DYNAMIC INTERACTIONS BETWEEN HOST AND PATHOGEN DURING ACUTE URINARY TRACT INFECTIONS
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ABSTRACT
Urinary tract infections (UTIs) have traditionally been viewed as acute and often self-limiting infections caused predominantly by noninvasive Escherichia coli. However, this concept has been challenged by recent findings demonstrating that an acute bladder infection results from a complex series of host–pathogen interactions that can lead to bacterial invasion and persistence and that ultimately can determine the course of the infectious disease. The ability of E. coli to gain a foothold in the bladder is greatly facilitated by type 1 pilus-mediated attachment to and invasion of bladder epithelial cells. Invasion allows uropathogenic strains of E. coli to exploit the intracellular environment by replicating within these epithelial cells while evading a multitude of host defenses. An intracellular location also provides them a safe haven from many common antibiotic therapies. However, attachment and invasion also activates a cascade of innate host defenses, leading to the death and exfoliation of bladder cells and the production of inflammatory mediators. The ability of uropathogenic E. coli to flux out of cells and colonize surrounding cells provides them a mechanism to subvert these defense mechanisms and persist in the bladder epithelium for weeks following the acute infection. The persistence of E. coli in bladder tissue may be relevant to more chronic diseases of the urinary tract such as recurrent UTIs and interstitial cystitis. UROLOGY 57 (Suppl 6A): 56–61, 2001. © 2001, Elsevier Science Inc.

From an evolutionary perspective, a bacterial infection can be viewed as a series of measures and countermeasures by the host and pathogen that ultimately determine the course of the infectious disease. Powerful host defense mechanisms can promote changes in the expression of virulence properties in bacteria, which can contribute to the activation of new host defenses. Recent studies of the interaction between uropathogenic Escherichia coli (UPEC) and host defenses in the bladder have provided unique insight into the pathogenic relations that can shape a urinary tract infection (UTI). The principles learned from this system will likely have general applications to infectious diseases at other mucosal sites.

The early phase of any bacterial infection is a battle between the innate defenses of the host and the virulence factors elaborated by the infecting bacterial pathogen. The primary objectives of innate host defenses are to control bacterial proliferation and dissemination early in the infection and to stimulate the development of an adaptive immune response. These goals are achieved through the collective efforts of a variety of cell types, including macrophages, dendritic cells, natural killer (NK) cells, mast cells, endothelial cells, and epithelial cells. The cascade of innate responses is triggered by the recognition of bacteria or bacterial-associated components through pattern recognition receptors.1 Upon recognition of an intruder, proinflammatory cytokines and chemokines are produced that lead to the recruitment and activation of inflammatory cells and to the induction of co-stimulatory molecules on the surface of professional antigen presenting cells. This latter response is critical in priming naive T-cells in draining lymph nodes, thus initiating the development of an adaptive immune response.

One goal of a pathogen is to resist the onslaught of early host defenses and establish a persistent infection. To prevent clearance by innate and adaptive defenses, microbes must either avoid immune recognition or have a means to resist the antibacterial effector mechanisms utilized by the host. In
addition, some bacteria have evolved mechanisms to capitalize on the destructive nature of the host inflammatory response to promote their spread within tissues. Irrespective of the mechanism, to survive, the bacteria must adapt to the environment created by the host–pathogen interaction.

**TYPE 1 PILI AND ACUTE URINARY TRACT INFECTIONS**

In the urinary tract, infection is initiated when UPEC binds to the superficial bladder epithelial cells that line the luminal surface of the bladder. The majority of UPEC express surface adhesive structures known as type 1 pili that facilitate bacterial interactions with bladder epithelial cells. Type 1 pili are composite fibers consisting of a long, thick cylindrical rod joined to a short, thin tip fibrillum. The FimH adhesin is located at the distal end of the linear tip fibrillum.

Although type 1 pili can mediate bacterial interactions with the bladder epithelium, the extent to which these structures contribute to disease in the bladder is only beginning to be appreciated. Initially, bacterial adherence mediated by type 1 pili is critical to prevent washout of the pathogen by the flow of urine and other substances that bathe the bladder mucosa. Subsequently, bladder epithelial cells internalize type 1-piliferous E. coli, an event which has been proposed to be an innate defense against this bacterium. Not surprisingly, UPEC has evolved to take advantage of uptake by host cells. The low pH and high salt concentration of urine make it a poor growth medium, and bacteria in the luminal space are more accessible to antibiotics and antibody, suggesting that an intracellular environment may be beneficial to the pathogen. Consistent with this concept is that the invasion of bladder epithelial cells is associated with a survival advantage in vivo.

The invasion process mediated by type 1 pili is a direct consequence of host-cell signaling cascades activated by the pilus adhesin—FimH—which can lead to host cell cytoskeletal rearrangements and the eventual internalization of adherent UPEC. Thus, bacteria use a host endocytic pathway to enter an environment that could potentially protect them from constitutive host defenses and antibiotics as well as provide them with a more nutrient-rich milieu. These findings are particularly interesting, as UPEC has traditionally been viewed as a strictly extracellular pathogen.

Although adherence and invasion provide the pathogen with an early safehouse within superficial bladder epithelial cells, this respite is short lived. The same events that allow bacteria to enter epithelial cells also alert the host to the presence of a pathogen and trigger a series of countermoves by the infected bladder epithelial cells. One arm of these induced defenses is the exfoliation of superficial bladder epithelial cells into the urine, carrying with them associated bacteria. The process of exfoliation is activated in response to type 1-piliferous K12 and uropathogenic E. coli strains, but not nonpiliferous isogenic mutants, demonstrating that type 1 pili are a critical urovirulence factor involved in triggering this response. However, at the present time the precise role of type 1 pili in this response is unclear. Exfoliation occurs via an apoptoticlike mechanism that involves the activation of caspases, cysteine proteases implicated in the execution of apoptosis, and DNA fragmentation. The importance of this response as a host defense is illustrated by the fact that inhibition of exfoliation with a pan-caspase inhibitor dramatically reduces bacterial clearance from the bladder early in the infection. Furthermore, exfoliation rates vary among different inbred mouse strains and those with the slowest exfoliation rates have the highest bacterial burden during the acute phase of the infection. Thus, exfoliation is a powerful means to eradicate both attached and internalized bacteria from the bladder epithelium. However, mouse studies have also shown that although bladder titers decrease after exfoliation, bacteria still remain in the bladder after this process is complete. This result implies that UPEC must have a means of dealing with this effective host defense.

On entering a superficial bladder epithelial cell, UPEC do not wait for their impeding clearance by exfoliation, but instead they began to proliferate, forming large intracellular inclusions termed bacterial factories (unpublished data). This phenomenon also occurs in vitro and is specific to strains of UPEC, as K12 strains expressing type 1 pili can invade but cannot replicate efficiently within bladder epithelial cells. It has recently been demonstrated that after replication, UPEC can flux out of dying host cells before the completion of exfoliation, thereby allowing the bacteria to escape clearance by this innate defense. As UPEC reemerge from bladder epithelial cells, they often have a filamentous morphology, which simultaneously allows them to make contact with adjacent and/or underlying epithelial cells. It is possible that filamentous UPEC facilitate bacterial interactions with neighboring and underlying bladder epithelial cells. Such observations have suggested that the ability of UPEC to replicate intracellularly and to subsequently flux out of bladder epithelial cells likely contributes to bacterial spread and persistence within the urinary tract.

In addition to inducing exfoliation, the presence of type 1-piliferous UPEC in the bladder also activates the production of inflammatory cytokines and chemokines by epithelial cells. Although the expression of type 1 pili has been shown to
enhance epithelial cytokine production in response to E. coli, only recently have the molecular details of this phenomenon been clarified using an in vitro system. Type 1 pili do not directly stimulate epithelial cytokine responses, but instead activate this response indirectly by mediating bacterial invasion of bladder epithelial cells.14

Intriguingly, it was shown that bacterial invasion leads to increased cytokine production by enhancing epithelial responsiveness to E. coli through a lipopolysaccharide (LPS)-dependent mechanism. These findings demonstrate that LPS recognition is, in fact, an important component of epithelial innate defense. The means by which invasion boosts epithelial responsiveness to E. coli is unclear; however, several recent studies suggest that either receptor clustering during the invasion process, endosomally localized LPS receptors, or cytoplasmic LPS receptors may explain this phenomenon.15,16

The glycosylphosphotidylinositol (GPI)-linked membrane protein CD14 has been recognized as a receptor important for host responses to LPS.17,18 However, this molecule does not possess a cytoplasmic domain and thus would be incapable of transducing an activation signal to the nucleus. New findings in the area of LPS recognition have identified toll-like receptor (TLR) 4 as the signaling component of the LPS receptor complex.19 Substantial evidence exists to suggest that TLR4 directly interacts with the lipid A moiety of LPS and subsequently activates a signal transduction cascade ultimately leading to the activation of nuclear factor-κB and the production of proinflammatory cytokines and chemokines.20 Further research into the role of CD14 and TLRs in epithelial responses to bacterial pathogens will be necessary to understand the means by which epithelial cells recognize pathogens and participate in host responses at mucosal surfaces.

The relevance of LPS as a critical player in driving the activation of bladder epithelial cells in response to type 1-pilated E. coli in vivo has been illustrated using the LPS-hyporesponsive mouse strain C3H/HeJ. C3H/HeJ mice have a point mutation in the cytoplasmic domain of TLR4.21 This mutation has been shown to produce a dominant negative form of TLR4 that is incapable of eliciting biologic responses to LPS.22,23 Previous studies using a mouse model of acute pyelonephritis have demonstrated that C3H/HeJ mice have severe defects in their ability to clear E. coli from the kidney in comparison to their TLR4 normal sister strain C3H/HeN.24 In addition, UPEC has been shown to establish a chronic, high-level infection in the bladders of C3H/HeJ mice, which diverges significantly from C3H/HeN mice by day 7 after infection.25 However, the early effects of a TLR4 mutation on host responses to UPEC in the bladder, when epithelial cells would be most likely to contribute to host defenses, have not been extensively investigated. In a recent study it was shown that C3H/HeJ and C3H/HeN mice have comparable bacterial titers in the bladder at 10 hours after infection.14 However, by 48 hours after infection, C3H/HeN mice have reduced the number of bacteria in the bladder by 90%, whereas C3H/HeJ mice have failed to reduce bacterial titers. Histologic analysis of bladder tissue at these same time points revealed that the type 1-piliferous strain of UPEC used in this study invaded and replicated efficiently within the bladder epithelial cells of both mouse strains, but only in the C3H/HeN mice were there signs of epithelial activation, such as neutrophil recruitment into the epithelium, as a consequence of these events. In fact, the epithelia of C3H/HeJ mice were inundated with bacteria, yet very few inflammatory cells were present in either the epithelium or the lamina propria, suggesting a role for bladder epithelial TLR4 in alerting the underlying tissue to the presence of pathogen.

As the inflammatory response triggered by the recognition of type 1-piliferous UPEC in the bladder can be detrimental to bacterial survival, uropathogens have likely developed strategies to alter this response. Consistent with this concept several type 1-piliferous strains of UPEC have been shown to elicit a significantly more dampened epithelial cytokine response than type 1-piliferous K12 strains of E. coli (unpublished observations). However, the mechanism by which uropathogenic strains of E. coli decrease the amount of cytokines produced by bladder epithelial cells is currently unknown.

**CHRONIC PHASE OF URINARY TRACT INFECTIONS**

As described above, innate host defenses in the bladder are extremely effective early in the infection, clearing up to 99% of the infecting bacteria. However, a significant population of bacteria, ranging from a few hundred to a few thousand organisms, is able to persist in the bladder tissue. Most of these bacteria are intracellular, as demonstrated by ex vivo gentamicin protection assays, suggesting that invasion of bladder epithelial cells is linked to bacterial persistence in the bladder.3 Interestingly, the bacteria that persist seem to enter a quiescent state, potentially within the underlying epithelium. Ironically, it is the exfoliation of superficial cells that exposes the underlying epithelium and thus facilitates the interaction between bacteria and this deeper cell layer. In light of this fact, exfoliation can be seen as a double-edged sword for the host during a UTI; clearing a significant portion
FIGURE 1. Model of urinary tract infection (UTI) pathogenesis. When uropathogenic Escherichia coli enter the bladder, the organisms face a formidable array of constitutive host defenses, including uromucoid (um), Tamm-Horsfall protein (THP), secretory immunoglobulin A (SIgA), the flow of urine, and challenging growth conditions. To overcome and evade these defenses the bacteria bind to and invade the superficial bladder epithelial cells largely via the expression of type 1 pili. However, these events trigger inducible host defenses such as superficial cell apoptosis/exfoliation and cytokine/chemokine production. These defenses, respectively, lead to the sloughing of infected cells into the urine and the recruitment of inflammatory cells. As a means of coping with exfoliation, uropathogenic strains of E. coli have evolved to be able to replicate and flux out of dying host cells before completion of the death program, potentially facilitating bacterial interactions with the underlying epithelium. In addition, clinical strains of E. coli are able to blunt the cytokine/chemokine response of bladder epithelial cells. The end result of these dynamic interactions during the acute infection is the establishment of a persistent reservoir of intracellular bacteria within the bladder epithelium. It is possible such bacteria contribute to UTI recurrence and interstitial cystitis. IL-6 = interleukin-6; IL-8 = interleukin-8.
of the bacterial burden, but exposing the underlying epithelium to the infecting pathogen.

Although the establishment of this reservoir occurs during the acute infection, the pathogenic relevance of this bacterial population is likely during the weeks to months after an acute UTI. It has been shown in mice that these bacteria can persist in the bladder for at least 6 weeks after infection, with only a 30% clearance rate during this time interval. This unexpected result suggests that this reservoir of bacteria somehow avoids or delays recognition by innate and adaptive immunosurveillance mechanisms. Of additional importance is the observation that these bacteria are resistant to antibiotic therapy. These properties of the UPEC reservoir may be due to the small number of organisms present, their location in the tissue, or their quiescent state. Also of interest is that the vast majority of mice with persistent bacterial reservoirs have sterile urine, indicating that bacterial persistence in the tissue can go undetected by uroanalysis. In summary, the bacterial reservoir established by UPEC could be described as an occult infection involving small numbers of bacteria which are at least partially resistant to host defenses and antibiotics.

RECURRENT URINARY TRACT INFECTIONS

After an initial episode of a UTI, 25% of women will experience a recurrence within the next 6 months. In addition, a significant percentage of “reinfections” are caused by a strain previously isolated from the individual. It is presently assumed that recurrent infections are caused by a re inoculation of the bladder with bacteria residing in the gastrointestinal tract. However, another potential mechanism to explain the high propensity of UTI recurrence is the existence of bacteria that can persist within the bladder tissue itself. In this scenario, re-emergence of bacteria from a quiescent state to an active replicative state, could lead a “new” infection. If this were the case, new approaches toward the clinical management of recurrent UTIs would be indicated.

INTERSTITIAL CYSTITIS

Interstitial cystitis (IC) is a complex inflammatory disorder of the bladder with an unclear etiology. The role of infectious agents in this disease have been questioned due to the absence of bacteria in the urine, the inability to detect bacteria in bladder biopsy samples by microscopy, and the ineffectiveness of antibiotics in treating the disorder. However, the possibility that a bacterial factor contributes to IC pathogenesis has not been excluded. As described above, the bacterial reservoir formed by UPEC during an acute UTI is associated with low levels of bacteria in the tissue, but not the urine, suggesting that bacteria would be difficult to identify using traditional microbiologic or imaging techniques. Furthermore, these bacteria appear to be resistant to antibiotics, consistent with the ineffectiveness of antibiotic therapy for treatment of IC. Although the role of persistent E. coli in IC remains to be determined, these results are the first direct evidence that such a bacterial stimulus could exist silently within the bladder tissue. Perhaps this explains the similar epidemiology of IC and UTIs at least in some patients.

CONCLUSION

The interplay between type 1-piliferous UPEC and innate host defenses in the bladder is a striking example of the dynamic evolution of host-pathogen interactions at a mucosal interface. As depicted in Figure 1, a UTI is the result a series of moves and countermoves by the host and pathogen, which ultimately determine the natural history of the infection. The ability of UPEC to persist within bladder tissue for weeks after the acute infection suggests a previously unrecognized role for these bacteria in chronic diseases of the urinary tract such as recurrent UTIs and IC. Further research into the complex pathogenesis of cystitis and other UTIs should lead to new and better therapies for acute and recurrent infections in the urinary tract.

REFERENCES