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

C. Svanborg, B. Frendéus, G. Godaly, L. Hang, M. Hedlund, and C. Wachtler

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.

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. Fimbriated bacteria associated with 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 fimbria-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]. Uropathogenic 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 IVα 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 coreceptors 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-
Figure 2. *Escherichia coli* P fimbriae utilize the Toll-like receptor (TLR4) 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 trans-
epithelial 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 recep-
tors with seven transmembrane domains that couple to het-
erotrimeric 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 che-
mokines 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 in-
flammation was examined in two ways: (1) MIP-2 antibody
treatment was shown to block transepithelial neutrophil mi-
gration, 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. In-
fec tion of the mIL-8Rh KO and control mice triggered an ep-
thelial 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 eventu-
ally accumulated under the epithelial barrier, and urine neu-
triphil 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 bac-
terial 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 ob-
erved, and flow cytometry showed reduced CXCR1 expression
in the children with pyelonephritis. This difference was re-
stricted to CXCR1; there was no apparent variation in CXCR2
staining [10].

Commentary

These studies provide new molecular handles on host resis-
tance to UTI and allow some conclusions about the mecha-
isms 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-re-
ceptors, such as TLR4, for transmembrane signaling and cell
activation. Following the secretion of chemokines and the ex-
pression of chemokine receptors, neutrophils migrate into the
tissues. If this process is fully functional, the patients may de-
velop symptoms and transient disease, but infection is cleared
with little tissue damage. On the other hand, deficient che-
mokine receptor function will cause neutrophil accumulation
followed by bacteremia and renal scarring. This “high-re-
ponder 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 car-
rrier 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 pro-
inflammatory 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 geneti-
cally regulated and is another decisive factor for the outcome
of host-parasite interaction in the urinary tract.
References