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The role of the epithelial cell in *Escherichia coli* induced neutrophil migration into the urinary tract

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The role of the epithelial cell in Escherichia coli *induced neutrophil migration into the urinary tract. W.W. Agace. ©ERS Journals Ltd 1996.*

ABSTRACT: Neutrophil influx to mucosal surfaces represents one of the earliest inflammatory responses to mucosal infection. We have been studying external interactions with urinary tract epithelial cells in an attempt to understand the molecular mechanisms behind this process.

Uropathogenic *Escherichia coli* induced urinary tract epithelial cells to secrete the neutrophil chemoattractant interleukin-8 (IL-8). IL-8 secretion was higher in response to isogenic strains expressing type 1 or P fimbriae that adhered to the epithelial surface. Deliberate colonization of the human urinary tract with *E. coli* induced the local production of IL-8 and levels correlated with urinary neutrophil numbers suggesting a role for IL-8 in neutrophil migration. *E. coli* induced neutrophil migration across urinary tract epithelial layers *in vitro*, and this process was blocked with anti-IL-8 antibody. IL-8's activity was localized to the epithelial surface. Furthermore, these cells were shown to constitutively express IL-8 receptor A and B messenger ribonucleic acid (mRNA), suggesting a possible role for IL-8 on epithelial cell function. *E. coli* enhanced the expression of intercellular adhesion molecule-1 (ICAM-1) on urinary tract epithelial cells, and neutrophil migration across urinary tract epithelial layers *in vitro* was dependent on epithelial ICAM-1 and neutrophil Mac-1 (CD11b/CD18) expression.

These results suggest that bacterial/epithelial cell interactions play a key role in the induction of neutrophil migration during mucosal infection, and show the necessity for host-derived chemotactic factors and cell adhesion events in *E. coli* induced transuroepithelial migration *in vitro*.

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Mucosal surfaces form barriers that prevent microbes from reaching internal organs. The antimicrobial defence of the mucosa is achieved through the co-ordinated action of innate and specific immune mechanisms. Despite this, the large majority of infections start at mucosal sites.

Neutrophil migration to the lung, the urinary and the intestinal tract occurs as part of the initial inflammatory response to mucosal bacterial infection. This process involves neutrophil adherence to the endothelial blood vessel wall and extravasation into the lamina propria. Finally, neutrophils must cross a basement membrane and epithelial layer into the luminal space. There is little information regarding the mechanisms of bacterial induced neutrophil migration to sites of mucosal infection, the resident mucosal cells involved in this response, and the chemotactic signals released by the mucosa at the onset of infection.

This review focuses on the importance of bacterial-epithelial interactions in the induction of neutrophil influx at the onset of urinary tract infection (UTI). The first part briefly reviews some of the cell adhesion molecules and chemotactic signals involved in neutrophil extravasation, whilst the second part examines the role of bacterial-epithelial interactions in the induction of neutrophil migration to the mucosa and some of the molecular mechanisms involved in this response.

Neutrophil extravasation

The first stage in neutrophil migration to sites of mucosal infection involves neutrophil migration across the endothelial vessel wall. Neutrophil migration from the vasculature occurs mainly through postcapillary venules or capillary beds. This migration is controlled by specific cell adhesion molecules on the neutrophil and endothelial surface, that are upregulated at sites of inflammation. The initial interaction between the neutrophil and endothelial vessel wall is mediated through selectin/ligand interactions and results in neutrophil rolling across the endothelial surface. Rolling is unlikely to occur in the capillary beds, since the smaller diameter of the capillary segments forces neutrophil deformation whilst passing through these vessels. Rolling is followed by tight adhesion, mediated by β_2 integrins on the neutrophil surface and intercellular adhesion molecules (ICAMs) on the endothelial surface. Chemoattractants are thought to play an important role in this second stage of adhesion by upregulating the avidity of β_2 integrins for their ligands and increasing β_2 integrin expression on the neutrophil surface. Such interactions result in a flattening of the neutrophil on the endothelial vessel surface. The mechanism of transendothelial migration is less well understood but is thought to require homologous binding of

platelet endothelial cell adhesion molecule-1 (CD31, PECAM-1) [1]. This molecule is a member of the immunoglobulin superfamily expressed on neutrophils and endothelial cells [2–4]. The majority of PECAM-1 on endothelial cells is concentrated to the endothelial intercellular junctions; however, approximately 15% is expressed on the apical surface. suggesting the existence of an apical-basal gradient through the intercellular junction. Anti-PECAM antibody inhibits transendothelial migration without interfering with the ability of the leucocytes to remain adherent to the apical surface of the endothelial cells.

Selectins

Selectins are involved in the initial event in neutrophil recruitment by mediating neutrophil rolling along endothelial vessel walls [5–7]. The selectin family of cell adhesion molecules consists so far of three members: L-selectin (lectin adhesion molecule-1 (LECAM-1), Leu 8), P-selectin (CD62), and E-selectin (CD62E, endothelial leucocyte adhesion molecule-1 (ELAM-1)).

These three selectins are C-type lectins, requiring calcium for their receptor-binding activity. E-selectin, P-selectin and L-selectin all bind to sialylated, fucosylated lactosamine structures, notably sialyl Lewis x (sLe^x) and sialyl Lewis a, an isomer of sLe^x, expressed on glycoproteins [8, 9]. L- and P-selectin have also been shown to interact with a wide number of sulphated polysaccharides, such as heparin, fucoidan and sulphatides (reviewed in [9]).

P-selectin is associated with α granules in resting platelets and Weibel-Palade bodies in endothelial cells [10, 11]. It is rapidly and transiently expressed on the endothelial cell surface when these cells degranulate upon activation. *In vitro* stimulation of endothelial cells with thrombin or histamine induces a rapid expression of P-selectin, which peaks at 5 min and is largely absent by 20 min [10–12]. Neutrophil adhesion to P-selectin does not require neutrophil metabolism but is dependent on extracellular calcium [13]. Whilst P-selectin itself appears incapable of inducing a signal to attaching neutrophils, a juxtacrine stimulation with endothelial associated platelet-activating factor (PAF) results in activation of surface β_2 integrins on the neutrophil surface and their high affinity binding to endothelial ligand [14]. *In vivo*, neutrophil migration into the peritoneum, in response to *Streptococcus pneumoniae* is severely impaired in P-selectin knockout mice, whilst migration into the lung in response to *S. pneumoniae* is not effected [15].

E-selectin is expressed by endothelial cells after stimulation with proinflammatory cytokines, such as interleukin (IL)-1 and tumour necrosis factor (TNF), and requires *de nova* protein synthesis. Levels of E-selectin peak after 4–6 h stimulation and decline to baseline levels by 24–48 h *in vitro* [16, 17]. In contrast to P-selectin, E-selectin binding to neutrophils directly activates integrin molecules on the neutrophil surface [18]. *In vivo*, anti-E-selectin antibody blocks neutrophil dependent immunoglobulin G (IgG) immune complex-induced damage in rats and neutrophil extravasation during antigen-induced acute airway inflammation and late phase airway obstruction in monkeys [19, 20].

L-selectin is constitutively expressed and functional on unactivated neutrophils. Its mediation of neutrophil rolling on activated endothelium occurs through an undefined inducible ligand [21]. L-selectin is rapidly shed upon neutrophil activation with chemotactic stimuli by proteolytic cleavage near the transmembrane domain [22, 23]. Cross-linking of L-selectin with monoclonal antibodies induces a rapid and transient increase in intracellular calcium and superoxide ions and an upregulation of surface β_2 integrin expression in neutrophils [24]. *In vivo*, anti-L-selectin antibody reduced neutrophil-mediated lung injury [25], and a soluble immunoglobulin chimera containing the murine L-selectin extracellular domain significantly decreased the number of neutrophils migrating to the peritoneum in response to the inflammatory irritant thioglycollate [26].

Integrins

Members of the integrin family are involved in the tight adhesion of neutrophils to the endothelial surface, that occurs subsequent to selectin/ligand mediated neutrophil rolling, and also in neutrophil migration through extracellular matrices. Integrins are transmembrane glycoproteins, each composed of an α subunit noncovalently associated with a β chain to form a heterodimer. The integrin α chain subunits form two groups based upon structural differences. One group is cleaved during synthesis to form one heavy and one light chain, that remain associated *via* a single disulphide linkage. The other group contains an inserted domain (I domain) that is thought to be involved in receptor-binding. Fourteen α and eight β subunits have been characterized so far, which in various combinations make at least 20 known integrins.

The integrins principally involved in neutrophil interactions with endothelial cells belong to the β_2 family [27–29]. The three members of this family share a common β subunit (CD18) that is associated with three different α subunits α_L, α_M, and α_X (CD11a, b and c). All three integrins are expressed on the neutrophil surface, the predominant form being CD11b/CD18 (Mac-1). Mac-1 and CD11a/CD18 (leucocyte function associated antigen-1 (LFA-1)) receptors on endothelial cells are members of the immunoglobulin superfamily (see below), whilst endothelial receptors for CD11c/CD18 have not yet been identified [29].

Mac-1 and CD11c/CD18 but not LFA-1 are also found in intracellular storage pools within neutrophils [30–32]. The majority of Mac-1 (75%) in resting neutrophils is localized in specific granules, the remainder being stored in secretory vesicles and plasma membranes [33]. Activation of neutrophils with many agonists, including chemotactic agents, results in a translocation of integrin molecules from the intracellular stores predominantly from secretory vesicles, to the neutrophil plasma membrane.

Activation of neutrophils also induces an increase in the avidity of expressed integrin for its receptor(s). It is this increased avidity and not *de novo* expressed integrin that results in the initial increased adhesion of neutrophils to activated endothelial cells [34]. *De novo* expressed integrin may, however, be utilized for subsequent locomotion events following further increases in

stimulus concentration [35]. The regulation of β_2 integrin avidity is not fully understood but is a rapidly reversible event thought to involve the cytoplasmic domain of the integrins β chain [36, 37]. The continuous modulation of integrins from low to high avidity state and vice versa appears vital for cell migration [38].

The importance of β_2 integrins is best demonstrated in patients suffering from the rare leucocyte adhesion deficiency (LAD) disorder. These patients suffer poor wound healing and recurrent, often fatal, bacterial infections as a result of deficient or much reduced expression of the common β_2 subunit CD18. This is associated with an inability of their phagocytes to adhere to endothelium and migrate to sites of infection. Studies in animal models have shown neutrophil migration in response to bacterial infection to be CD18-dependent or CD18-independent, depending on the site of infection and the bacterial stimulus. Anti-CD18 antibody blocked neutrophil migration in response to intraperitoneal and intrabroncheal instillation of *Escherichia coli*, and intraperitonial instillation of *S. pneumoniae*. In contrast neutrophil migration in response to intrabroncheal instillation of *S. pneumoniae* was CD18-independent [39]. Furthermore, neutrophil migration into the peritoneum in response to *Salmonella typhimurium* was CD18-dependent, but CD18-independent in response to *Listeria monocytogenes* [40]. Whilst the mechanism of CD18-independent neutrophil migration remains to be defined, these results indicate the level of complexity involved in neutrophil migration to sites of bacterial infection.

Immunoglobulin superfamily

Two members of the immunoglobulin superfamily, intercellular adhesion molecule-1 and intercellular adhesion molecule-2 (ICAM-1, ICAM-2) act as endothelial receptors for the neutrophil β_2 integrins. Whilst ICAM-1 is constitutively expressed by a wide variety of cells in addition to endothelial cells, the tissue distribution of ICAM-2 appears to be restricted to blood vessel endothelium and some lymphoid cells [41]. ICAM-1 (CD54) consists of five immunoglobulin (Ig)-like domains, with the LFA-1 binding site located at the N terminal domain (domain 1) and the Mac-1 binding site located within the 3rd Ig-like domain [42, 43]. Endothelial ICAM-1 expression increases after stimulation with proinflammatory cytokines, such as IL-1 and TNF, reaching maximal levels after 24 h. This increase in expression requires *de novo* protein synthesis [17].

ICAM-2 contains two Ig-like domains that are most homologous with the two N terminal domains of ICAM-1, and has binding sites for LFA-1 and Mac-1 [44, 45]. In contrast to ICAM-1, ICAM-2 expression is not enhanced following stimulation with proinflammatory cytokines [41, 46]. The inducibility of ICAM-1 and constitutive expression of ICAM-2 by endothelial cells suggests that ICAM-1 may be the relevant endothelial ligand for β_2 integrins during an inflammatory response, while ICAM-2 may be of more relative importance in the unstimulated state or early on during a response before ICAM-1 expression is increased. *In vivo*, anti-ICAM-1 antibody inhibits neutrophil-dependent lung vascular injury that occurs after occlusion and reperfusion, intravenous infusion of cobra venom factor, or after deposition of IgG and IgA immune complexes [47–49]. ICAM-1 knockout mice show reduced neutrophil migration to the peritoneum in response to sterile inflammatory stimuli [50], and are resistant to high-dose endotoxin lethal shock [51]. Resistance is associated with a significant decrease in neutrophil infiltration into the liver at 24 h. The role of ICAM-2 in neutrophil migration *in vivo* has not been studied.

Chemokines

Neutrophil migration from the vasculature to the mucosal surface is likely to occur in response to chemoattractants and inflammatory mediators released from the site of infection. While numerous chemotactic molecules have been described, recent evidence has indicated a key role for the chemotactic cytokine family (chemokines) in leucocyte migration. Chemokines are a relatively new and expanding group of low MW (8–10 kDa) cytokines, that are able to attract and activate leucocytes (for review see [52]).

Each member of the chemokine group contains two internal disulphide loops and four conserved cysteine residues. The positioning of the first two cysteines provides the basis for the division of the chemokine family into two subgroups. Members of the α chemokine (CXC) group contain a single amino acid insert between the first two cysteines, which in the β chemokine (CC) group are adjacent. Members of the α chemokine group include IL-8, neutrophil-activating protein-2 (NAP-2), platelet factor-4 (PF-4), connective tissue-activated peptide III (CTAP-III), epithelial cell neutrophil-activating protein-78 (ENA-78), *Gro*-α, *Gro*-β, *Gro*-γ. Most of these chemokines act as chemotactic and activating agents for neutrophils, inducing cytosolic free calcium changes, chemotaxis, shape changes and exocytosis. A major determinant of activity towards neutrophils is the necessity for the Glu-Leu-Arg (ELR) motif preceding the first cysteine in the N-terminal domain [53]. Members of the β chemokine group include monocyte chemoattractant protein 1, 2 and 3 (MCP-1, MCP-2 and MCP-3), macrophage inflammatory protein-1α and 1β (MIP-1α MIP-1β) and "regulated upon activation in normal T-cells, expressed and secreted" (RANTES). None of these, with the possible exception of MIP-1α, show stimulation for neutrophils but they are all potent activators of monocytes (for review see [52]).

Interleukin-8

IL-8 is the best characterized member of the α chemokine group. IL-8 is produced by most cells in response to inflammatory stimuli, such as IL-1α, IL-1β, TNF-α, immune complexes and bacteria (for review see [52]). The unglycosylated protein is secreted after cleavage of a 20 amino acid signal sequence [54]. Further N terminal processing yields numerous biologically active truncation analogues ranging in size from 69–79 amino acids in length [54, 55]. Processing requires protease release by the cells and appears to be cell-specific, since some cells (human umbilical vein endothelial cells (HUVEC) and fibroblasts) predominantly produce the 77 amino acid protein, while others (monocytes and macrophages)

produce both the 77 and 72 amino acid protein [54, 56–59]. Interestingly, the potency of IL-8 has been shown to increase with progressive N terminal cleavage [60]. IL-8 exists as a noncovalently bound homodimer in concentrated form or upon crystallization [61, 62]. However, IL-8 exists as a monomer at physiologically relevant concentrations *in vitro*, and the monomeric form is sufficient for receptor-binding and full *in vitro* biological activity [63, 64].

The *in vitro* biological activities of IL-8 on neutrophils include integrin receptor upregulation and increased avidity, chemotaxis, shape change degranulation, and formation of bioactive lipids [65–70]. The role of IL-8 in neutrophil chemotaxis appears complex. *In vitro* IL-8 can mediate neutrophil chemotaxis through numerous extracellular matrices, including fibrin gels, matrigel (reconstituted basement membrane proteins), fibronectin, fibrinogen and collagen IV [71]. In addition, endogenous IL-8 secreted by stimulated endothelial layers is required for neutrophil migration across endothelial vessel walls [72]. *In vivo*, intradermal injection of IL-8 was shown to induce a rapid and long-lasting neutrophil influx in rabbits [73, 74]. The maximum rate of neutrophil migration was reached within 30 min and remained detectable for at least 8 h [74]. In contrast to these pro-inflammatory effects of IL-8, GIMBRONE *et al*. [57] reported that exogenous IL-8 inhibited neutrophil adherence to lipopolysaccharide (LPS)-activated HUVEC cells. Recent studies have shown that intravascular IL-8 inhibits extravasation *in vivo*. Human IL-8 transgenic mice, that have elevated serum IL-8 levels, and animals injected intravenously with IL-8 have impaired neutrophil migration extravascularly [75, 76]. The mechanism behind the leucocyte adhesion inhibition (LAI) activity of IL-8 is unknown; however, its effects appear to be localized to the neutrophil and not the endothelial cell surface. IL-8 induced shedding of L-selectin has been proposed to play a role by preventing activated neutrophils from initiating the rolling event [77]. This is not likely to be the whole story, since IL-8 when delivered intravenously decreased the migratory capacity of rabbit neutrophils without inducing a significant loss of L-selectin [78]. The LAI activity of IL-8 may be related to its ability to cause rapid changes in the confirmation of neutrophil actin microfilaments [79].

Since soluble IL-8 prevents neutrophil adhesion to the endothelial surface, a model for chemokine-induced neutrophil migration involving an attractant gradient bound to the endothelial surface has been proposed [80, 81]. IL-8 binds to the surface of postcapillary venules *in vivo* and to extracellular matrix proteins, endothelial cells, keratinocytes and dermal cells, suggesting that a cell-bound IL-8 gradient could potentially be set up from inflammatory loci [82–84].

IL-8 receptors

Early binding studies of IL-8 to human neutrophils indicated the presence of two high affinity IL-8 receptors [85]. Molecular cloning has identified both IL-8 receptors (IL-8RA and IL-8RB) as members of the seven transmembrane guanine-nucleotide-binding (G) protein linked receptor family [86, 87]. IL-8RB, but not IL-8RA, also has high affinity for other members of the α chemokine family, including NAP-2, *Gro*, and melanoma growth-stimulating activator (MGSA) [88]. These two receptors share 77% amino acid sequence homology; however, they have low amino acid identity at their amino terminus, which is thought to be involved in receptor subtype specificity [86, 87, 89]. Subsequent studies have shown that many cell types, in addition to neutrophils, including T-cell populations, basophils, endothelial cells, monocytes and fibroblasts, express transcripts for one or both IL-8 receptors (for reviews see [52, 90]).

The development of monoclonal antibodies specific for each receptor has led to some initial observations regarding their relative importance in neutrophil migration. A recent study, examining IL-8 receptor internalization rates upon binding with ligand, showed IL-8RB to have a 2–5 fold higher affinity for IL-8 than IL-8RA on human neutrophils. Binding of IL-8 to both receptors resulted in ligand internalization, degradation of IL-8 and reappearance of receptor on the cell surface [91]. Receptor reappearance for IL-8RA was rapid (100% within 90 min), whilst reappearance for IL-8RB was slow (40% within 180 min). At sites distant from the inflammatory loci, where IL-8 concentrations are likely to be at their lowest, IL-8RB may receive the IL-8 signal first and initiate neutrophil migration. IL-8RA, since it reappears on the neutrophil surface quicker than IL-8RB, may mediate the IL-8 signal as the neutrophils approach the site of inflammation. In a further *in vitro* study, blocking of IL-8 binding to either IL-8RA or IL-8RB differentially effected IL-8-induced neutrophil chemotaxis. Monoclonal anti-IL-8RA antibody blocked the majority of IL-8-induced neutrophil chemotaxis, whilst 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 IL-8RA [92]. These results indicate that IL-8RA may be the important neutrophil IL-8 receptor in IL-8 mediated chemotaxis.

Other receptors for IL-8 include the duffy antigen and proteoglycans. The duffy antigen acts as a chemokine receptor on the surface of erythrocytes and is also expressed on kidney venules [93, 94]. The *in vivo* role of this receptor is not known but IL-8 bound to erythrocytes is incapable of activating neutrophils, suggesting that erythrocytes may act as a sink for IL-8 by reducing the amount of soluble IL-8 in serum [95]. Chemokines, including IL-8, also bind to proteoglycans [81]. These are proteins that have been post-translationally modified by the addition of glycosaminoglycan side chains at serine residues. This results in the formation of highly charged molecules with long polysaccharide chains consisting of repeating disaccharide units. Proteoglycans are found at the endothelial surface and within the extracellular matrix, and have been proposed to play an important role in presenting chemokines to leucocytes [81]. Indeed, the activity of IL-8 is enhanced when it is bound to heparan sulphate or heparin [96].

Escherichia coli-epithelial interactions in the induction of neutrophil migration

Infections of the urinary tract have provided a useful model in examining mechanisms of bacterially-induced

mucosal inflammation. Uropathogenic *E. coli* become established in the host at a site outside the urinary tract (often the large intestine). The bacteria then spread *via* the vaginal and periurethral areas into the urinary tract and establish bacteriuria [97–99]. Once in the urinary tract, bacteria induce a rapid mucosal inflammatory response that includes the influx of large numbers of neutrophils into the urine [100, 101]. Indeed neutrophils in the urine (pyuria) has been used as a diagnostic parameter for UTI since the start of the century [102].

E. coli interactions with urinary tract epithelial cells

Since epithelial cells dominate the urinary mucosal surface and are the first cellular contact with mucosal pathogens, they have become a focus of attention as mediators of the mucosal inflammatory response to infection. The initial interaction of *E. coli* with urinary tract epithelial cells is mediated by specific fimbrial structures expressed on the bacterial surface. Most uropathogens express P and type 1 fimbriae. P fimbriae adhere to Galα1-4Galβ and GalNAcβ-3Galα1-4Galβ-containing oligosaccharide sequences in the globoseries of glycolipids that are present on epithelial and nonepithelial components of the urinary bladder and ureters, and are the dominating nonacid glycolipids on kidney tissue [103, 104]. Adherence is mediated by papG adhesins located at the fimbrial tip [105]. Several G adhesins exist that all share specificity for the globoseries of glycolipids but differ in isoreceptor specificity, binding to epithelial cells, and disease association [106–110]. Type 1 fimbrial adherence is mediated by the FimH adhesin, located along the fimbrial shaft and at the fimbrial tip [111]. This adhesin recognizes terminally located D-mannose moieties on cell-bound and secreted glycoproteins [112–116]. In contrast to the papG adhesins, the deoxyribonucleic acid (DNA) sequences of FimH adhesins are highly homologous. Minor variations in these sequences may, however, result in different adherence phenotypes [117, 118]. While type 1 fimbriated bacteria bind to urinary tract epithelial cells, the identity of the type 1 receptors on the epithelial surface remain to be identified.

Early studies showed that urinary tract epithelial cell lines originating from the human bladder and kidney secreted IL-6 in response to uropathogenic *E. coli* [119]. The magnitude of this response was influenced by the properties of the bacterial strain [120]. The production of IL-6 by bladder and kidney epithelial cell lines was higher in response to P and type 1 fimbriated strains than nonfimbriated isogens, and was associated with an increased adherence of these strains to the epithelial surface. Furthermore, co-incubation of urinary tract epithelial cell lines with D-threo-1-phenyl-2-decamoylamino-3-morpholino-1-propanol PDMP, a structural analogue of ceramide, depleted expression of the globoseries of glycolipids by these cells, resulting in an inability of P fimbriated *E. coli* to adhere to the epithelial surface. This led to a significant reduction in IL-6 secretion in response to P fimbriated but not type 1 fimbriated *E. coli* [121]. Together these results suggested that epithelial cells were playing an important role in the induction of mucosal inflammation, and that the properties of the bacterial pathogen may influence this response.

Epithelial cell IL-8 production in response to *E. coli*

To examine whether epithelial cells are involved in the induction of neutrophil migration during UTI, we determined whether these cells produced cytokines with chemotactic activity for neutrophils and whether *E. coli* could stimulate their production. We also compared the ability of *E. coli* strains expressing P and type 1 fimbriae to stimulate epithelial cytokine production.

Normal urinary tract epithelial cells isolated from bladder scrapings of patients undergoing cystoscopy for bladder neck obstruction stained for intracellular IL-8 after stimulation with *E. coli* (fig. 1) [122]. Furthermore, indirect immunofluorescence studies examining intracellular cytokine levels in bladder (J82) and kidney (A-498) epithelial cell lines showed that uropathogenic *E. coli* induced an increase in IL-8 producing cells, which peaked after 2 h stimulation and remained higher than controls at 6 and 24 h [123]. The production of IL-8 by these cells in response to bacteria was accompanied by increased expression of IL-8 messenger ribonucleic acid (mRNA) [124].

The role of adherence in the induction of epithelial IL-8 responses was examined by stimulating epithelial cells with isogenic *E. coli* strains expressing either P or type 1 fimbriae (table 1) [122]. The increased IL-8 secretion was associated with increased bacterial adherence to the epithelial cell surface. IL-8 secretion by the bladder and kidney epithelial cell lines was greatest in response to the highly adherent type 1 fimbriated isogen. Furthermore, addition of α-methyl-D-mannoside, a receptor analogue for the type 1 fimbrial adhesin, reduced type 1 mediated adherence and epithelial IL-8 production. The P fimbriated isogen adhered only to the bladder epithelial cells and induced higher IL-8 secretion in these cells than the parent strain (table 1). The low adherence properties of this strain were due to its atypical P fimbrial adhesin. Subsequent studies have shown that *E. coli* strains expressing the major P fimbrial adhesin ($PapG_{IA2}$) associated with

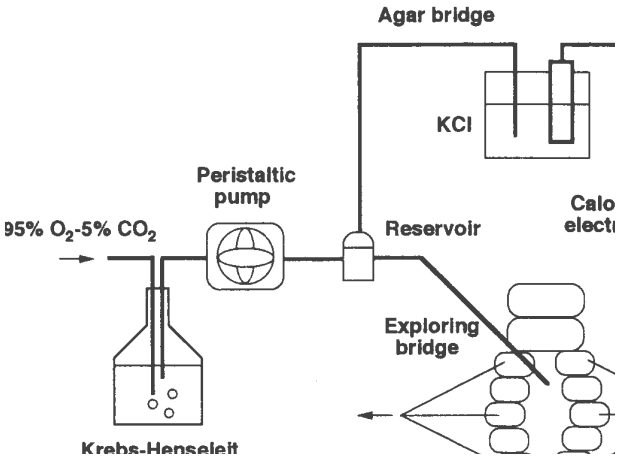

Fig. 1. – Intracellular staining of interleukin-8 in freshly isolated urinary tract epithelial cells after stimulation with *E. coli*.

Table 1. – Secretion of interleukin-8 (IL-8) by urinary tract epithelial cells (kidney A-498 and bladder J82) after stimulation with isogenic strains of *Escherichia coli*

Cell line	*E. coli*	Adherence bacteria·cell^{-1}	IL-8 ng·mL^{-1}
A-498	None		0.2
	Nonfimbriated	0.2	2.1
	P fimbriated	0.3	2
	Type 1 fimbriated	100	4.3
J82	None		1.3
	Nonfimbriated	0.2	4.6
	P fimbriated	3.4	9
	Type 1 fimbriated	100	14.5

Epithelial cells were stimulated for 24 h with *E. coli* (10^8 bacteria·mL^{-1}).

severe UTI, bind well to urinary tract epithelial cells and induce higher levels of cytokine secretion in these cells than nonfimbriated isogens [121, 125].

These results represent early observations in the field of epithelial cytokine production in response to bacteria. *E. coli* had previously been shown to stimulate epithelial IL-6 secretion; however, the ability of epithelial cells to produce a range of cytokines, including neutrophil chemoattractants, in response to bacterial stimulation had not been recognized. Subsequent studies have confirmed that mucosal epithelial cells produce a specific range of proinflammatory cytokines in response to bacterial stimulation, and have shown the ability to secrete IL-8 to be common to lung, intestinal, gastric and urinary tract epithelium (for review see [126]).

The mechanism of bacterial induced IL-8 production differs depending on the bacterial stimulant and origin of the epithelial cells. Several possibilities by which adherence and fimbrial expression enhances the urinary tract epithelial cytokine response have been suggested. Firstly, attachment may increase the concentration of bacterial products at the epithelial surface that activate cell cytokine responses. Secondly, fimbriae may directly activate the cells through fimbriae-receptor interactions. Adhesin positive P fimbriae have been shown to induce epithelial IL-6 production, albeit at lower levels than whole bacteria [120]. The ceramide signalling pathway was recently implicated in the epithelial IL-6 response to P fimbriated *E. coli*. P fimbriated bacteria induced the release of free ceramide and the formation of ceramide-1-phosphate in urinary tract epithelial cells [125]. IL-6 production was completely blocked by serine threonine kinase inhibitors. In contrast, serine threonine kinase inhibitors had no effect on epithelial IL-6 secretion in response to a type 1 fimbriated isogen. P and type 1 fimbriated *E. coli*, therefore, stimulate epithelial IL-6 production *via* different intracellular pathways. Whether similar intracellular signals are involved in bacterial-induced IL-8 production remains to be determined.

Bacterial adherence also enhances IL-8 production in epithelial cells from other mucosal sites. Purified Pseudomonas pilin induces a dose-dependent IL-8 secretion, but at lower levels than whole bacteria. Furthermore the Pseudomonas flagellin, which may also serve as an adhesin for epithelial attachment, and a low molecular mass product suggested to be the Pseudomonas auto-inducer (PAI) stimulate epithelial IL-8 secretion [127, 128]. Bacterial invasion appears to be a prerequisite for the induction of IL-8 by colon epithelial cells, since only invasive Salmonella, Shigella, Yersinia, Listeria and Escherichia species stimulate IL-8 production in these cells [129]. In contrast, low and highly adhesive *Helicobacter pylori* strains, soluble *H. pylori* extracts and concentrated *H. pylori* supernatants induce IL-8 production in gastric epithelial cell lines. Other Gram-negative organisms, including *Pseudomonas aeruginosa* and an *E. coli* strain that failed to induce IL-8 production in colon epithelial cells, also induce IL-8 production in these cells [130, 131]. Common to all mucosal epithelial cells is their low responsiveness to LPS [120, 129, 132, 133]. It has been suggested that the poor epithelial response to LPS is due to a lack of CD14 expression by these cells. Indeed, addition of soluble CD14 has been reported to enhance epithelial sensitivity to LPS [134]. However, this has yet to be shown for urinary tract epithelial cells.

Evidence of local IL-8 production during mucosal bacterial infection

The production of IL-8 by *E. coli* stimulated urinary tract epithelial cells suggested the possible involvement of IL-8 in the neutrophil influx during UTI. To examine whether *E. coli* stimulate IL-8 production in the human urinary tract, and the relationship between IL-8 and urinary neutrophil numbers, urine samples were collected from patients that had been deliberately colonized with *E. coli* in the urinary tract and measured for IL-8 [122].

Deliberate colonization of the urinary tract with nonvirulent strains of *E. coli* has been used as a treatment for patients suffering from recurrent UTI who are refractory to other therapy [135]. Three *E. coli* strains were used in the patient colonizations: a wild type ABU strain, which had been carried by a girl with asymptomatic bacteriuria for 3 yrs without deterioration of renal function (this strain did not express P or type 1 fimbriae); and two transformants containing the *pil* DNA sequences encoding type 1 fimbriae and the *pap*J96 DNA sequences encoding P fimbriae, respectively. Colonizations were performed with mixtures of the two transformants or all three strains.

IL-8 was detected in the urine of all patients after colonization with *E. coli* but not in precolonization samples. The lack of IL-8 in serum samples taken during the colonization period suggested a mucosal production of this cytokine [122]. Levels of urinary IL-8 varied greatly from patient to patient, and showed no correlation with previously recorded urinary IL-6 levels or bacterial numbers [101, 135]. In contrast, urinary IL-8 levels correlated strongly with neutrophil numbers (fig. 2). Peak urinary IL-8 levels corresponded with peak neutrophil numbers in all patients. Furthermore, patients with low levels of urinary IL-8 had few neutrophils in their urine. The strong correlation with urinary neutrophil numbers suggested that IL-8 was involved in the induction of neutrophil influx and that neutrophils were a possible second source of this chemokine during UTI. Recent results

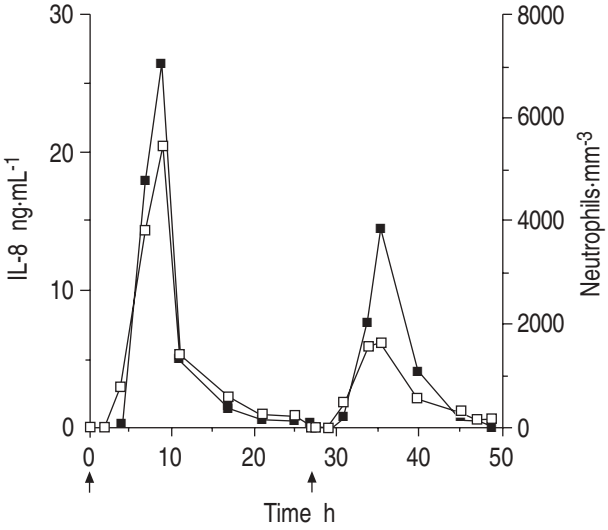

Fig. 2. – Correlation between urinary neutrophil numbers and urinary interleukin-8 (IL-8) levels after deliberate colonization of the human bladder with *Escherichia coli*. The arrows depict the times of colonization. —■— : neutrophils; —□— : IL-8.

have shown that all three colonization strains stimulate neutrophil IL-8 production *in vitro* (fig. 3). IL-8 production by neutrophils was not a result of LPS stimulation alone, since levels of IL-8 secreted in response to LPS were lower than to whole bacteria (fig. 4). Further preliminary data indicate that phagocytosis *per se* does not result in the high levels of IL-8 secretion observed after bacterial stimulation, since type 1 fimbriae coated fluorescein-labelled 0.75 µm microspheres were phagocytosed by neutrophils and induced levels of IL-8 secretion similar to that of LPS (authors, unpublished observations).

Together, these results show that IL-8 is produced at the urinary tract mucosa in response to *E. coli* infection and suggest that IL-8 is an important neutrophil chemoattractant during UTI. The cellular origin of mucosal IL-8 is not clear; however, since all three colonization strains stimulated urinary tract epithelial IL-8 production, it is likely that epithelial cells are involved in the initial IL-8 response to colonization [122]. The production of IL-8 by neutrophils in response to these strains suggests that these cells are a second source of urinary IL-8 once at the site of infection.

Since mixed inoculations of the bacterial strains were used to colonize patients, it was not possible to examine the role of bacterial adherence in the activation of the mucosal IL-8 response and neutrophil influx. However, a subsequent study has shown that monocolonization of the human urinary tract with the same ABU strain used in the above study [122], containing a single copy plasmid encoding the Pap_{IA2} P fimbriae, induced significantly higher urinary IL-8 levels and neutrophil influx than monocolonization with the nonfimbriated parent strain (H. Connell, personal communication). Thus, bacterial adherence appears to influence epithelial IL-8 production *in vitro* and IL-8 secretion and neutrophil influx into the urinary tract *in vivo*.

IL-8 is also present at mucosal sites during natural infection. Urinary IL-8 levels are elevated in adults and children with UTI caused both by Gram-positive and Gram-negative bacteria [136–138]. High levels of IL-8 are found in the sputum of patients with cystic fibrosis that are chronically infected with *P. aeruginosa*, and IL-8 producing cells are found in the intestinal epithelium during Shigella infection and in patients suffering *H. pylori* associated gastritis [139–141]. The local production of IL-8 is, therefore, a common occurrence during mucosal bacterial infection.

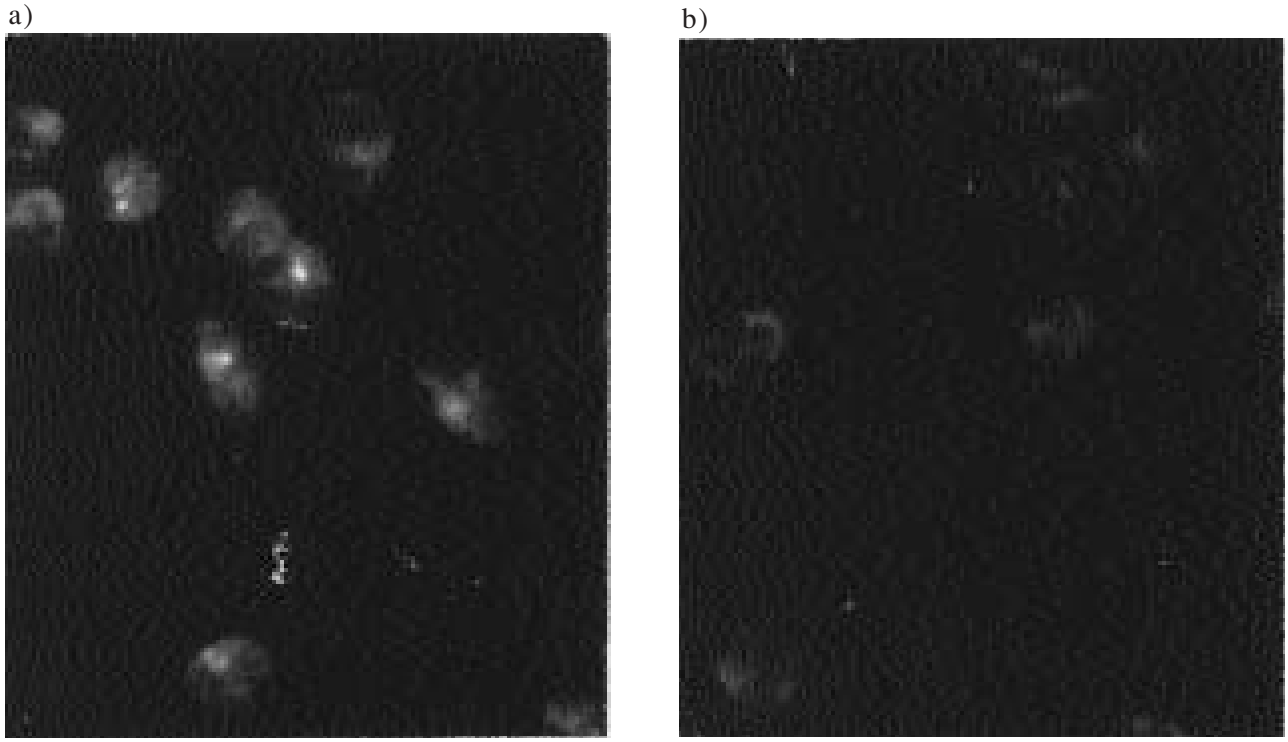

Fig. 3. – Interleukin-8 (IL-8) production by neutrophils in response to *Escherichia coli*. Staining was performed with anti-IL-8 antibody after stimulation with: a) *E. coli*; or b) medium alone.

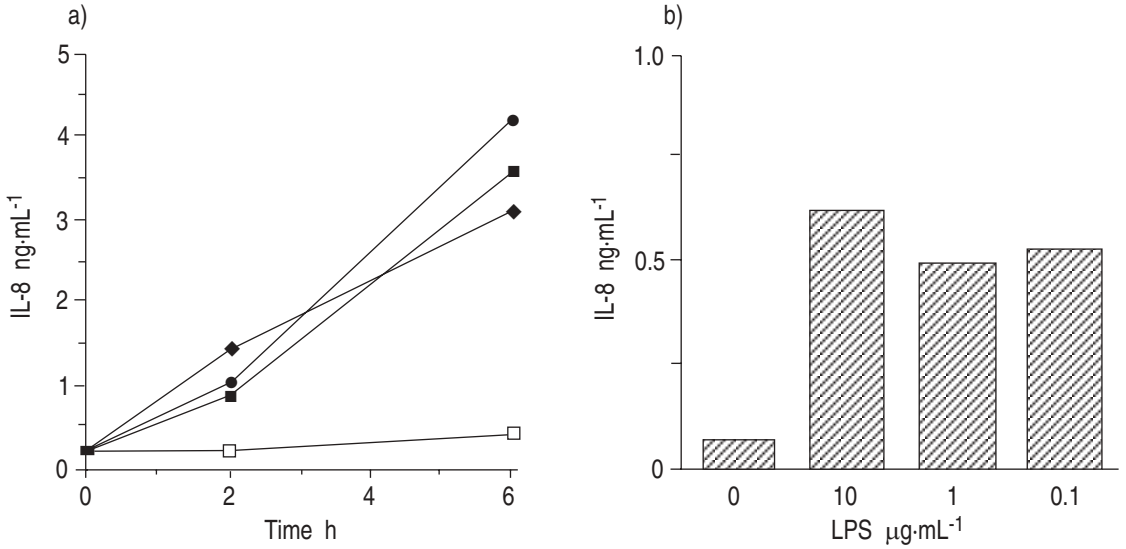

Fig. 4. – a) Secretion of interleukin-8 (IL-8) by neutrophils after stimulation with isogenic *Escherichia coli* strains (10^6 neutrophils·mL^{-1}: 10^8 bacteria·mL^{-1}). —□— : control (medium alone); —◆— : nonfimbriated *E. coli*; —●— : Type 1 fimbriated isogen; —■— : P fimbriated isogen. b) IL-8 secretion by neutrophils (10^6·mL^{-1}) after stimulation with LPS (*E. coli* serotype O:111) for 8 h. LPS: lipopolysaccharide.

E. coli induced neutrophil migration across urinary tract epithelial layers

Neutrophil migration to sites of infection or inflammation requires two events; 1) the production of a chemotactic signal originating from the inflammatory site; and 2) specific cellular interactions mediated through cell adhesion molecules expressed on the neutrophil surface and their ligands on the extracellular and cellular matrix. In order to examine whether *E. coli* induces neutrophil migration across epithelial cell layers and the role of IL-8 and cell adhesion molecules in this response, we developed an *in vitro* model based on the transwell system (fig. 5). Transwell inserts were placed into cluster dishes to form a two chamber system, separated by a polycarbonate membrane. The polycarbonate membrane contained 3 µm pores, allowing the passage of cells from the top to the bottom chamber.

Kidney and bladder epithelial cell lines were grown to confluency on the underside of the membrane supports and prestimulated by addition of *E. coli* to the bottom well for 0, 4 or 24 h [142]. After stimulation, medium in the top well was removed and replaced with new medium containing neutrophils. Neutrophil migration was measured by taking samples from the bottom well 3 h after their addition to the top well, and expressing the number of migrated neutrophils as a percentage of neutrophils added to the top well. In further experiments, epithelial layers were prestimulated for 24 h with IL-1α, LPS or preconditioned medium (obtained by incubating *E. coli* in cell medium in the presence of gentamycin for 24 h and removing the bacteria with a 0.22 µm filter).

Preincubation of epithelial layers with *E. coli* for 24 h induced a marked increase in transuroepithelial neutrophil migration, which was stimulant dose- and time-dependent [142]. *E. coli* failed to induce neutrophil migration when added to the epithelial layers at the same time as the neutrophils. Furthermore, *E. coli* were not themselves chemotactic for neutrophils in this model, since they failed to enhance neutrophil migration across filters in the absence of an epithelial layer. Together, these results indicate a requirement for epithelial cell activation in the induction of transuroepithelial neutrophil migration. *E. coli* LPS (0.1–10 µg·mL^{-1}) and pre-conditioned medium failed to induce transuroepithelial neutrophil migration, suggesting that whole bacteria were required for full induction of this response.

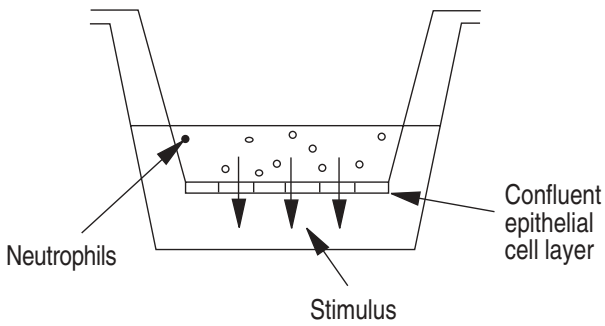

Fig. 5. – Transwell model used to study neutrophil migration across confluent urinary tract epithelial cell layers.

Table 2. – The role of IL-8 in *Escherichia coli* and IL-1α induced transuroepithelial neutrophil migration

	Neutrophil migration %		
Antibody 10 µg·mL^{-1}	Medium	*E. coli*	IL-1α
None	23	71	82
Monoclonal anti-IL-8	7	19	16
Control	23	62	68
Washed cell layers			
None	18	63	76
Monoclonal anti-IL-8	12	15	15
Control	17	49	52

Epithelial cell layers were prestimulated with *E. coli* (10^8 bacteria·mL^{-1}) or IL-1α (1 ng·mL^{-1}) for 24 h. IL: interleukin; Control: monoclonal mouse immunoglobulin-G negative antibody.

Role of IL-8 in transuroepithelial neutrophil migration

Since *E. coli* induced neutrophil migration across urinary tract epithelial cell layers required epithelial cell activation, we examined whether epithelial IL-8 production was involved in this process.

Anti-IL-8 antibody was added to *E. coli* and IL-1α stimulated urinary tract epithelial layers 30 min prior to addition of the neutrophils. These antibodies completely blocked *E. coli* and IL-1α induced transuroepithelial migration (table 2) [143]. Furthermore, IL-8 was sufficient to induce neutrophil migration across urinary tract epithelial layers, since addition of recombinant IL-8 to the lower well of unstimulated cell layers induced similar levels of migration as occurred across *E. coli* and IL-1α stimulated cell layers. Several observations suggested that IL-8 was inducing transuroepithelial migration while bound to the epithelial surface and not as a soluble gradient. Firstly, neutrophil migration occurred across *E. coli* and IL-1α stimulated epithelial cell layers that had been washed free of soluble IL-8, and this process was blocked with anti-IL-8 antibody (table 2). Secondly, staining of *E. coli* or IL-1α stimulated epithelial layers with anti-IL-8 antibody localized IL-8 within the layers and at the epithelial surface (fig. 6). Thirdly, fluorescein-labelled IL-8 bound to the epithelial cells and binding was blocked with polyclonal anti-IL-8 antibody.

This study provided *in vitro* evidence that IL-8 can induce neutrophil migration across uroepithelial layers, and that uropathogenic *E. coli* induce migration, in part, by activating epithelial IL-8 production. IL-8 appears to play a major role in *E. coli* and IL-1α induced neutrophil migration, since antibodies to IL-8 blocked all of the induced migration in response to these stimulants. These *in vitro* observations, are consistent with *in vivo* studies implicating IL-8 in the induction of neutrophil migration to sites of mucosal bacterial infection. IL-8 levels correlated strongly with neutrophil numbers in the urine of patients deliberately colonized in their bladders with *E. coli* and in patients with natural UTI [122, 136]. Anti-IL-8 antibody blocked, on average, 50% of the chemotactic activity of infected urine [136]. IL-8 levels were also shown to correlate with neutrophil numbers in bronchiolar lavage fluids from patients with *P. aeruginosa* infection, and IL-8 represented the majority of chemotactic activity in sputum samples obtained from patients with chronic airway disease [139, 144]. Taken together with the results of the present study, these observations suggest a role for mucosal IL-8 in establishing an early bound chemotactic gradient across the mucosal epithelium into the urine.

Both the bladder and kidney epithelial cell lines constitutively produced mRNA for IL-8RA and IL-8RB [143]. However, it seems unlikely that IL-8RA and IL-8RB are involved in presenting IL-8 to passing neutrophils as part of a haptotactic gradient, since binding of IL-8 to these receptors, at least in neutrophils, results in a rapid internalization of receptor bound IL-8 [91]. The expression and functional activity of these receptors on epithelial cells and the effects that IL-8 has on urinary tract epithelial cells remains to be determined. Since proteoglycans have been proposed to present chemokines to neutrophils on endothelial cells (see above) [81], they are likely candidates in binding IL-8 at epithelial surfaces and in building IL-8 haptotactic gradients across urinary tract epithelial surfaces. The role of these molecules and possible additional IL-8 receptors in transuroepithelial neutrophil migration requires further study.

It remains to be seen whether epithelial cells from other mucosal sites are able to bind IL-8, and express mRNA for IL-8 receptors. If this is the case, the results of the present study suggest IL-8 may be playing an important role in directing neutrophil migration across the mucosal epithelium, not only in the urinary tract but also at other mucosal sites.

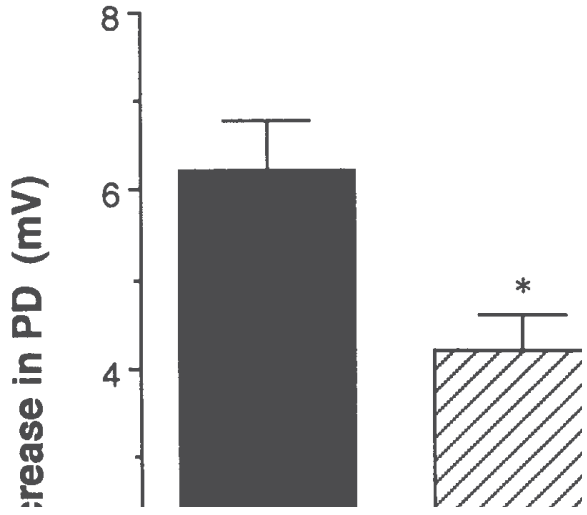

Fig. 6. – Stimulated urinary tract epithelial layers stained with anti-interleukin-8 (IL-8) antibody (green) and propidium iodide (red) and visualized with a confocal laser scanning microscope. The arrow depicts IL-8 at the epithelial surface.

Role of cell adhesion molecules in transuroepithelial neutrophil migration

To examine the role of cell adhesion molecules in *E. coli* induced transuroepithelial neutrophil migration, we screened normal urinary tract epithelial cells and epithelial cell lines for the expression of ICAM-1, ICAM-2, E-selectin and P-selectin. We studied the ability of *E. coli* to upregulate cell adhesion molecule expression and blocked the function of these molecules in the transwell model with monoclonal antibodies.

Urinary tract epithelial cell lines and normal urinary tract epithelial cells constitutively expressed ICAM-1, but not E-selectin, P-selectin or ICAM-2 [142]. ICAM-1 expression on the urinary tract epithelial cells was augmented after stimulation with *E. coli* (fig. 7), with maximal levels being reached after 24–48 h of stimulation. Epithelial ICAM-1 expression was required for transuroepithelial cell migration *in vitro*, since anti-ICAM-1 antibody and anti-ICAM-1 Fab fragments blocked *E. coli* and IL-1α induced transuroepithelial neutrophil migration (table 3). In contrast, anti-human leucocyte antigen-1 (HLA-1) antibody, which bound to the epithelial cells to a similar degree as the anti ICAM-1 antibody, had no

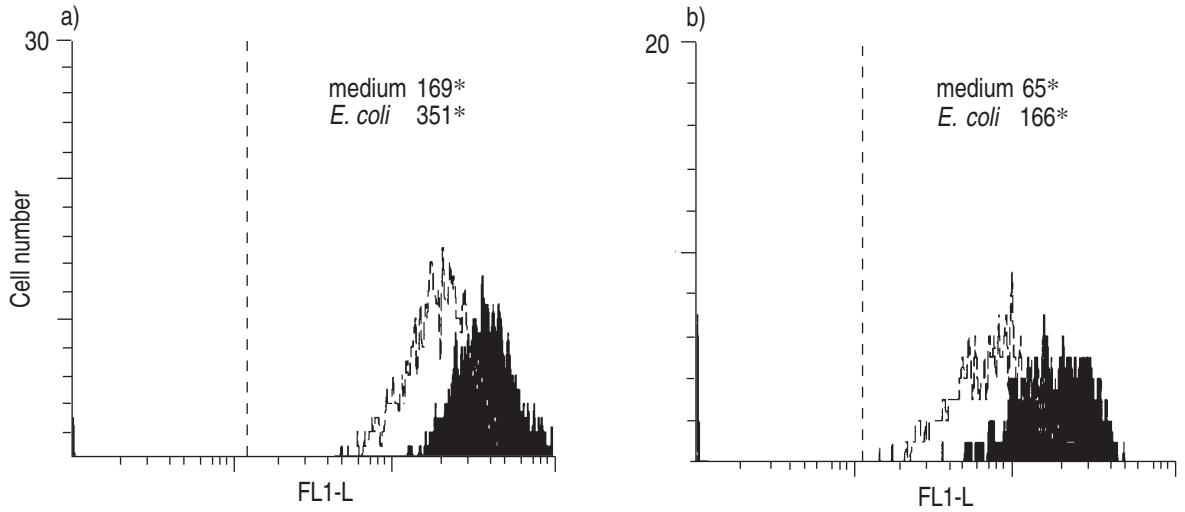

Fig. 7. – Flow cytometry analysis of ICAM-1 expression on urinary tract epithelial cells after stimulation with medium or *Escherichia coli* for 24 h. a) A-498 kidney epithelial cells; b) J82 bladder epithelial cells. (Of cells incubated with the monoclonal mouse immunoglobulin-G negative antibody, 98% fell to the left of the dotted line (data not shown)). ☐: medium; ■: *E. coli*. ICAM-1: intercellular adhesion molecule-1; FL1-L: Log fluorescence intensity; *: mean fluorescence.

effect on transuroepithelial neutrophil migration. The neutrophil ligand for ICAM-1 was tentatively identified as Mac-1, since anti-CD11b and anti-CD18 but not anti-CD11a antibody also blocked migration (table 3).

Previous studies have shown that mucosal epithelial cells express ICAM-1. Epithelial cells from the lung, and the intestine constitutively express ICAM-1, and expression is augmented by proinflammatory cytokines [145–147]. The expression of ICAM-1 by human bronchial epithelium *in vitro* correlates with increased binding of activated neutrophils to monolayers of bronchial epithelial cells. This attachment can be inhibited with anti-ICAM-1 antibody [145]. ICAM- 1 is also expressed by urinary tract epithelial cells. ICAM-1 is constitutively expressed by primary tubular epithelial cells in culture, and expression is augmented by proinflammatory cytokines [148]. In addition cytokine stimulated primary urothelial epithelial cells, proliferating ureter epithelial cells, and numerous bladder epithelial cell lines express ICAM-1 [149–151]. Weak ICAM-1 expression is observed in normal kidney epithelium *in vivo* and is upregulated during renal disease [152–154]. ICAM-1 is also expressed on bladder urothelial cells in patients with interstitial cystitis [151], and on normal bladder urothelium in patients after instillation of Calmette-Guérin bacillus (BCG) [155].

Table 3. – The role of cell adhesion molecules in Escherichia coli induced transepithelial neutrophil migration

	Neutrophil migration %		
Antibody (10 μg·mL^{-1})	Medium	*E. coli*	IL-1α
None	8	53	52
Anti-ICAM-1	2	8	15
Control	11	45	48
None	27	79	63
Anti-CD11a	27	73	53
Anti-CD11b	9	23	16
Anti-CD18	3	21	13

Epithelial cells layers were prestimulated with *E. coli* (10^8 bacteria·mL^{-1}) or interleukin-1α (IL-1α) (1 ng·mL^{-1}). ICAM-1: intercellular adhesion molecule-1; Control: monoclonal mouse immunoglobulin negative antibody.

Preliminary results from our group examining ICAM-1 expression *in vivo* indicate constitutive expression of ICAM-1 in the glomeruli, endothelial vessels, and the basolateral side of kidney tubular epithelium in the mouse (authors unpublished observation) and strong ICAM-1 staining in the kidney glomeruli and weak ICAM-1 staining throughout the kidney tubular and bladder epithelium in the human (H. Long, unpublished observation). Whilst the results presented here show that *E. coli* can upregulate the expression of cell adhesion molecules on the surface of urinary tract epithelial cells and that these cell adhesion molecules are required for neutrophil migration across urinary tract epithelial layers *in vitro*, the kinetics of ICAM-1 expression by kidney and bladder epithelium and its role in neutrophil migration *in vivo* during bacterial infection are yet to be determined.

Concluding remarks

It is now becoming clear that mucosal inflammation and, particularly, neutrophil influx plays a key role in the clearance of bacteria from the urinary tract at the onset of infection. C3H/HeJ mice that are hyporesponsive to LPS, have significantly higher bacterial numbers in their bladders and kidneys 24 h after intravesical infection with *E. coli*, compared to normal C3H/HeN mice [156]. This corresponds with a significantly lower inflammatory response (neutrophil influx and urinary IL-6 levels) in C3H/HeJ mice [100, 157]. Macrophages from C3H/HeJ mice were recently shown to be nonresponsive to the lipid second messenger ceramide, that has been implicated in TNF-α, IL-1β, interferon-γ (IFN-γ) and more recently P fimbriated *E. coli* signalling pathways [125, 158]. The inability of C3H/HeJ mice to mount an inflammatory response to *E. coli* infection may, therefore, be due to an insensitivity to numerous alternative inducers of this pathway other than LPS. C57BL/10ScCr

LPS hyporesponder mice also have significantly higher bacterial numbers in their bladders and kidneys after 24 h infection compared to normal C57BL/6J mice [159]. In both C3H and C57 mouse backgrounds the presence of neutrophils in the urine corresponds with clearance of infection. Furthermore, treatment of C3H/HeN mice with the anti-inflammatory drugs, dexamethasone, diclofenac and indomethacin, severely inhibited their ability to clear infection [160]. A role of neutrophil influx in the clearance of UTI has also been reported by MILLER *et al*. [161], who showed that treatment of rats with antineutrophil serum led to a 1,000 fold increase in bacterial numbers in infected kidneys [161]. These results suggest that the inflammatory response, particularly neutrophil influx to infection, is important in the clearance of infection.

The work presented in this review has highlighted the importance of epithelial cells and bacterial/epithelial interactions in the induction of neutrophil influx during mucosal infection and has shown the necessity for host-derived chemotactic factors and cell adhesion molecules for neutrophil influx across epithelial layers *in vitro*. Based on this data, we propose the following model of *E. coli* induced neutrophil migration during UTI. *E. coli* first induce the secretion of cytokines, including IL-8, and the upregulation of adhesion molecules at the mucosal epithelial lining. The degree of IL-8 production will be influenced by the adherence and presumably other virulence properties of the bacteria. 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 bladder mucosa has shown IL-8 to be present within the mucosal tissue during inflammation (H. Long, personal communication). Finally mucosal IL-8 bound to the urinary tract epithelial cell surface induces neutrophil migration across the epithelium into the luminal space. Neutrophil Mac-1 and epithelial ICAM-1 expression are also necessary for migration across the urinary tract epithelium *in vitro*. Strong IL-8 staining is observed in the inflamed human urinary tract epithelium (H. Long, personal communication). Once at the site of infection, neutrophils may secrete IL-8 and, therefore, help to regulate the migration process (fig. 8).

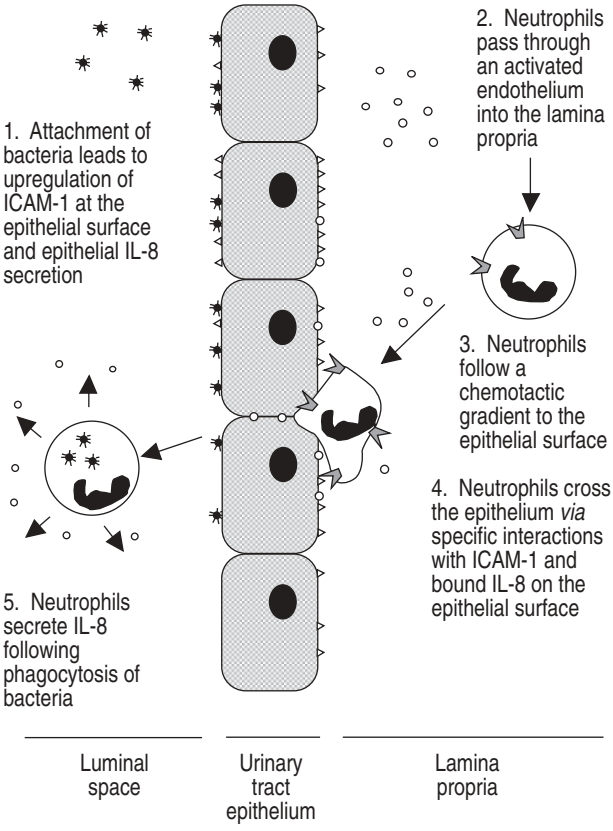

Fig. 8. – Schematic model *Escherichia coli* induced neutrophil migration across urinary tract epithelium. ○ : interleukin-8 (IL-8); ⊱: intercellular adhesion molecule-1 (ICAM-1); ⋗ : Mac-1; ✶ : *E. coli*. ICAM-1: intercellular adhesion molecule-1; IL-8: interleukin-8.

Further work examining the importance of the α chemokines and cell adhesion molecules in *E. coli* induced neutrophil influx to the urinary tract *in vivo* is underway, using antibodies to specifically block the function of these molecules. Results obtained from these studies should help us obtain a clearer understanding of the mechanisms of neutrophil migration to the urinary tract mucosa and their role in the different stages of the disease process.

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