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Antigen-Specific Responses Accelerate Bacterial Clearance in the Bladder

Praveen Thumbikat,* Carl Waltenbaugh,† Anthony J. Schaeffer,* and David J. Klump²§†

Urinary tract infections (UTIs) cause patient morbidity and have a substantial economic impact. Half of all women will suffer a UTI at least once, and 25% of these women will have recurrent infections. That 75% of previously infected women do not become reinfected strongly suggests a role for an adaptive immune response. The goal of this study was to characterize the adaptive immune responses to uropathogenic Escherichia coli (UPEC), the predominant uropathogen. A novel murine model of UTI reinfec- tion was developed using the prototypic cystitis UPEC isolate NU14 harboring a plasmid encoding OVA as a unique antigenic marker. Bacterial colonization of the bladder was quantified following one or more infections with NU14-OVA. Animals developed anti-OVA serum IgG and IgM titers after the initial infection and marked up-regulation of activation markers on splenic T cells. We observed a 95% reduction in bacterial colonization upon reinfec- tion, and splenic leukocytes showed Ag-specific proliferation in vitro. Adoptive transfer of splenic T cells or passive transfer of serum from previously infected mice protected naïve syngeneic mice from UPEC colonization. These findings support our hypothesis that adaptive immune responses to UPEC protect the bladder from reinfec- tion and form the basis of understanding susceptibility to recurrent UTI in women. The Journal of Immunology, 2006, 176: 3080–3086.

UTIs (e.g., from atypical anatomy) are caused by diverse uropathogens. A variety of virulence factors has been implicated in uropathogenic E. coli (UPEC) pathogenesis, including the type 1 pilus that mediates attachment and colonization of mucosal surfaces (4), hemolysin that induces calcium waves in urothelial cells (5), cytotoxic necrotizing factor that alters ρ GTPase function (6), and cytotoxicity of distending toxin that induces cell cycle arrest (7, 8). In addition to the actions of these virulence factors, the morbidity suffered by UTI patients is due to acute inflammation that results from an innate response (9). Both IL-6 and the chemokine CXCL8 are secreted by the urothelium in direct response to bacterial Ags, including LPS. In culture, LPS induces TLR4 signaling that is required for NF-κB activation and CXCL8 secretion by urothelial cells (10, 11). CXCL8 induces a rapid influx of neutrophils into the bladder, resulting in phagocytosis and clearance of bacteria. Although innate immune responses rapidly clear the majority of UPEC from the bladder, this is not a sterilizing immunity in murine models. For example, the UPEC isolate UTI89 was found to establish reservoirs within the urothelium that were resistant to antimicrobials and persisted for weeks (12). These reservoirs most likely resulted from UPEC invasion of urothelial cells, as has been demonstrated previously with the UPEC isolate NU14 (13). Although human UPEC urothelial reservoirs remain to be demonstrated, the murine model raises the question of the mechanisms used by human hosts to eradicate such infected cells.

Approximately one of every two women will suffer at least one symptomatic UTI during her lifetime (14), and most women will successfully resist subsequent UPEC infections. This suggests the development of a strong adaptive immunity in these individuals. Previous attempts to examine anti-UPEC responses have included the adoptive transfer of splenocytes from wild-type mice into naive SCID mice, thereby conferring enhanced resistance to subsequent colonization by UPEC (15). Protective immunity has also been demonstrated in mice immunized s.c. or i.p. with the FimH adhesin protein (16) and in cynomolgus monkeys immunized i.m. with the PapG adhesin protein of P.fimbriae (17). Immunity correlated with Ab levels and with resistance to UPEC challenge. These vaccination studies do not, however, mimic the normal route of host Ag encounter during UTI pathogenesis and, as such, may not reflect the true host response to UPEC infection.

We have developed a novel murine reinfec- tion model of UTI that recapitulates the sequence of events that lead to the development of an adaptive immune response to UPEC. Using this model, we show that the murine host, in response to UPEC infection through natural routes, is capable of initiating an adaptive immune response that includes the activation and recruitment of T cells to the bladder and the production of specific Abs. This study also shows that adaptive responses are sufficient to significantly reduce UPEC re- colonization of the bladder and can be transferred to naïve hosts.

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Abbreviations used in this paper: UTI, urinary tract infection; eGFP, enhanced GFP; EMB, eosin-methylene blue; L-amp, Luria broth agar with ampicillin; UPEC, uropathogenic E. coli.
Materials and Methods

**Bacterial strains**

NU14 is a clinical isolate of *E. coli* originally obtained from the urine of a patient with cystitis (16). Bacteria were propagated in Luria broth at 37°C under static conditions that promote expression of type 1 pili (18). The extent of type 1 pilus expression was determined by mannose-sensitive hemagglutination (19, 20) of guinea pig erythrocytes (Cleveland Scientific).

**Generation of OVA-producing NU14**

The OVA coding sequence or TET-C (C fragment of tetanus toxoid, as a control) was expressed in NU14 (NU14-OVA and NU14-TET). This was done by transformation with the plasmids pniR15.OVA or pniR15.TET (21) by electroporation. The parental NU14 strain is ampicillin sensitive; transformants were selected by their resistance to ampicillin. Western blot analysis confirmed expression of OVA in *E. coli* after overnight culture under anaerobic conditions for induction of the nirX promoter. Bacteria were lysed in 2× sample buffer (6% Tris-Cl (pH 6.8), 20% glycerol, 4% SDS, 2% 2-ME), and heat denatured at 100°C for 5 min. Cell lysates and purified OVA (positive control) were resolved on a 10% SDS polyacrylamide gel and transferred to nylon membranes (Pierce Biotechnology). Membranes were incubated at room temperature with 1% blocking reagent (Kirkegaard & Perry Laboratories) for 1 h and a 1/500 dilution of murine anti-OVA mAb (22) was added to OVA-elicited mouse serum (gift of M. Okabe, Osaka University, Japan) and expressed under conditions that minimize reflux to the kidneys (23). During the process of developing and characterizing the model, we have confirmed, under these inoculation conditions, the lack of kidney infection by plating kidney homogenates onto nutrient agar. The results show that infection of kidney homogenates onto nutrient agar. The results show that infection was localized to the urinary bladder and does not ascend to the kidneys. To ensure that the inoculum was retained within the bladder, mice were debarbed with water for 30 min before anesthesia, the pelvic region was massaged before instillation to displace any urine previously collected in the bladder, and mice were maintained under anesthesia for 30 min following instillation to minimize voiding activity. At intervals after initial challenge, mice were rechallenged and euthanized the following day. At the time of sacrifice, blood was collected by cardiac puncture for serum separation. Urine was recovered by catheterization at the time of sacrifice and stored at −70°C for subsequent determination of anti-OVA Abs. Spleens and lymph nodes were harvested and processed for isolation of lymphocytes. Bladders were harvested for determination of colonization. Bladder mucosa was prepared in sterile saline, serial dilutions were plated onto eosin-methylene blue (EMB) agar, and discrete colonies within the range of 20–200 were counted for calculation of CFU/bladder. EMB agar is selective for Gram-negative enteric rods, but Luria broth agar with ampicillin (L-amp) plates are selective for pniR15.OVA and were used routinely in duplicate plating to confirm that the plasmid was retained in each experiment. All in vivo experiments were conducted following the guidelines of the Northwestern University Institutional Animal Care and Use Committee.

**Isolation of spleen and lymph node cells**

Spleens and inguinal, renal, and lumbar lymph nodes were removed aseptically from euthanized mice and passed through a 70-μm nylon mesh to obtain single cell suspensions. Erythrocytes were lysed using a mouse erythrocyte lysing kit (R&D Systems). Isolated lymphocytes were resuspended in appropriate buffer for subsequent processing.

**Flow cytometry**

Cells were washed twice in PBS/0.3% w/v BSA supplemented with 0.1% w/v sodium azide. Nonspecific binding of Abs to FcRs was blocked by preincubating cells with mAb 2.4G2 (BD Biosciences) directed against the FcεRII/II CD16/CD32 (0.5 μg/10^6 cells/100 μl). Cells were washed and incubated with 0.5 μg of mAb per 10^6 cells for 30 min at 4°C, and washed again twice. Four-color analyses were performed using a FACSCalibur (BD Biosciences). Data were analyzed using FlowJo software (Tree Star).

The following reagents and mAb were obtained from BD Biosciences: PerCP-conjugated anti-CD3ε mAb 145-2C11, PE-conjugated anti-CD4 mAb GK1.5, allopolycoycanin-conjugated anti-CD8α mAb 53-6.7, and FITC-conjugated anti-CD69 mAb H1.2F3.

**Cell and serum transfers**

Sera and spleen cells were harvested from NU14-OVA-infected C57BL/6 mice or instilled with saline 14 days previously. T cells were isolated using T cell enrichment columns (R&D Systems), according to the manufacturer’s instructions. Spleen cells (1 × 10^7) or enriched T cells (5 × 10^6) were rechallenged in 500 μl of PBS and injected into the lateral tail veins of naive syngeneic C57BL/6 mice. Other naive C57BL/6 mice received 200 μl of undiluted preimmune or naive serum.i.v. All groups were challenged transurethrally 24 h later with NU14-OVA and sacrificed 1 day later. In one series of experiments, T cells were isolated from female enhanced GFP (eGFP) mice that had been infected with NU14-OVA and adoptively transferred into naive, syngeneic C57BL/6 mice to examine trafficking of specific T cells to the bladder.

**In vitro analyses of T cell response to OVA**

Spleen cells from naive or NU14-OVA-challenged mice were cultured in 200 μl of RPMI 1640 containing 5% FBS ≥ 20 μg of OVA or saline. At day 3, cells were centrifuged, supernatants were discarded, and plates were stored at −70°C. Briefly, the CyQUANT proliferation assay (Molecular Probes) was performed by thawing frozen cells and adding a lysis buffer containing the nucleic acid-binding CyQUANT GR dye. Proliferative response was measured as a direct increase in fluorescence measured using a GeminiXS fluorescence microplate reader (Molecular Devices) with monochromaters set for 480 nm excitation and 520 nm emission maxima. Cell numbers were quantified based on a standard curve generated from known counts of spleen cells.

In vitro cytokine stimulation assays were performed on spleen cells from naive, NU14-OVA-challenged mice at day 7 postinfection or NU14-OVA-rechallenged mice 1 day after the second challenge (day 15). Briefly, spleen cells were cultured in 200 μl of DMEM/F12 supplemented with Nutridoma (a serum substitute; Roche), 1-glutamine, and gentamicin. Cells were cultured in the presence of 18 μM OVA or PBS for 1–3 days. Supernatants were collected daily and frozen at −70°C. Dilutions of the supernatant were used in a mouse Th1/Th2 ELISA as per manufacturer’s protocol (eBioscience), and results were expressed in pg/ml.

**Ig ELISA**

Total and OVA-specific Abs were detected by ELISA. Briefly, for anti-OVA ELISA, 96-well microwell plates (Corning) were coated with OVA (10 μg/ml in carbonate-bicarbonate buffer (pH 9.5)) overnight, then blocked with milk blocking buffer (Kirkegaard & Perry Laboratories). The plates were washed; serum or urine samples were incubated overnight at 4°C and washed; and bound Abs were detected using HRP-conjugated goat anti-mouse IgG (H + L) (Jackson ImmunoResearch Laboratories) or goat anti-mouse IgA and goat anti-mouse IgM (Bethyl Laboratories). The anti-OVA mAb, OVA-3 (IgG2b; Brookwood Biomedical), was used as a standard to quantitate total IgG in the sample, while IgM and IgA were expressed in relative absorbance units at 450 nm. Total IgG, IgM, and IgA were measured by coating plates with a capture Ab, followed by addition of serum/urine, and detected using an appropriate HRP-conjugated secondary Ab (Bethyl Laboratories). Reference mouse serum was used as a standard to quantitate total tIgs. The color reaction for ELISA was developed by adding 3,3′,5′-tetramethylbenzidine substrate (Pierce Biotechnology).

**Immunohistochemistry and immunofluorescence**

Paraffin-embedded 5-μm sections were prepared from samples fixed in 10% neutral buffered Formalin. The sections were deparaffinized using standard methods and rehydrated in graded ethanols. Nonenzymatic Ag retrieval was performed by treatment with 0.01 M sodium citrate (pH 6.0) at 92°C for 20 min, and sections were blocked with blocking solution (1% BSA, 0.1% Triton X-100 in PBS), followed by incubation with FITC-conjugated anti-CD4 (clone RM4-5; Santa Cruz Biotechnology), anti-CD8 (clone 2.43; Santa Cruz Biotechnology), or biotinylated anti-CD45R Ab (clone RA3-6B2; BD Biosciences) in blocking solution at room temperature in a humidified chamber. Biotinylated Ab was
detected by incubation with streptavidin-conjugated Alexa-fluor 594 (Molecular Probes). Finally, slides were washed with PBS, mounted using diaminopropylidide mounting medium, cover slipped, and visualized using a fluorescence microscope. Serial H&E sections (Northwestern University pathology core) were also prepared and examined.

Statistical analyses
Data were analyzed using Prism software (version 4.0; GraphPad). Values are presented as mean ± SEM. The statistical significance of differences in the measured mean T cell frequencies between groups was calculated using Student’s two-tailed t test for two groups, or the one-way ANOVA, followed by Dunnett’s posttest comparison. A p value <0.05 was considered significant.

Results
NU14-OVA colonizes the bladder and undergoes normal clearance
The prototypical cystitis isolate NU14 was transformed with a plasmid encoding OVA or a control protein to generate the strains NU14-OVA and NU14-TET, respectively. The NU14-OVA strain expresses OVA under anaerobic conditions (Fig. 1A), indicating that the strain is likely to express OVA within the anaerobic environment of the bladder. The OVA-nonexpressing NU14-TET...
strain was used as a specificity control in subsequent experiments. Following transurethral inoculation into the bladder, the kinetics of NU14-OVA clearance was quantified by plating bladder homogenates on selective EMB or on Luria broth agar with ampicillin (L-Amp). Bladder bacterial counts fell significantly by 3 days postinfection and reached a minimum by 5 days postinfection (Fig. 1B). No further reduction in bacterial numbers was found at the 7-day time point. Subsequent time points showed bacterial numbers at or below the level of detection (data not shown). Similar bacterial counts were obtained at all time points on EMB and L-Amp agar, showing that the NU14-OVA strain retains the OVA plasmid for extended periods in vivo.

Reinfection leads to accelerated clearance of NU14-OVA from the bladder

To assess whether an initial exposure to UPEC alters subsequent responses to challenge infection, NU14-OVA was used to infect naive mice (1°), to infect mice twice at a 2-wk interval (2°), or infect mice three times at 2-wk intervals (3°). Bladder homogenates were prepared from all animals the day after the final infection, and bacterial CFUs were determined (Fig. 2). Bacterial numbers were significantly lower in mice with prior NU14-OVA exposure (2° and 3°) compared with previously naive animals (1°). These data demonstrate that initial bladder infection results in an enhanced host response to subsequent bladder infection, leading to accelerated bacterial clearance. Repeated NU14-OVA challenge did not enhance resistance significantly. A similar clearance pattern was observed with the parent NU14 strain (data not shown), suggesting that the immune response is not directed against OVA.

NU14-OVA infection induces leukocyte infiltration

Little or no leukocytic accumulation was seen in the bladder wall 24 h after primary infection with NU14-OVA (Fig. 3A), with no visible change until 7 days postinfection (Fig. 3B). Leukocyte aggregates were larger after a second infection (Fig. 3C) and appeared more pronounced after a third infection (Fig. 3D). Staining with anti-CD45R Ab revealed an accumulation of CD45R+ cells in bladders of reinfe
ced mice that were not observed in tissues 24 h after initial infection (Fig. 3, compare E and F). Furthermore, CD4+ and CD8+ cells were observed among the infiltrate (Fig. 3, G and H). These data show that NU14-OVA induces leukocyte infiltration and suggest that an adaptive immune response may mediate the accelerated clearance of UPEC upon reinfection.

Infection with NU14-OVA evokes an Ab response

To examine a possible humoral response against UPEC, mice were infected one or more times with NU14-OVA, and serum and urine Ab responses were examined. We also characterized the specific contributions of IgM and IgG responses and examined the possible influence of UPEC infection on anti-OVA Ab titers (day 14 vs day 15). Infected mice showed no anti-OVA IgG response in serum during the first 7 days after a primary infection (data not shown), but developed significant titers by day 15 that showed no further increase at day 29 (Fig. 4A). At day 15, the urine of these animals contained 8960 ± 690.6 pg/ml anti-OVA IgG (n = 3, data not shown), but no anti-OVA IgM or IgA was detected. A rise in serum anti-OVA IgM and IgG, but not IgA, was demonstrable at both days 14 and 15, with no significant difference between the two time points (Fig. 4, B and C). Analysis of total serum and urine IgG, IgM, and IgA did not reveal significant differences between the uninfected and NU14-OVA-infected animals (Fig. 5). The ability to induce a specific IgG and IgM response suggests that UPEC, when introduced through its natural route, can evoke a host humoral immune response.

NU14-OVA infection activates T cells

We next sought to determine whether UPEC infection elicited specific T cell responses. Female C57BL/6 mice received transurethral infection with NU14-OVA or PBS, and 5 days later their splenic lymphocytes were analyzed by flow cytometry. Primary
bladder infection greatly increased CD69 expression by CD3⁺ spleen (T) cells (Table I) and included both CD4⁺ and CD8⁺ T cell subsets. We examined in the in vitro lymphocyte proliferative response to OVA following infection of mice with NU14-OVA or NU14-TET (Table II). Splenocytes from NU14-OVA-infected animals showed significant proliferation in response to OVA stimulation that was not observed in spleens of mock-infected controls or mice infected with NU14-TET. The use of OVA as the stimulating foreign Ag allowed for the determination of specific responses not likely to have been compromised by pre-existing immune responses to antigenic epitopes shared by normal enteric and uropathogenic strains of E. coli. These data suggest that UPEC infection leads to the generation of Ag-specific T cell responses. We also examined the cytokine response of in vitro OVA-stimulated spleen cells derived from naïve and NU14-OVA-infected animals. Our results show enhanced secretion of IL-2 by spleen cells derived from NU14-OVA-infected animals 7 days after infection (Fig. 6). This significant rise in IL-2 secretion in these animals is consistent with the increased proliferation of splenocytes from NU14-OVA-infected animals (Table II). IL-4, IL-10, and IFN-γ production was not detected, suggesting a lack of Th1/Th2 skewing at this early time point or an exodus of skewed T cells from the spleen to the site of infection. Our data suggest that UPEC infection leads to cellular activation consistent with the development of an adaptive immune response.

Transfer of protection with T cells or serum

To examine the protective role of humoral and cell-mediated responses, naïve mice were inoculated transurethrally with NU14-OVA, 24 h after receiving transfer of serum, splenocytes, or column-enriched splenic T cells from preimmune syngeneic hosts. Bacterial colonization of the bladder was reduced significantly in animals that received serum, splenocytes, or T cells from previously infected mice (Fig. 7A), whereas serum or T cells from naïve donors did not confer protection. We next examined whether T cells trafficked to the bladder of infected mice. C57BL/6 mice expressing an eGFP transgene were infected transurethrally with NU14-OVA and used as donors for column-enriched splenic T cells. Bladder sections from naïve recipient mice can be seen with GFP⁺ T cells (Fig. 7B) within the bladder 24 h after NU14-OVA infection. In contrast, adoptive transfer of T cells from uninfected female eGFP mice did not result in significant numbers of eGFP T cells in the bladders of donor mice (data not shown). Taken together, these data suggest that both humoral and cell-mediated mechanisms confer enhanced host resistance to bladder infection.

Discussion

One in three women will be afflicted with at least one urinary tract infection before the age of 24 years (24). Approximately 50% of women have at least one symptomatic UTI (14), and nearly 25% of these individuals have recurrent episodes (25). In this study, we developed and characterized a novel murine model of reinfection that may represent the response of 75% of patients who successfully resist future infection with UPEC. Our results suggest a significant adaptive immune response to UPEC that is sufficient to protect the host against a subsequent challenge.

The innate immune response to type 1 pilated E. coli infection is initiated by bladder epithelial cell activation through LPS interaction with CD14 and TLRs (11). The release of chemokines leads to the recruitment of neutrophils that mediate bacterial clearance from the bladder. This response results in progressive resolution of bacterial colonization in different strains of mice, including the C57BL/6 strain used in this study (26). Our result in mice infected with the NU14 cystitis strain, modified to express OVA, shows that the host innate response is intact and results in spontaneous reduction in bacterial colonization over time. The use of OVA as a foreign Ag allowed for the elimination of cross-reactivity with bacterial Ags derived from other mucosal sites like the gut. Furthermore, the use of OVA for in vitro proliferation and Ab assays permitted quantitation of defined antigenic immune response as opposed to the wide array of nonspecific responses most likely against less defined bacterial Ags.

Reinfection of mice with UPEC leads to accelerated clearance of bacteria from the bladder. These findings show that initial Ag exposure results in a robust, effective bacterial clearance upon subsequent challenge. Within the constraints of our experimental system in which bladders were harvested 24 h after the last infection, multiple reinfections did not lead to sterilizing immunity of the bladder. At this juncture, we cannot rule out the possibility that other time points beyond 24-h postinfection may result in complete clearance of UPEC. The protective response observed in this study appears to parallel the classic paradigm of adaptive immune response with an initial Ag exposure evoking an enhanced and accelerated secondary response to the same Ag.

Cellular aspects of the immune response to UTI are easily detected and are often used clinically to determine the presence of infection. Macroscopic urinalysis to detect urinary leukocyte esterase, a product of inflammatory cells and microscopic detection

Table I. CD69 induction by NU14-OVA

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Saline (CD3⁺ Spleocytes (%))</th>
<th>OVA (CD3⁺ Spleocytes (%))</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mock (n = 3)</td>
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<td>1.6 ± 0.2</td>
<td>63.5 ± 4.8</td>
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<tr>
<td>NU14-OVA (n = 3)</td>
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<td>0.7 ± 0.1</td>
<td>50.4 ± 3.9</td>
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<tr>
<td>NU14-TET (n = 3)</td>
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<td>0.5 ± 0.2</td>
<td>13.2 ± 0.9</td>
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</tr>
</tbody>
</table>

Table II. OVA induces proliferation of NU14-OVA leukocytes

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Saline</th>
<th>OVA</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
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<td>3</td>
<td>2.2 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>0.0422</td>
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<tr>
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<td>0.0153</td>
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<tr>
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<td>3.0 ± 0.4</td>
<td>2.5 ± 0.4</td>
<td>0.1164</td>
</tr>
</tbody>
</table>

FIGURE 6. In vitro stimulation of splenocytes derived from NU14-OVA-infected animals after 7 days shows release of IL-2 that is significantly different from naïve animals (p ≤ 0.001) and from a second infection (D15) with NU14-OVA (p ≤ 0.01). Cytokine measurements were done using an ELISA.
of cellular response in the form of pyuria, both attest to the presence of leukocytes in UTI infection. In humans, leukocyte migration into the bladder wall has been attributed to the CXCL8 chemokine (27–31). Although much of the initial inflammatory infiltrate is comprised of neutrophils, our studies demonstrate that in secondary infections there is recruitment of B and T lymphocytes to the bladder wall. Interestingly, both CD4 and CD8 T cell subsets appear to be recruited, which may have important implications with regard to the ability to clear infection. UPEC that have been demonstrated to be capable of entry and persistence in urothelial cells (12) may serve as targets for CD8 T cells through their cytotoxic activity, while extracellular UPEC may be removed by CD4-dependent mechanisms. Our study shows activation of T cells beginning as early as 5 days after bladder infection that proliferate in response to specific antigenic challenge in vitro. Furthermore, in vitro cytokine assays demonstrate an increase in IL-2 secretion from splenocytes stimulated with OVA that is consistent with the increased proliferation. However, secretion of cytokines that point to a Th1/Th2 bias was not measurable within the limits of our study, conducted 7 days after an NU14-OVA infection. We speculate that there is a lack of Th1/Th2 skewing at this early time point after NU14-OVA infection. Alternately, it is possible that activated T cells may have largely migrated to the site of infection, and as such are not present in large numbers in the spleen. The significant reduction in IL-2 secretion at day 15 following a secondary infection may further reflect this phenomenon as T cells migrate out of the spleen to the bladder to combat an active infection. Nonetheless, our results in toto suggest that UPEC infection is capable of activating a cellular response that may be required for protection.

UPEC infection elicits Ab responses in animal models and humans. Both IgG and secretory IgA have been found in urine and act as neutralizing Abs (32–34). A variety of vaccine candidates has been tested in both animal models and humans with the purpose of evoking protective humoral responses (16, 17). This study demonstrates that an initial UTI infection in mice elicits a significant systemic humoral immune response that is well developed by 14 days postinfection. Both IgG and IgM Abs specific for OVA were detectable in serum, although overall quantities of each Ig were unchanged. A second encounter with UPEC does not significantly enhance the anti-OVA humoral response, but it is possible that Ab affinity increased as often occurs upon repeated antigenic challenge. It is likely that these and other mucosal Abs play an important role in protection against UPEC infection.

Protection against UPEC infection can be adoptively transferred from immune mice to naive mice. This can be achieved by transfer of either T cells or serum, suggesting that cellular and/or humoral arms of adaptive immunity protect against UPEC reinfection. Serum used for the transfer was derived from mice 1 day after reinfection and was shown to have significant titers of OVA-specific IgG and IgM that may play a role in conferring protection. Resistance to UTI in mice has been shown previously to be enhanced by the transfer of spleen cells (T and B lymphocytes) from immunocompetent mice into T and B cell immunodeficient (SCID) mice (15). However, these investigators report that αβ T cell-deficient mice have colonization levels of UPEC equivalent to those observed in immunocompetent controls. We speculate that the differences observed in our study may be attributed to the fact that we used fully immunocompetent mice, and our reinfection model reflects the average healthy human who develops an innate and adaptive immune response to UPEC. Because the site of UPEC infection is within the bladder, it is likely that effector cells would be specifically recruited to the site of infection. The ability of adaptively transferred T cells to traffic to the bladder suggests specific recruitment that is consistent with a role for T cells in protection against UPEC infection.

The observation that transfers of immune serum and T cells thwart bacterial colonization suggests that the adaptive immune response to bladder infection is multifaceted. Immediately after infection, an innate immune response is activated and is characterized by recruitment of neutrophils and other professional phagocytes to the bladder mucosa. Urothelial cells play a critical role in this early activation mechanism through TLR4-mediated signaling and proinflammatory mediators like CXCL-8 and IL-6. These immune cells mediate significant clearance of bacteria from the bladder during the first week of an infection. Concurrently, we observe the development of both cellular and humoral immune responses that peak ~2 wk after initial UPEC challenge. The cellular response appears to be driven by Ag-specific activated T cells, while the humoral immune response is characterized by Ag-specific IgG and IgM Abs in serum and urine. These separate adaptive immune responses, in combination with an innate immune response, cause significantly accelerated clearance of UPEC in subsequent infections. The similar protection against UPEC conferred by transfer of either T cells or immune serum suggests overlapping kinetics in the development of these distinct arms of immunity to UPEC. We speculate that the cellular and humoral immunity may be directed at different stages of the UPEC life cycle in UTI pathogenesis,
perhaps to specifically target both bacteria within the bladder lumen and bacteria that have established intracellular reservoirs.

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Disclosures
The authors have no financial conflict of interest.

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