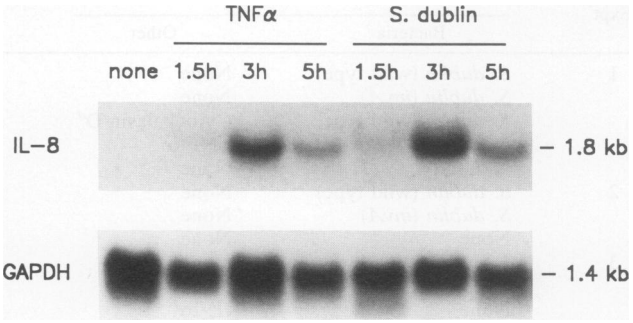

FIG. 2. Induction of IL-8 mRNA in HeLa epithelial cells after entry of *S. dublin*. Cultures of HeLa epithelial cells were infected with *S. dublin* at a bacterium/cell ratio of 50:1 and incubated for 30 min to allow bacterial entry to occur. After removal of the extracellular bacteria, the cultures were incubated in the presence of gentamicin for an additional 60 min (i.e., 1.5 h after infection), 2.5 h, and 4.5 h, after which RNA was extracted. Forty micrograms of total RNA per lane was size fractionated on a formaldehyde-agarose gel, blotted onto nitrocellulose, probed with a ^{32}P-labeled cDNA fragment for human IL-8 and, after the blots were stripped, a cDNA fragment for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a control, and exposed to X-ray film. Exposure times were 7 days for the IL-8-probed blot and 12 h for the GAPDH-probed blot. Parallel cultures were stimulated with 100 ng of TNF-α per ml for the indicated periods of time. Similar results were obtained with T_{84} cells.

ies (10 μg/ml) to cultures of T_{84} or HeLa cells had no effect on increased IL-8 secretion following bacterial entry or on the number of intracellular *S. dublin* or *L. monocytogenes* bacteria. The same concentration of anti-TNF-α antibodies completely blocked the IL-8 response of T_{84} cells to TNF-α (10 ng/ml) added to the cultures.

DISCUSSION

These studies demonstrate that human intestinal and cervical epithelial cells secrete IL-8 in response to bacterial entry. Bacterial LPS was not the stimulus for IL-8 secretion since none of the cell lines produced IL-8 in response to LPS stimulation, and IL-8 secretion was stimulated by *L. monocytogenes*, a gram-positive bacterium. Moreover, the intracellular location of the invading bacteria does not appear to be a critical factor for IL-8 secretion since shigellae and listeriae enter and grow in the cytoplasm of nonphagocytic cells (24, 27), while salmonellae remain in vacuoles inside the host cell (33). Actin polymerization, an early event after bacterial entry (7), is not a sufficient signal for IL-8 production since enterohemorrhagic *E. coli* O157 bacteria induce actin polymerization when they attach to and efface epithelial cells (35) but do not stimulate IL-8 secretion. Currently, it is not known whether other cellular events that are associated with bacterial entry, i.e., changes in intracellular free calcium levels (11, 23, 26) or in the phosphorylation of host proteins (9), are essential for increased IL-8 production by epithelial cells.

The ability of the cells tested in these studies to respond to bacterial entry with increased IL-8 secretion appears to fall into two categories. HeLa and WI-38 cells made large amounts of IL-8 in response to all tested invasive bacteria, while T_{84} cells responded well to *S. dublin* and *L. monocytogenes* but to a lesser extent to *S. dysenteriae* and *Y. enterocolitica*. These differences are not related to the invasiveness of the different bacteria since comparable numbers of intracellular bacteria were found in all cases. Moreover, for all bacteria that stimulated IL-8 secretion, there was a strict relationship between the number of intracellular bacteria and the amount of secreted IL-8. It is currently unknown what mechanisms underlie the quantitative differences in the IL-8 response to different invasive bacteria.

It has been reported that coculture of two urinary epithelial cell lines with uropathogenic *E. coli* caused an increase in the proportion of cells expressing immunoreactive IL-8 (1). Although cell entry by the bacteria was not assessed in these studies, those *E. coli* strains are known to adhere to epithelial cells but not to enter them. However, it is conceivable that urinary epithelial cells responded to contact with surface molecules on the *E. coli* such as LPS. In support of this, we found recently that some intestinal epithelial cell lines (e.g., SW620 and HT29) secrete IL-8 following LPS stimulation (6), and LPS stimulation of renal and urinary bladder epithelial cells induces secretion of IL-8 (28) and IL-6 (12), respectively. Of note, the epithelial cells used in our studies are not responsive to LPS, a situation more likely to be encountered in the human colon where LPS is abundant even under normal conditions.

Since differentiated epithelial cell functions can be lost or altered during the malignant transformation process, extrapolation of the findings with transformed epithelial cells to physiologic conditions may appear limited at first glance. However, several lines of evidence suggest that our findings are representative of normal physiologic responses of epithelial cells. We have recently shown that freshly isolated human intestinal epithelial cells have the capacity to secrete IL-8 (6). Moreover, we note that the T_{84} epithelial cells used in these studies have retained multiple functions of their normal counterparts, including formation of tight junctions and vectorial chloride secretion (20), and that WI-38 fibroblasts, which are normal, nontransformed human fibroblasts, also responded to bacterial entry with increased IL-8 secretion. Thus, essentially identical findings were obtained with three cell lines of different origin. This indicates that the observed cellular IL-8 response to bacterial entry likely is a normal physiologic response in vivo.

Recently, Pace et al. demonstrated that entry of *S. typhimurium* into Henle 407 epithelial cells induces the production of peptidoleukotrienes (23). Moreover, production of leukotrienes was required for successful entry, and efficient entry of an invasion-deficient *Salmonella invA* mutant could be restored by the addition of leukotriene D_4 to the cultures (23). Since peptidoleukotrienes have potent vasoactive functions, release of these leukotrienes in response to bacterial entry may also have a function in inducing or modulating an acute inflammatory response and could further enhance the function of chemoattractants released locally.

Our data suggest that IL-8 secreted by epithelial cells may be the initial signal for the acute inflammatory response following bacterial invasion of mucosal surfaces. In support of this, the ability of IL-8 to initiate an acute inflammatory response is known (5, 16), and IL-8 production by epithelial cells has been documented in vivo, i.e., by keratinocytes in psoriatic lesions (10) and by renal epithelial cells during acute allograft rejection (28). Moreover, as shown herein, IL-8 secretion following bacterial entry is predominantly basolateral, indicating that secreted IL-8 will accumulate in the mucosa underlying the epithelial cell layer where IL-8-responsive effector cells reside. For instance, in experimental oral *Salmonella* and *Listeria* infection, polymorphonuclear leukocytes collect under infected epithelial cells within a few hours of cell entry, even before bacteria have penetrated through the epithelial cell layer into the underlying tissue (24, 33, 34).

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