Enhanced Susceptibility to Urinary Tract Infection in the Spinal Cord-Injured Host with Neurogenic Bladder

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Neurogenic bladder predisposes to recurrent urinary tract infections (UTI) and renal failure, and susceptibility is commonly ascribed to urinary stasis from elevated residual urine volumes. *Escherichia coli* UTI was modeled in the spinal cord-injured (SCI) rat with the hypothesis that SCI animals would require fewer bacteria to establish infection, have an exaggerated inflammatory response, and have delayed clearance of infection compared to normal-voiding controls. T10 SCI rats and controls had median infectious doses (ID₅₀) of 10² and 10⁵ CFU, respectively. Mean residual volumes in the SCI animals did not correlate with susceptibility to initiation of UTI or outcome. In the acute infection, control and SCI rats developed acute cystitis and pyelitis without acute differences in histopathological scores of inflammation. However, *in vivo* imaging of infected animals revealed persistently higher levels of bacteria in the SCI urine and bladders than were seen for controls over 2 weeks. Likewise, at 2 weeks, acute and chronic inflammatory infiltrates persisted in the bladders and kidneys of SCI rats, whereas inflammation largely resolved within the controls. Together these data demonstrate that SCI rats exhibit delayed clearance of infection and exaggerated inflammatory responses in bladders and kidneys; however, the severity of residual volumes does not predict increased susceptibility to UTI. These studies suggest that host-dependent mechanisms that are discrete from alterations in bladder physiology influence UTI susceptibility with the SCI-neurogenic bladder. This model will allow elucidation of SCI-neurogenic bladder-mediated changes in host response that yield UTI susceptibility and may lead to new preventative and therapeutic options.

Neurogenic bladder refers to bladder dysfunction that is secondary to disruption of the central or peripheral nervous system pathways that regulate bladder storage and emptying. In children, the most common causes of neurogenic bladder are neural tube defects, such as spina bifida (myelomeningocele), while in adults, the most common cause is trauma-related spinal cord injury (SCI). Regardless of etiology, all patients with neurogenic bladder are at significantly increased risk for recurrent urinary tract infections (UTI) compared to the normally voiding population, with an annual incidence as high as 20 to 25% (1).

A number of risk factors have been postulated for the increased susceptibility to UTI in patients with neurogenic bladder. They include urinary stasis due to elevated postvoid residual volumes, bladder overdistention, high-pressure voiding, vesicoureteral reflux, nephrolithiasis, and the use of chronic indwelling catheters. Many patients with neurogenic bladder perform scheduled clean intermittent catheterizations (CIC) to assist with bladder emptying. However, these individuals still have a 4-fold-increased risk of UTI compared to those who do not perform CIC (2, 3). Given the frequency of UTI, many neurogenic bladder patients are exposed to repeated courses of therapeutic antibiotics, thereby increasing the incidence of multidrug-resistant bacterial infections in this population. Compounding these challenges in medical management, the use of prophylactic oral antibiotics to prevent UTI among those with neurogenic bladder has been shown to increase the risk of developing subsequent UTI with antibiotic-resistant bacteria without a significant benefit in reducing the incidence of UTI (4, 5).

Nonantibiotic prophylaxis and alternative treatments for recurrent UTI are greatly needed for patients with neurogenic bladder.

Efforts to identify new treatments have been hindered by the lack of a defined animal model of experimental UTI in the neurogenic bladder host. Among existing models of SCI, the rat model of spinal cord transection or contusion is the most well established. Most studies have used this animal model of SCI to examine spinal cord regeneration and functional recovery (reviewed in references 6 and 7). A number of groups have investigated the neurogenic bladder phenotype in SCI rats and have demonstrated that the urodynamic profiles in these animals are similar to those seen in humans with SCI or spinal dysraphism (8–10). This suggests that the SCI rat may be a useful system for modeling UTI with neurogenic bladder. Development of such a model would allow one to distinguish between mechanical and physiological mechanisms that increase the susceptibility to UTI with neurogenic bladder. Specifically, such a model would allow the exploration of discrete host factors that influence susceptibility to UTI in the neurogenic bladder population, as well as the innate and adaptive immune responses to infection and how they differ from spinally intact hosts. The model would also be a translational plat-

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form for the development of prophylaxis and treatment strategies for UTI in this high-risk population.

The aim of this study was to create a tractable animal model of experimental UTI with neurogenic bladder and to test the hypothesis that the severity of bladder voiding dysfunction correlates with susceptibility to acute UTI. We employed the well-defined technique of complete T10 spinal cord transection in rats to create neurogenic bladder and then infected these animals by transurethral inoculation with a uropathogenic strain of *E. coli* to induce UTI. Contrary to our hypothesis, we obtained evidence that the degree of voiding dysfunction, as assessed by degree of urinary stasis and urinary residual volumes, does not closely correlate with susceptibility to infection, implicating an altered host response in the failure to block initiation and clearance of UTI with neurogenic bladder. By examining both the acute and late phases of UTI, we have demonstrated that SCI rats are not only more susceptible to UTI than their normally voiding counterparts, but they also take longer to clear their infections. Furthermore, SCI rats display a heightened inflammatory response to infection at late time points.

We propose that our model of UTI in the SCI rat resembles the infectious process in humans with neurogenic bladder and will serve as a useful tool both to study the mechanisms of increased susceptibility to recurrent UTI in this population and to develop novel treatments.

MATERIALS AND METHODS

Animals and spinal cord injury. Six- to twelve-week-old female Sprague-Dawley rats (Charles River Laboratories, Wilmington MA), initially weighing 175 to 200 g, were used according to experimental protocols approved by the Duke University Institutional Animal Care and Use Committee. All surgeries were performed under a dissecting microscope with animals maintained under 2% inhaled isoflurane anesthesia. Spinal cord injury was induced by performing T10 laminectomy followed by complete spinal cord transection at this level with iridectomy scissors. Gelfoam (Medisave Pharmacy, Canada) was inserted into the space between the cranial and caudal ends of the cord to ensure separation and hemostasis. Muscle and skin were then approximated using absorbable suture. For control animals undergoing sham surgery, exposure of the T10 lamina was performed, but neither laminectomy nor cord transection was performed. All animals received meloxicam (5-mg/kg of body weight subcutaneous injection daily for 3 days) for postoperative analgesia and enrofloxacin (10 mg/kg subcutaneous injection twice daily up to 72 h prior to infection) for antibiotic prophylaxis.

Urine collection. SCI rats underwent manual expression of their bladders via Credé maneuver twice daily for the duration of each experiment. Daily residual urine volumes were always determined from morning volumes to avoid diurnal variability in urine production. Because control rats which have undergone sham surgery still have intact micturition pathways and minimal residual urine volumes (P. C. Dolber, unpublished), these animals did not undergo regular Credé maneuver. To obtain urine from control rats at designated times postinfection, these animals were first anesthetized with 2% inhaled isoflurane and administered 2 ml phosphate-buffered saline (PBS) intravenously (i.v.) via the tail vein to facilitate urine production. After 30 min, urine was collected via either Credé maneuver or transurethral catheterization, as indicated in Results.

Bacterial strains and growth conditions. UTI89 is a clinical cystitis isolate from humans previously described by Mulvey et al. (11). Acute infections were carried out to determine the median infectious dose (ID_{50}) using a strain of UTI89 transformed with pCOM-GFP (referred to as UTI89-GFP) and selected with 50 μ g/ml kanamycin sulfate. For *in vivo* imaging experiments, an isogenic strain of UTI89 carrying the complete *lux* operon (*luxCDABE*) from *Photobacterium luminescens* was used for in-

fection (referred to as UTI89-*lux*). UTI89-*lux* was also selected with 50 μ g/ml kanamycin sulfate. UTI89-*lux* and UTI89-GFP demonstrated similar growth characteristics *in vitro* (data not shown).

For all animal infections, UTI89 was cultured at 37°C overnight in Luria broth (LB) supplemented with 50 μ g/ml kanamycin sulfate. Bacteria were then back diluted 1/100 into fresh LB and grown for an additional 18 h at 37°C before being diluted in PBS to the stated inocula used for infection. UTI89 was subsequently isolated from infected urine and homogenized organs by plating on LB agar containing 50 μ g/ml kanamycin sulfate.

Experimental UTI, determination of ID_{50} , and *in vivo* bioluminescent imaging. All animal infections were performed under 2% inhaled isoflurane. Briefly, anesthetized rats were transurethrally catheterized with a lubricated, sterile 22-gauge angiocatheter. Urine was then aspirated prior to intentional inoculation, and this urine was plated on both LB agar supplemented with 50- μ g/ml kanamycin sulfate and McConkey agar to confirm sterility of urine prior to infection. After the aspiration of test urine, 100 μ l of *E. coli* UTI89 at the stated inoculum was slowly instilled via the angiocatheter into the bladders of rats over 1 min to avoid vesicoureteral reflux. The results displayed for each experiment were combined from 2 to 3 biological replicates. The rare number of SCI animals ($n = 2$) that developed spontaneous infections of their urine with other Gram-negative organisms prior to UTI89 inoculation were excluded from our studies.

To determine the ID_{50} of UTI89, SCI and control rats were transurethrally inoculated with various concentrations of UTI89-GFP (10^2 , 10^3 , 10^5 , or 10^7 CFU). At 24 h postinfection (hpi), rats were transurethrally catheterized, and urine was aspirated and collected. Rats were subsequently euthanized, and their bladders, kidneys, spleens, and livers were collected. One-half of each organ was fixed in 10% neutral buffered formalin for histologic processing (see below). The remaining one-half of each organ was homogenized in PBS–0.02% Triton X-100 using a hand-held electric tissue dissociator. To enumerate CFU/ml urine or CFU/organ, 10-fold dilutions of urine or homogenized organs were made in sterile PBS and plated on LB agar supplemented with 50 μ g/ml kanamycin sulfate to select for UTI89. Dilutions were also plated on McConkey agar to confirm bacterial counts of lactose-fermenting Gram-negative bacteria, consistent with the *E. coli* UTI89 infection.

In vivo bioluminescent-imaging studies of infected rats were performed using the Ivis Spectrum imaging system (Xenogen, Caliper Life Sciences, Hopkinton, MA). Briefly, animals were maintained under 2% inhaled isoflurane in the Ivis chamber and imaged for a maximum of 1 min in both the dorsal and ventral positions at various times postinfection with UTI89-*lux*. Preinfection images were initially obtained to measure the level of background luminescence from each animal. Imaging at each time point was performed prior to urine collection from SCI and control rats. Living Imaging software (Xenogen) was used to measure the number of photon emissions from defined regions of interest over the bladder (on ventral images) and over each kidney (on dorsal images). The average radiance (photons/s/cm²/sr) emitted from each bladder and kidney at times postinfection was normalized by first subtracting out the background luminescence from a region of interest defined over each animal's thorax and then subtracting out the background luminescence of either the bladder or kidney obtained from preinfection images. All animals were euthanized immediately after the final imaging time point at 14 days postinfection (dpi), and organs were processed as described above to enumerate CFU of UTI89 and to perform histologic analysis.

Histopathology. Bladders and kidneys were longitudinally cut in half, and one-half of each organ was fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned (5 to 7 μ m thick), and stained with hematoxylin and eosin. The severity of inflammation in the bladders and kidneys was determined in a blinded fashion using the following histologic grading criteria, described previously (12, 13): for bladder, 0, normal; 1, focal subepithelial inflammatory cell infiltration; 2, diffuse subepithelial inflammatory cell infiltration with edema; 3, marked subepithelial

FIG 1 SCI rats develop a neurogenic bladder phenotype characterized by elevated residual urine volumes. Following complete T10 spinal cord transection, SCI rats fail to empty their bladders completely. SCI rats underwent manual emptying of their bladders twice daily, with measurement of morning residual urine volumes. Residual urine volumes stabilized by 2 weeks postinjury, with a mean volume of 2.94 ml (range = 0.15 ml to 10.6 ml) on postoperative day 14. Spinally intact control rats had no measurable residual urine volumes over the 2-week course postinjury and are not shown. Individual circles represent individual SCI animals, and the dashed black line highlights the mean residual urine volumes for each day postinjury.

inflammatory cell infiltration with neutrophils in and on bladder mucosal epithelium with or without associated necrosis; 4, meets criteria for grade 3, plus inflammatory cell infiltrate extends into muscularis propria; and 5, full-thickness inflammatory cell infiltration with loss of surface epithelium/necrosis; for kidneys, 0, normal; 1, mild pyelitis with focal infiltration of low to moderate numbers of neutrophils confined to the uroepithelium and subepithelium of the renal pelvis with or without edema; there can be focal necrosis of individual epithelial cells, but urothelium is intact overall; 2, moderate pyelitis with diffuse infiltration of moderate numbers of neutrophils in uroepithelium and in adjacent underlying parenchyma; diffuse damage to uroepithelium; 3, severe pyelitis with moderate damage (i.e., necrosis/ulceration) to the uroepithelium and moderate inflammation extending into the inner medulla only; 4, acute pyelonephritis extending into the outer medulla and/or involving <50% of the kidney; 5, acute pyelonephritis extending into the renal cortex and/or involving >50% of the kidney. All images were taken at the Duke University Light Microscopy Core Facility using the Zeiss Axio Imager system with Metamorph software (Molecular Devices, Inc., Sunnyvale, CA).

Statistical analysis. Data were assumed to follow a non-Gaussian distribution. All data were analyzed using unpaired, nonparametric statistics (Mann-Whitney test or Fisher's exact test, as indicated in Results).

RESULTS

SCI rats develop a neurogenic bladder phenotype characterized by elevated residual urine volumes. Prior studies of T10 SCI in rats have demonstrated phenotypes of hind limb paralysis and neurogenic bladder characterized by decreased voiding efficiency, high micturition pressures, high bladder capacities, and elevated residual urine volumes (10). These phenotypes are similar to that seen in humans with thoracic SCI. To begin to characterize our experimental model of UTI in SCI rats, we first evaluated the reproducibility and stability of neurogenic bladder in spinal cord-injured animals ($n = 42$) using residual urine volumes as a non-invasive marker of the phenotype. As shown in Fig. 1, the average morning residual urine volume increased over the first week postinjury and peaked in volume between 6 to 8 days postinjury.

Average residual urine volumes declined over the second week, consistent with development of spinal micturition reflexes which occur at ~1 week post-SCI (14). By 14 days postinjury, residual urine volumes had stabilized at a mean of ~2.94 ml (range, 0.15 ml to 10.6 ml), which is approximately twice the bladder capacity of spinal cord-intact rats (15). Notably, mean residual urine volumes in SCI rats remain stable at ~2.5 to 3.0 ml for at least an additional 2 weeks (i.e., up to 4 weeks post-SCI; data not shown). In contrast to SCI rats, control rats that underwent sham surgery ($n = 29$) had minimal residual urine volumes over the 2-week postsurgical period (data not shown). These results suggest that complete T10 spinal cord transection in rats results in a durable neurogenic bladder phenotype that can be easily and reliably followed by measurement of daily residual urine volumes.

SCI rats are exquisitely susceptible to acute *E. coli* urinary tract infection compared to control rats. Patients with neurogenic bladder are at significantly increased risk of UTI compared to the spinal cord-intact population, although the precise etiology for this increased susceptibility has not been experimentally well defined (1). We hypothesized that like their human counterparts, rats with SCI would be more susceptible than controls to acute bacterial cystitis and ascending UTI. To address this hypothesis, we determined the ID_{50} for UTI using a human-uropathogenic strain of *E. coli*, UTI89. Lower ID_{50} values for a group of animals correspond to increased susceptibility to infection.

After residual urine volumes had stabilized at 2 weeks postinjury, SCI and control rats were transurethrally inoculated over a range of doses of UTI89 (10^2 to 10^7 CFU). At 24 hpi, urine, bladders, and kidneys were harvested, and the CFU/ml urine or CFU/organ of UTI89 was enumerated. As shown in Fig. 2, SCI rats were more susceptible to acute lower UTI than their control counterparts. The ID_{50} for control rats was ~ 10^5 CFU of UTI89, with 4 of 7 control rats (57%) establishing infection in their urine and bladders at this inoculum. At an inoculum of 10^3 , only one of four (25%) control rats, compared to four of four (100%) SCI rats, was infected ($P = 0.048$, Fisher's exact for urine and bladders). Three of six SCI rats (50%) were still infected at an inoculum of 10^2 CFU of UTI89, thus approximating the ID_{50} in the SCI animals to be 3 logs lower than the approximate ID_{50} for the control animals. The incidence of ascending infection of the kidneys appeared to be similar between SCI and control rats at the higher inocula tested (10^5 and 10^7 CFU). As the inoculum decreased, the number of animals establishing infections in their kidneys also decreased among SCI and control rats, paralleling the lower rates of urine and bladder infections. Notably, at an inoculum of 10^2 CFU of UTI89, infection in the urine and bladder of SCI rats occurred in the absence of ascending kidney infections. These results suggest that this animal model of UTI in the SCI rat recapitulates the clinical scenario of increased susceptibility to lower UTI in the neurogenic bladder population compared to that in the normally voiding population.

The severity of UTI in SCI rats does not directly correlate with residual urine volumes. Precise mechanisms accounting for the increased susceptibility of SCI patients to UTI are unknown, although a major postulate is the presence of elevated postvoid residual urine volumes that can lead to urinary stasis and promote bacterial growth. To determine if residual urine volumes influence the susceptibility to acute UTI in our animal model, we calculated the Spearman rank correlation coefficient (Spearman's rho) between the morning residual urine volumes in SCI rats at the time

FIG 2 SCI rats are more susceptible to acute *E. coli* UTI than control rats. To determine the median infectious dose (ID_{50}) of experimental *E. coli* UTI in SCI versus spinally intact control rats, animals were transurethraly inoculated with 10^2 to 10^7 CFU of the prototypic *E. coli* cystitis strain, UTI89 (x axis). Urine, bladders, and kidneys were harvested at 24 hpi and plated to enumerate CFU of UTI89 (y axis). The ID_{50} for control rats is $\sim 10^5$ CFU (A), while the ID_{50} for SCI rats is $\sim 10^3$ CFU (B). At the lowest inoculum compared (10^3 CFU), there was a statistically significant difference between the numbers of control animals and SCI animals that established infections within their urine and bladders ($P = 0.048$, Fisher's exact test). Individual symbols represent individual animals, solid black lines represent the geometric mean for each group, and the dashed black line represents the level of detection for the assay.

of infection (14 days postinjury) and bacterial CFU recovered from urine and bladders at 24 hpi. Spearman's rho was calculated for each bacterial inoculum tested. Contrary to our original hypothesis that higher residual urine volumes would be associated with more-severe infections, we detected no significant correlation between these two variables (Table 1). Although this finding does not exclude the possibility that elevated residual urine volumes *per se* predispose to UTI, it does suggest that as a marker of voiding dysfunction, urine retention volumes do not predict susceptibility to infection. Severity of urine retention does not appear to fully account for the increased susceptibility to UTI with neurogenic bladder.

SCI rats have higher prolonged subacute bacteriuria and bladder infection than control rats despite a 3-log-lower initial inoculum. The 3-log difference in the inoculum of UTI89 necessary to establish acute infection in SCI versus control rats suggested that there are discrepancies in the initial establishment of infection between these two groups of animals. We reasoned that this difference in susceptibility to infection may persist throughout the course of infection, in part because SCI rats lack the normal voiding mechanism to clear bacteria from their urine. To evaluate the temporal course of infection in real time, we used a bioluminescent strain of UTI89 that carries the complete *lux* operon (*luxCDABE*) from *Photobacterium luminescens* (referred to as UTI89-*lux*). Two weeks after SCI or sham surgery, SCI rats or control rats were transurethraly inoculated with UTI89-*lux*. To

ensure 100% infection of both SCI and control rats but still allow a reasonable comparison of the inflammatory response to infection, we inoculated SCI and control rats with UTI89-*lux* at a dose corresponding to ~ 1.5 logs above their respective ID_{50} values (i.e., 3.5×10^3 CFU for SCI rats and 3.5×10^6 CFU for control rats, as determined by the findings shown in Fig. 2).

In vivo imaging of infected animals was performed at different times postinfection to track the temporospatial course of UTI89 infection in SCI versus control rats over 2 weeks. Images from a representative time course are shown in Fig. 3A. Regions of interest were drawn around each bladder and kidney, and the average radiance (photons/s/cm²/sr) emitted from these organs is plotted in Fig. 3B and C. Urine was manually expressed and collected from infected SCI and control rats at different times postinfection, and the number of CFU of UTI89 in the urine was enumerated over 2 weeks (Fig. 3D). Consistent with results of our acute infection studies (Fig. 2), both SCI and control animals develop detectable infections in their urine and bladders by 24 hpi at the respective inocula used. After 24 hpi, control rats started to clear their infections as evidenced by a decline from the peak of infection in both the number of organisms measured in the urine (Fig. 3D) and the average radiance detected from bladders (Fig. 3B) over the 2-week period. At 14 days postinfection (dpi), control rats had an average of 3.734×10^5 CFU of UTI89 per ml urine (SD, $\pm 8.262 \times 10^5$ CFU/ml) (Fig. 3D) and an average radiance of 2.459×10^5 photons/s/cm²/sr (SD, $\pm 3.528 \times 10^5$) (Fig. 3B) from their bladders. In contrast, at the lower inoculum used for SCI rats, the peak of infection in the bladders and urine occurred at ~ 72 hpi. However, once the infection peaked, bacteriuria and bladder radiance were persistently elevated in SCI rats for the remainder of the 2 weeks. At 14 dpi, SCI rats had an average of 1.099×10^8 CFU/ml urine (SD, $\pm 1.949 \times 10^8$ CFU/ml) (Fig. 3D) and an average radiance of 1.414×10^6 photons/s/cm²/sr (SD, $\pm 1.570 \times 10^6$) (Fig. 3B) from their bladders. Statistical analysis approached but did not reach significance in comparison of SCI and control animals at each time point (lowest P value = 0.057). *In vivo* imaging also demonstrated infections within the kidneys of SCI and control

TABLE 1 Spearman's rho for relationship between residual urine volumes at the time of UTI89 infection and the number of CFU in urine and bladders of SCI rats at 24 hpi (for each given bacterial inoculum)

Inoculum	Spearman's rho		Significant correlation?
	Urine	Bladder	
10^2	-0.143	0.029	No
10^3	0.400	0.400	No
10^5	-0.200	-0.543	No
10^7	0.018	-0.082	No

FIG 3 SCI rats maintain high levels of UTI89 infection in urine and bladders for at least 14 days postinfection. (A) Control and SCI rats were transurethraally inoculated with a bioluminescent strain of UTI89 carrying the complete *lux* operon (*luxCDABE*) from *Photobacterium luminescens*. The inoculum used for each group was ~ 1.5 logs above their respective ID_{50} values (control, 3.5×10^6 CFU; SCI, 3.5×10^3 CFU). *In vivo* imaging with the IVIS Spectrum imaging system (Xenogen) was performed to determine the temporospatial course of UTI89 infection. Animals were imaged in both the ventral (V) and dorsal (D) positions to maximize detection in the bladders and kidneys, respectively. Representative images are shown in panel A. Uninf., uninfected. Regions of interest were identified over the bladders and kidneys of each animal for each time point, and the average radiance of emitted photons (in photons/s/cm²/sr) from bladders (B) or kidneys (C) of control ($n = 4$) versus SCI ($n = 3$) animals is plotted. Because of the high levels of background interference detected over the kidneys, the threshold level of detection for kidneys was set at 5×10^3 p/s/cm²/sr. The average CFU in urine collected from control ($n = 7$) and SCI animals ($n = 7$) over 2 weeks postinfection is shown in panel D. Squares in panels B and D represent the arithmetic mean values for all animals in each group, and error bars represent standard deviations from the means. Symbols in panel C represent values for individual animals. Closed symbols with black lines represent control animals, whereas open symbols with gray lines represent SCI animals. Panel E shows the numbers of CFU present in the urine, bladders, and kidneys of control and SCI animals at 14 dpi. Individual symbols represent individual animals, solid black lines represent the geometric mean for each group, and the dashed black line represents the level of detection for the assay.

animals over 2 weeks and 1 week, respectively (Fig. 3A and C). Similar to the results for lower urinary tract infections, SCI rats took longer than control rats to reach peak levels of infection within their kidneys. However, infections within the kidney remain detectable by *in vivo* imaging for only 48 to 72 h.

Because *in vivo* imaging may not be sensitive enough to detect low numbers of bioluminescent organisms within the upper urinary tract (16), SCI and control animals were subsequently sacrificed at 14 dpi, and the number of CFU of UTI89 was enumerated from the urine and from bladder and kidney homogenates. Consistent with the *in vivo* imaging studies, SCI rats had CFU of UTI89 in their urine and bladders ~ 3 logs higher than numbers for con-

trol rats at 14 dpi ($P = 0.1649$ for urine; $P = 0.1282$ for bladders) (Fig. 3E). The percentage of SCI rats with high burdens of organisms in the bladder ($>10^5$ CFU/ml or organ) was significantly greater than that for controls ($P = 0.02$, bladder; $P = 0.29$, urine [by Fisher's exact test]). Interestingly, although *in vivo* imaging at 14 dpi no longer detected signals from the kidneys of either SCI or control rats, enumeration of bacteria from kidney homogenates revealed that the majority of SCI animals (5/7) and control animals (6/7) continued to have detectable numbers of UTI89 in at least one kidney at this late time point. The SCI animals had on average approximately 1 log higher kidney CFU at 14 dpi ($P = 0.239$), although they fell into 2 groups, with approximately half of

the kidneys with low to undetectable counts and the other half with elevated counts relative to those of the controls. The degree of kidney infection did not correlate with residual volumes (data not shown). From these data, it appears that *in vivo* imaging is less sensitive at detecting luminescent UTI89 organisms in the kidneys after acute infection than the more accurate detection in the bladders and urine of live animals. This difference in detection may be related to different oxygen levels within bladders versus kidneys, since the luciferase reaction is dependent upon intracellular ATP levels, which can be affected by the oxygen saturation in tissues (17). Alternatively, there may be a difference in the attenuation of signal from surrounding tissues. Together, these results suggest that SCI rats are not only more susceptible than spinal cord-intact rats to the acute initiation and establishment of *E. coli* UTI, but SCI animals also take longer to clear this infection once established. Furthermore, this difference in susceptibility is more pronounced in the lower urinary tract than in the upper tracts.

SCI rats display exaggerated inflammatory responses to subacute infection. One possible explanation for the increased susceptibility to acute UTI and the delayed clearance of infection among SCI rats is that these animals elicit an altered inflammatory response to infection compared to control rats. To begin to address this hypothesis, we performed a pathological analysis of infected bladders and kidneys after both acute (24 hpi) and subacute (14 dpi) infection. At 24 hpi, tissue pathology indicated that both SCI and control animals mount a robust acute inflammatory response to infection in their bladders and kidneys across a wide range of inocula. Representative images of infected bladders and kidneys from SCI and control animals at 24 hpi are shown in Fig. 4A to F. Neutrophilic infiltrates are present within the lamina propria and extend into the transitional epithelium in both infected SCI and control bladders (Fig. 4A and D). Likewise, neutrophils are present within the transitional epithelial layer of the renal pelvis and within the lumen of collecting ducts in both SCI and control rats (Fig. 4B and E).

Histologic analysis also revealed the presence of intracellular bacterial communities (IBC) at the luminal surface of bladders in both SCI and control rats (Fig. 4C and F). IBC are collections of bacteria that serve as intracellular foci for rapid bacterial replication protected from both the host inflammatory response and antibiotics (18–20). IBC have previously been shown to develop in the bladders of infected mice and humans; however, they have yet to be found in hosts with neurogenic bladder. Although quantifying differences in IBC number and density in SCI versus control bladders is limited by the asynchronous cycle of IBC formation and their asymmetric distribution within bladders, at the very least these results suggest that the neurogenic bladder epithelium remains competent for intracellular replication by UTI89.

Grading of inflammation in the bladders and kidneys of SCI and control rats at each inoculum was performed at 24 hpi according to the criteria outlined in Materials and Methods. Mean inflammatory scores plus or minus standard deviations are plotted in Fig. 4G. At higher inocula, there was a trend toward higher inflammatory scores for the bladders of control animals than for those of SCI animals (3.00 ± 1.55 versus 1.67 ± 1.03 at 10^5 CFU and 2.50 ± 0.58 versus 1.82 ± 0.98 at 10^7 CFU), although the observed differences did not reach statistical significance ($P = 0.180$ for 10^5 CFU; $P = 0.264$ for 10^7 CFU). At an inoculum of 10^3 CFU, it is not surprising that there was minimal inflammation in the bladders of control rats, since the majority of these animals

FIG 4 Both SCI and control rats develop cystitis and pyelitis during acute UTI89 infection. (A to F) Twenty-four hours after infection with UTI89, bladders and kidneys from SCI and control rats were fixed, embedded, sectioned, and stained with hematoxylin and eosin. Both control (bladder [A] and kidney [B] [magnification, $\times 40$]) and SCI (bladder [D] and kidney [E] [magnification, $\times 40$]) animals mounted robust acute inflammatory responses predominated by neutrophils (black arrows; highlighted in insets). Intracellular bacterial communities (IBC) were seen in the bladders of both control (C) (magnification, $\times 100$) and SCI (E) (magnification, $\times 100$) rats (white arrows; highlighted in insets). (G) Histologic grading of inflammation was performed by a pathologist who was blinded to the identity of samples. Error bars represent standard deviations from the means. “**” indicates a statistically significant difference between groups, with a P value of 0.029.

failed to establish an infection (Fig. 2A). Likewise, there was no statistically significant difference in the inflammatory response generated in the kidneys of control versus SCI rats at higher inocula ($P = 0.297$ for 10^5 CFU; $P = 0.057$ for 10^7 CFU). Only at an inoculum of 10^3 CFU, when the majority of control kidneys fail to establish an infection, is there a statistically significant difference between inflammatory scores for the kidneys of SCI versus control animals ($P = 0.029$). Interestingly, a trend toward higher inflam-

mation within the kidneys of SCI animals with decreasing inocula was observed (0.55 ± 1.01 at 10^7 CFU versus 1.00 ± 0.95 at 10^5 CFU versus 1.25 ± 1.17 at 10^3 CFU).

Histopathology was also performed with organs of SCI and control animals harvested at 2 weeks postinfection. As previously described, for these experiments, each group of animals was inoculated with 1.5 logs above the ID_{50} of UTI89 (i.e., 3.5×10^3 CFU for SCI rats and 3.5×10^6 CFU for control rats). As shown in Fig. 5A, control animals had essentially resolved all inflammation in their bladders at this late time point, despite prior data indicating sustained bacteriuria throughout the 2-week infection and measurable CFU in the bladders at the 14-dpi endpoint. In contrast, SCI animals continued to exhibit inflammation within their bladders at 14 dpi (Fig. 5D). In fact, there was evidence of mild chronic inflammation with scattered foci of mononuclear cell aggregates, resembling lymphoid follicles, within the lamina propria of SCI bladders (bracket). An interesting difference in the late inflammatory response between SCI and control rats was also noted within the kidneys. At 14 dpi, inflammation in the kidneys of control rats was mild and confined to the renal pelvis (Fig. 5B and C). In contrast, acute and chronic inflammatory infiltrates, including microabscesses, were seen extending from the renal pelvis into both the medulla and cortex of SCI kidneys (Fig. 5E and F). Objective grading of inflammation at 14 dpi is seen in Fig. 5G. The degree of bladder inflammation between control and SCI rats (0.00 ± 0.00 versus 1.75 ± 0.50 , respectively) was significantly different ($P = 0.029$) at this late time point. Despite qualitative differences in the inflammatory responses in kidneys of SCI animals versus those for control animals, inflammatory scores are not significantly different (0.63 ± 0.92 versus 2.17 ± 2.48 ; $P = 0.345$). Overall, these histologic data suggest that although control and SCI rats mount similar inflammatory responses early in infection, discrete differences in inflammation do emerge late in infection and are more pronounced within the lower urinary tract.

DISCUSSION

The urologic complications faced by patients with SCI or spina bifida are numerous and varied. They can include sexual dysfunction, urinary incontinence, and urolithiasis. However, one of the most significant urologic issues is UTI. Close to 60% of SCI patients develop bacteriuria or UTI within the first year postinjury, while 80% experience at least one UTI by their 16th year postinjury (21). Recurrent UTI account for major morbidity and mortality, with at least 15% of SCI patients dying from urinary sepsis (1, 22). In addition to the devastating health impact, there is also a social and financial impact associated with recurrent UTI. Several quality-of-life studies have cited bladder dysfunction and severe UTI as having a negative impact on quality-of-life outcomes (23). Likewise, UTI-related complications are a major cause of hospital readmissions in this population (24, 25), thereby increasing health care costs.

Despite the paramount significance of UTI in the neurogenic bladder population, effective preventive measures are limited. This is due in part to a limited understanding of the precise mechanisms accounting for the increased susceptibility to UTI in these patients. Several animal models of UTI exist (26); likewise, a variety of animal models of spinal cord injury have been described (6, 7). However, our study uniquely marries these two features into a tractable animal model of experimental *E. coli* UTI in a neurogenic

FIG 5 Significant differences in the inflammatory response to UTI89 are seen in the bladders of control versus SCI rats in late infection. (A to F) Two weeks after infection with UTI89, bladders and kidneys from SCI and control rats were fixed, embedded, sectioned, and stained with hematoxylin and eosin. Inflammation completely resolves in bladders of control animals (A) (magnification, $\times 40$), while mild inflammation is confined to the renal pelvis of these animals after 2 weeks of infection (magnification, $\times 40$ [B] or $\times 100$ [C]; renal pelvis is designated with a bracket; the black arrow and inset highlight neutrophils and mononuclear cells). In contrast, bladders of SCI animals (D) (magnification, $\times 40$) display chronic inflammatory infiltrates resembling lymphoid aggregates (designated with a bracket; the black arrowhead and inset highlight mononuclear cells) and kidneys (magnification, $\times 40$ [E] or $\times 100$ [F]) exhibit focal collections of acute inflammation that extend out to the cortex (black arrows and inset highlight neutrophils; white arrows designate glomeruli within the renal cortex). (G) Histologic grading of inflammation was performed by a pathologist who was blinded to the identities of samples. Error bars represent standard deviations from the mean. The symbol “Ψ” represents a mean inflammatory score of 0. “**” indicates a statistically significant difference between groups, with a P value of 0.029.

bladder population. An important strength of this model lies in its ability to recapitulate the association between neurogenic bladder and increased susceptibility to UTI that is seen in SCI and spina bifida patients. This feature allows one to use this model to inter-

rogate the molecular mechanisms that predispose the neurogenic bladder population to recurrent UTI. Equally exciting is the prospect that this model can be used to study novel prophylactic and therapeutic strategies to combat UTI in this high-risk group. Both lines of investigation are currently in progress.

An interesting finding in our model was the degree of biologic variability in the amount of residual urine volumes present after SCI. During the first 4 days after SCI, residual urine volumes have a narrow distribution. However, by the second week postinjury, residual urine volumes demonstrated greater variation. This was initially surprising given that all surgeries performed were complete transections of the spinal cord at the same thoracic cord level by the same surgeon. However, the wide variation in residual volumes has been reported by others (14, 27). Several theories have been proposed for this variability in recovery of bladder function after SCI. Some groups propose that differences in local neurotrophic factors may alter sprouting of primary afferent neurons, which influences bladder functional outcomes; others suggest that the timing and nature of the inflammatory reaction resulting from SCI may alter bladder function (14, 27–30). While this variability adds complexity to our model, it makes our model more applicable to the human disease state, in which there is noticeable diversity in the degree of bladder dysfunction among SCI and spina bifida patients.

Moreover, the wide range in residual urine volumes among SCI animals at the time of infection allowed examination of a potential association between the amount of residual urine volume (i.e., degree of urinary stasis) and the severity of infection. It has long been presumed that urinary stasis promotes bacterial growth and subsequently increases the risk of developing UTI. Studies by Stern et al. (31) and Truzzi et al. (32) demonstrated a positive correlation between elevated postvoid residual volumes and positive bacterial urine cultures. A natural corollary would be that as the residual urine volume rises, the severity of UTI also increases. However, our studies showed no such association. We acknowledge that our studies do not eliminate the possibility that the simple presence of any amount of residual volume and urinary stasis, rather than the absolute volume of residual urine *per se*, may influence susceptibility to infection. Nevertheless, our results also raise the intriguing possibility that other host factors, such as an altered host epithelium or altered host response, and/or other urodynamic parameters, such as bladder compliance or voiding pressures, may play more important roles in influencing susceptibility to UTI.

Several epidemiologic studies have demonstrated an increased incidence of pyelonephritis and renal scarring among SCI and spina bifida patients. Some studies quote an incidence as high as 34% (33). To our surprise, we did not see a difference in the incidence of ascending kidney infections between SCI and control animals despite the increased susceptibility of SCI rats to lower UTI. This perhaps highlights the important distinction that needs to be made between a detectable infection of the kidney and genuine pyelonephritis. While both SCI and control rats appear to be equally susceptible to ascending bacterial infection of the kidneys, our data at 2 weeks postinfection suggest that SCI rats may develop a more pronounced inflammatory response to the same degree of infection. Although one might argue that the persistently high levels of UTI89 in the bladders of SCI rats may be a persistent source of ascending infection and a potent stimulus of the host immune response, this seems less plausible given that the

kidneys of SCI rats trend toward increased inflammation despite having bacterial counts similar to those in the control animals. This suggests the possibility that SCI rats actually develop a dysregulated response to infection that promotes pyelonephritis and subsequent renal scarring. This seems plausible given that we and others have found disruption in the normal host inflammatory pathways following SCI injury (34); R. Chaudhry and P. C. Seed, unpublished data). This change in gene expression after SCI may predispose to a dysregulated inflammatory response to subsequent infection. We have recently determined that the SCI rat fails to mount a strong proinflammatory response during acute *E. coli* UTI relative to spinal cord-intact controls (Chaudhry and Seed, unpublished). Further studies over a longer time course, together with an evaluation of the cytokine response to infection, may help to clarify this hypothesis further.

This study focused on susceptibility differences between SCI and control animals to an initial, acute UTI. However, a significant challenge among neurogenic bladder patients is their propensity for recurrent and/or persistent UTI. Our temporal course of infection reveals that SCI rats have delayed clearance of UTI89 compared to that of control rats. This may set the stage for persistent infection. However, because many patients receive antibiotic treatment for symptomatic UTI, a relevant question to address with our model is whether the temporospatial course of infection differs between SCI and control rats even after early antibiotic intervention. The increased incidence of recurrent UTI in neurogenic bladder patients despite antibiotic treatment would suggest that even after bacteria are cleared from the urine, neurogenic bladder may harbor larger numbers of quiescent intracellular reservoirs (QIRs) of *E. coli* that can reemerge to cause UTI weeks to months later. Studies by Mysorekar and Hultgren hint at this possibility (35). These authors have demonstrated that *E. coli* bacteria are more likely to form QIRs in previously damaged bladders, as might be seen in the chronically irritated bladders of neurogenic bladder patients. Equally exciting is the prospect that susceptibility to recurrent infection may be influenced by the diversity of endogenous bacteria that colonize the bladders of SCI and spina bifida patients. These hypotheses can be readily addressed with our model. Knowledge of the mechanisms of increased susceptibility to UTI in the neurogenic bladder population can then be used to tailor development of new therapies aimed at preventing or attenuating UTI.

It should be noted that our studies were conducted exclusively with UTI89. Infection with a different *E. coli* isolate or a different uropathogen altogether could potentially yield different results with regard to urine, bladder, and kidney titers as well as inflammatory responses to infection. Our model can be readily adapted to study infection with different cystitis or pyelonephritis strains of bacteria and thereby assess the discrete host-pathogen interactions which are necessary to establish acute versus chronic infections within the lower versus upper urinary tracts in hosts with neurogenic bladder.

Finally, there are data that demonstrate an increased propensity for malignant transformation within the neurogenic bladder (36, 37). This is thought to be secondary to repeated inflammatory insults. Our model could also potentially be used to examine molecular changes that occur over time in the neurogenic bladder following recurrent UTI that predispose to cancer.

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