Toll-like Receptor Signaling and Chemokine Receptor Expression Influence the Severity of Urinary Tract Infection

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Urinary tract infections (UTIs) vary in pathogenesis and severity. After their ascent into the urinary tract, bacteria may establish asymptomatic bacteriuria (ABU), cause acute cystitis, or cause acute pyelonephritis. Research during the last few decades has established that the site of infection and the disease severity are influenced by bacterial virulence. In the 1940s, hemolysin was shown to identify *Escherichia coli* that cause extraintestinal infections [1]. "Uropathogenic" *E. coli* strains were later shown to belong to a restricted set of serotypes or clones [2], and acute pyelonephritis and ABU strains were shown to differ in surface antigen repertoire [3]. Studies in the 1970s started to involve host cell interactions with attachment to the urinary tract mucosa [4]. We proposed that the disease severity was a direct result of bacterial virulence and that tissue attachment is a first critical step. The special virulence of the uropathogenic clones has subsequently been shown to include numerous virulence factors encoded on the pathogenicity islands (see Middendorf et al., this issue).

The variation in urinary tract virulence reflects the ability of bacteria to trigger mucosal and systemic host responses. Through different molecular interactions, bacteria may trigger epithelial cell responses, cause cell detachment, and invade or kill cells by apoptosis (for review see [5]). Inflammation has received special attention because it determines the severity of UTI and the clearance of infection [6] (also see Agace et al. in [5]).

We have studied how the inflammatory response is initiated and how it determines the resistance to UTI. Herein we argue that individuals differ in the ability to respond to UTI. We propose that pyelonephritis occurs more readily in "high responders" and that their abnormalities exaggerate the damaging rather than the protective aspects of inflammation. The "low responders," on the other hand, have suppressed inflammatory signals, allowing bacteriuria to establish without harming the host. Genetic factors are proposed to explain these differences.

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Current Knowledge

The inflammatory response proceeds in three main steps. First, the bacteria stimulate uroepithelial cells to produce inflammatory mediators [6, 7]. Second, chemokines and chemokine receptors direct inflammatory cells to the site of infection [8–10]. Third, the quality of the local inflammatory response determines if bacterial clearance or tissue damage will result [6, 11].

Attachment, Transmembrane Signaling, and Cell Activation by Fimbriated Bacteria

The epithelial cells form the first line of defense and act as arbitrators of subsequent cell activation. Fimbriae-associated surface lectins attach uropathogenic *E. coli* to glycoconjugate receptors on the host cell surface. In the 1980s, we showed that attachment enhances cytokine responses to uropathogenic *E. coli* and that isolated P or type 1 fimbriae activate receptor-bearing epithelial cells [6].

Fimbriae may enhance the host response by activating receptor-specific signaling or by delivery of other bacterial products with host response–inducing ability. We have shown that the fimbrial receptor specificity determines the transmembrane signaling pathway involved in cell activation. The evidence for P fimbriae–mediated signaling through glycosphingolipid (GSL) receptors is summarized by Hedlund et al. in this issue and in [12]. Type 1 fimbriae use other signaling mechanisms to activate cytokine responses.

Difference in lipopolysaccharide (LPS) delivery between P- and type 1–fimbriated E. coli. LPS is thought to be the principal component of gram-negative bacteria that alerts the host to systemic infection. LPS binding protein transfers LPS to a binding site on CD14, and cell activation occurs through the recruitment of Toll-like receptors (TLRs) [13, 14]. Uroepithelial cells are $CD14^{-}$ [15] and respond poorly to free LPS [12, 15], yet LPS may still participate in epithelial cell activation if fimbriae deliver LPS to the tissues and route them to CD14-independent signaling pathways. This was investigated by mutational inactivation of the *msbB* gene that encodes an acyl transferase coupling myristic acid to the lipid IV_A precursor [16]. The msbB+ and the msbB− mutants were transformed with the *pap* or the *fim* sequences encoding P fimbriae and type

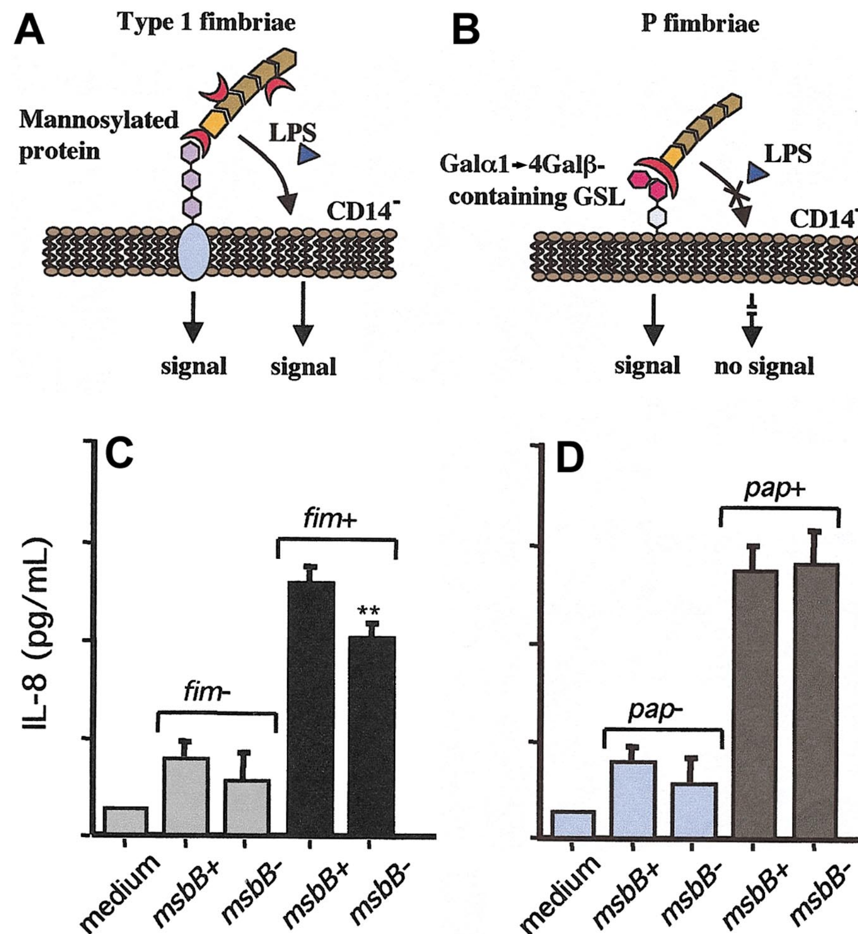


Figure 1. P- or type 1–fimbriated bacteria differ in lipopolysaccharide (LPS) delivery. Type 1 fimbriae deliver 1 LPS (msbB)-dependent and one msbB-independent signal to epithelial cells in vitro. In contrast, P fimbriae activate epithelial cells in a strictly fimbriae-dependent but msbB-independent manner. Human kidney A498 cells were challenged with isogenic mutants differing in msbB, fim, or pap genotype, and the interleukin (IL)-8 response was quantified in cell supernatants. The fimbriated strains triggered high responses, but only the fim+ mutants showed a difference related to msbB genotype (**, $P < .01$), [17]. GSL = glycosphingolipid.

1 fimbriae, respectively, and examined for cell activation (figure 1).

Nonfimbriated strains were poor host response inducers in vitro, regardless of LPS genotype. Responses to the P-fimbriated transformants were insensitive to the *msbB* mutation, demonstrating the LPS independence of the P fimbrial signal. In contrast, responses to the type 1–fimbriated transformants were partly reduced by the *msbB* mutation, demonstrating some LPS dependence of the type 1 fimbrial signal.

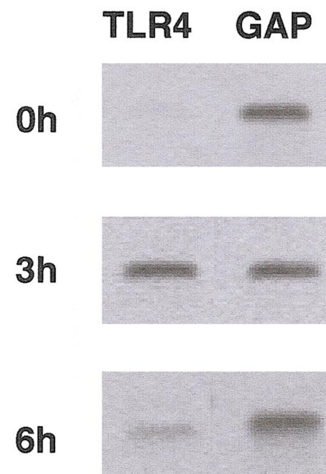
P fimbriae activate a TLR4-dependent signal. Subsequent studies demonstrated that P fimbriae recruit TLR4 as co-receptors in cell activation. Results of experimental UTI in TLR4-proficient C3H/HeN and TLR4-deficient C3H/HeJ mice carrying a point mutation in TLR4 [18] are shown in figure 2. The *pap*+ strains triggered inflammation in C3H/HeN mice, regardless of *msbB*+ genotype, confirming the P fimbriae dependence and LPS independence of the signal. Responses were virtually absent in the C3H/HeJ mice, suggesting that their TLR4 deficiency rendered the epithelial cells unable to respond to the P-fimbriated bacteria. In vitro studies demonstrated that human uroepithelial cells contain mRNA for several TLR species and that TLR4 is up-regulated by P-fimbriated strains. By confocal microscopy, TLR4 was shown to co-localize with the GSL P fimbriae receptors in caveolae. We propose that P fimbriae bind to the receptor GSLs and recruit TLR4 for signal transduction. It is interesting that P fimbriae utilize an LPS-like cell-activation mechanism to activate cells that lack CD14 and are refractory to LPS itself [19].

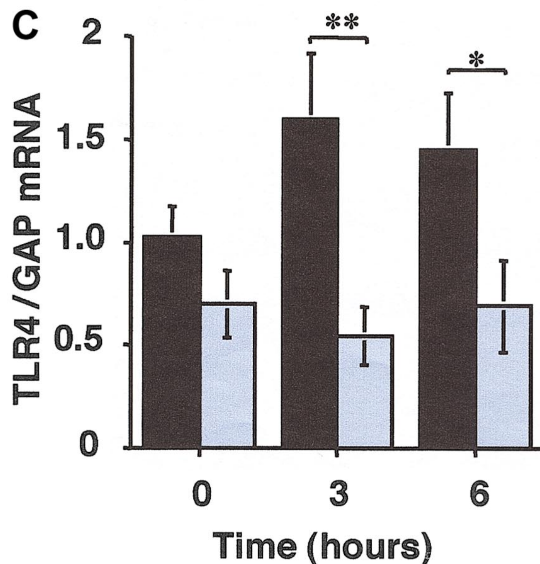
Type 1–fimbriated E. coli trigger a lectin-dependent and TLR4-independent and an LPS- and TLR4-dependent signal. The in vitro studies suggested that type 1 fimbriae deliver both an LPS-dependent and an LPS-independent signal [20]. This was con-

A TLR4 dependence of the host response to P fimbriae

E. coli fimbriae	Lipid A genotype	Urine neutrophil numbers	
		HeN	HeJ
P	*msb+*	253	29 *
	msb-	218	25 *
Type 1	*msb+*	348	160**
	msb-	160	193

The host response was determined as neutrophil numbers in urine collected at 6 h after inoculation. * = $P < .0001$, ** = $P < .05$.





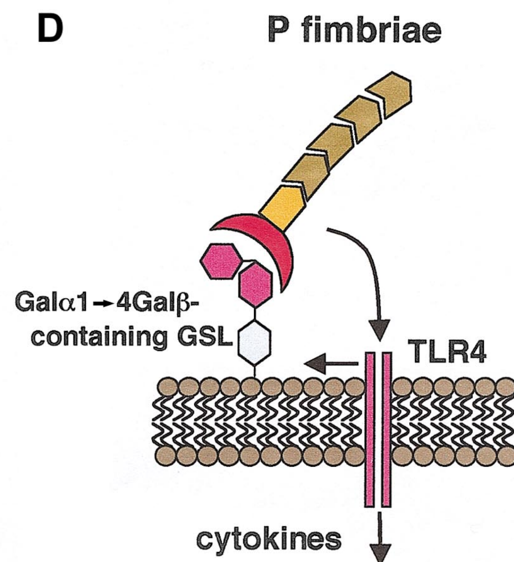


Figure 2. *Escherichia coli* P fimbriae utilize the Toll-like receptor (TLR)4 pathway for cell activation. *A,* TLR4-proficient and -deficient mice were inoculated with mutants differing in P or type 1 fimbrial expression and msbB genotype. Responses to the P-fimbriated mutant were abrogated in the TLR4− mice. *B, C,* Infection with P-fimbriated *Escherichia coli* up-regulates TLR4 mRNA expression in human kidney epithelial cells. **, $P < .01$; *, $P < .05$. *D,* We propose that the glycosphingolipid (GSL) receptors for P fimbriae recruit TLR4 as co-receptors.

firmed by experimental UTI in C3H/HeN mice. The *fim+, msbB+* transformant induced a rapid inflammatory response, but the response to the *fim+, msbB−* strain was significantly lower and delayed. In C3H/HeJ mice, there was an intermediate response, but the early, LPS-dependent response was absent. We conclude that type 1 fimbriae trigger the epithelial cytokine response by one LPS- and TLR-dependent pathway and one lectin-dependent but LPS- and TLR-independent pathway (figures 1, 2).

Chemokines, Chemokine Receptors, and Neutrophil Recruitment

In response to chemotactic signals from the infected urinary tract mucosa, neutrophils leave the blood vessels, traverse the lamina propria to the epithelial barrier, and cross the polarized epithelial cell layer into the lumen, resulting in "pyuria." This process is strictly regulated through the sequential elaboration of chemokines and chemokine receptors [6]. Interleukin (IL)-8

was identified as the main human chemokine supporting transepithelial neutrophil migration, and macrophage inflammatory protein (MIP)-2 was identified as a mouse homologue [9–11].

IL-8 and other chemokines mediate their biological responses by binding to cell-surface chemokine receptors. The two high-affinity IL-8 receptors (IL-8R), CXCR1 (IL-8RA) and CXCR2 (IL-8RB), are members of the large family of serpentine receptors with seven transmembrane domains that couple to heterotrimeric G proteins for signal transduction [20]. CXCR1 has greater ligand specificity than CXCR2 and binds with high affinity to IL-8 and GCP. CXCR2 binds multiple CXC chemokines in addition to IL-8, including epithelial cell–derived neutrophil-activating protein-78, neutrophil-activating peptide-2, and growth-related protein-α. Uroepithelial cells express CXCR1 and CXCR2, and infection increases the expression of both receptors. As a consequence, there is higher IL-8 binding and enhanced IL-8–dependent neutrophil migration across the infected epithelial cell layers. Antibodies to IL-8 or to the CXCR1 receptor inhibited this increase by 60% ($P < .004$), but anti-CXCR2 antibodies had no effect, suggesting that CXCR1 was most essential in this process [9].

The relevance of these molecular interactions for in vivo inflammation was examined in two ways: (1) MIP-2 antibody treatment was shown to block transepithelial neutrophil migration, and the neutrophils were trapped under the epithelium, apparently unable to cross the mucosal barrier into the lumen, and (2) the murine IL-8R homologue knock-out (mIL-8Rh KO) mice had an aberrant neutrophil response to UTI.

Mice express one main CXC chemokine receptor that binds several IL-8–like CXC chemokines, including MIP-2. The IL-8Rh KO mice carry a mutation in the murine gene, and their neutrophils fail to migrate in response to the CXC chemokines but have intact sensitivity for other activation pathways. Infection of the mIL-8Rh KO and control mice triggered an epithelial MIP-2 response in control mice, and chemokine receptor expression increased in control but not in mIL-8Rh KO mice. In control mice, there was a rapid neutrophil response followed by clearance of infection. The neutrophil influx was delayed in the mIL-8Rh KO mice, and once in the mucosal compartment, the cells were trapped. Massive numbers of neutrophils eventually accumulated under the epithelial barrier, and urine neutrophil numbers remained low throughout infection. These results demonstrated that CXCR1 is required for neutrophil migration across infected human epithelial cell layers in vitro and that the mIL-8R is needed for neutrophils to cross the infected mucosa of the urinary tract in vivo [9–11].

The Local Inflammatory Response Determines Bacterial Clearance and Tissue Damage

The mIL-8Rh mutation had two important effects on bacterial clearance and tissue integrity [10]. First, IL-8Rh KO mice are extremely susceptible to UTI. Normal mice cleared infection within 3–7 days, but bacterial numbers increased in the IL-8R KO mice, which developed symptoms and bacteremia. Second, neutrophil accumulation was disastrous for tissue integrity. The kidneys of surviving mice showed signs of renal scarring, with small kidneys, abscesses, and fibrosis [11].

Patients Prone to Acute Pyelonephritis Have Low CXCR1 Expression

We compared the neutrophil CXCR1 receptor expression in children with pyelonephritis to that in age-matched controls. By confocal microscopy, fewer CXCR1-positive cells were observed, and flow cytometry showed reduced CXCR1 expression in the children with pyelonephritis. This difference was restricted to CXCR1; there was no apparent variation in CXCR2 staining [10].

Commentary

These studies provide new molecular handles on host resistance to UTI and allow some conclusions about the mechanisms that control host-pathogen interactions in the urinary tract. “High responder” individuals express receptors that allow fimbriae to bind to the mucosal surface and to recruit co-receptors, such as TLR4, for transmembrane signaling and cell activation. Following the secretion of chemokines and the expression of chemokine receptors, neutrophils migrate into the tissues. If this process is fully functional, the patients may develop symptoms and transient disease, but infection is cleared with little tissue damage. On the other hand, deficient chemokine receptor function will cause neutrophil accumulation followed by bacteremia and renal scarring. This “high-responder phenotype” was found in mIL-8Rh KO mice and in patients with recurrent UTI and low CXCR1 expression [10] and (Frendéus et al., this issue).

The “low responder” scenario is best illustrated by the TLR4-deficient C3H/HeJ mouse. Experimental infection of these mice caused little or no chemokine response and no neutrophils were recruited. The mice were unable to clear the infection but did not develop symptoms. Instead, they developed a chronic carrier state resembling ABU. We propose that ABU patients may have a TLR4 deficiency or may down-regulate the signaling mechanisms that control interactions with LPS and other proinflammatory molecules in the high responders.

The contribution of bacterial virulence to disease severity is well established (see the introduction). We propose that the tendency to develop ABU or pyelonephritis is regulated also at the host level. This review emphasizes the new and emerging information that the propensity for a host response is genetically regulated and is another decisive factor for the outcome of host-parasite interaction in the urinary tract.

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