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Pascale S. Guiton  
Washington University School of Medicine in St. Louis

Thomas J. Hannan  
Washington University School of Medicine in St. Louis

Bradley Ford  
Washington University School of Medicine in St. Louis

Michael G. Caparon  
Washington University School of Medicine in St. Louis

Scott J. Hultgren  
Washington University School of Medicine in St. Louis

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Enterococcus faecalis Overcomes Foreign Body-Mediated Inflammation To Establish Urinary Tract Infections

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Department of Molecular Microbiology and Microbial Pathogenesis, Center for Women's Infectious Disease Research, and Department of Pathology and Immunology, Washington University in St. Louis, Saint Louis, Missouri, USA

Urinary catheterization elicits major histological and immunological changes that render the bladder susceptible to microbial invasion, colonization, and dissemination. However, it is not understood how catheters induce these changes, how these changes act to promote infection, or whether they may have any protective benefit. In the present study, we examined how catheter-associated inflammation impacts infection by Enterococcus faecalis, a leading cause of catheter-associated urinary tract infection (CAUTI), a source of significant societal and clinical challenges. Using a recently optimized murine model of foreign body-associated UTI, we found that the implanted catheter itself was the primary inducer of inflammation. In the absence of the silicone tubing implant, E. faecalis induced only minimal inflammation and was rapidly cleared from the bladder. The catheter-induced inflammation was only minimally altered by subsequent enterococcal infection and was not suppressed by inhibitors of the neurogenic pathway and only partially by dexamethasone. Despite the robust inflammatory response induced by urinary implantation, E. faecalis produced biofilm and high bladder titers in these animals. Induction of inflammation in the absence of an implanted catheter failed to promote infection, suggesting that the presence of the catheter itself is essential for E. faecalis persistence in the bladder. Immunosuppression prior to urinary catheterization enhanced E. faecalis colonization, suggesting that implant-mediated inflammation contributes to the control of enterococcal infection. Thus, this study underscores the need for novel strategies against CAUTIs that seek to reduce the deleterious effects of implant-mediated inflammation on bladder homeostasis while maintaining an active immune response that effectively limits bacterial invaders.
tions that *E. faecalis* is commonly recovered from patients with foreign body-associated infections or under immunosuppressive therapies and suggests that although immunosuppressive approaches for the management of CAUTIs may help limit the deleterious consequences of urinary catheterization for bladder biology, they may inadvertently predispose patients to increased bacterial colonization and dissemination leading to adverse side effects and more severe infections.

**MATERIALS AND METHODS**

**Bacterial strain and growth conditions.** *E. faecalis* strain OG1RF, resistant to rifampin and fusidic acid (23, 24), was used in this study. Unless otherwise specified, experiments were performed using an overnight bacterial culture grown in brain heart infusion broth (BHI) (Becton, Dickinson, Franklin Lakes, NJ) from a single colony of OG1RF grown on BHI agar plates supplemented with 25 μg/ml of rifampin and 25 μg/ml of fusidic acid (BHRIF25). Liquid cultures were grown statically at 37°C for 18 h.

**Inhibitors and chemicals.** The drug treatments used in this study, their relevant modes of action, and references for dosage and effectiveness are described in Table 1. Vehicles were saline or dimethyl sulfoxide (DMSO) as indicated.

**Mouse strains.** Six- to 7-week-old female wild-type C57BL/6Ncr mice purchased from the National Cancer Institute (NCI) were used in this study. Experiments were performed following a 1-week adaptation in the animal facility. All studies and procedures were approved by the Animal Studies Committee at Washington University School of Medicine.

**Animal implantation and infection.** Animals were transurethrally implanted and inoculated as previously described (18). Briefly, 7- to 8-week-old female mice were anesthetized by inhalation of isoflurane and immediately following implantation, or at time of infection for nonimplanted animals. 50 μl of −1 × 10^7 to 2 × 10^7 CFU of bacteria in phosphate-buffered saline (PBS) was introduced in the bladder lumen by transurethral inoculation, when indicated.

**Bladder weight determination and PPE.** When indicated, bladders were aseptically removed, bisected, and blotted dry. Bladders were placed in preweighed Eppendorf tubes and weighed. Bladder weight (in grams) was determined as the difference between bladder and Eppendorf tube weight and that of the empty Eppendorf tube. Plasma protein extravasation (PPE) in the bladder was determined using the Evans blue technique (25) in nonimplanted and implanted animals at 6 h after treatments with the indicated chemicals listed in Table 1. Mice were anesthetized by inhalation of isoflurane, and 30 mg/kg of body weight Evans blue (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was administered intravenously (i.v.). Animals were sacrificed 15 min later by cervical dislocation. The bladder was then excised, bisected, and blotted dry before weighing. Bladders were then placed in 1 ml of 100% formamide (Sigma-Aldrich) and incubated at 60°C for 24 h for Evans blue extraction. The extracted Evans blue from each bladder was quantified by colorimetric measurement at 620 nm with an automated microplate reader. The dye content extrava-sated in each bladder was determined from an Evans blue standard curve and expressed as μg/g of tissue.

**Bacterial titer determination on implants and organs.** Animals were sacrificed at indicated time points by cervical dislocation after anesthesia inhalation, and the bladders and kidneys were aseptically harvested. Subsequently, the silicone implant was retrieved from the bladder when present, placed in PBS, sonicated for 10 min, and then vortexed at maximum speed for 3 min. The bladder and kidneys from each mouse were homogenized in PBS. Samples were serially diluted and plated onto BHI agar plates containing rifampin. CFU were enumerated after 24 h of incubation at 37°C. In all cases, experiments were performed at least twice with *n* = 5 to 10 mice/condition/experiment.

**Cytokine profiling.** Bladder homogenates from nonimplanted and implanted animals with or without bacterial infections were microcentrifuged at 14,000 × g for 5 min, and supernatants were frozen at −80°C until the time of the assay. Assays were carried out according to the manufacturers’ protocols using the Bio-Plex Pro mouse cytokine 23-plex assay kit from Bio-Rad Laboratories (Hercules, CA).

**Histopathology.** For histological analyses, bladders were fixed in methacarn (60% methanol, 30% chloroform, and 10% glacial acetic acid) or formalin for 1 to 2 h at room temperature and dehydrated in 70% ethanol overnight at 4°C. They were then embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H/E) for light microscopy.

**Flow cytometry.** Single-cell bladder suspensions were made from minced bladder tissues subjected to collagenase/DNase I digestion for 90 min at 37°C and then passed through a 40-μm filter, and cells were washed as described previously (26). Staining of surface markers was performed in FcR block with fluorochrome-conjugated monoclonal antibodies (MAbs). Cells were counterstained with propidium iodide (PI) prior to flow cytometry, and only live (PI-low) cells were included in the analysis (gating strategies are shown in Fig. S1 in the supplemental material). To specifically characterize the immune infiltrates, specific combinations of MAbs were chosen which distinguish granulocytes (CD11b^+^ Gr1^hi^ Ly6G<sup>hi</sup> Ly6C<sup>lo</sup>), monocytes/macrophages (F4/80<sup>+</sup>), dendritic cells (CD11c<sup>+</sup>), basophils (cKit<sup>+</sup> FceR<sup>+</sup> I<sup>+</sup>), eosinophils (SiglecF<sup>+</sup>), mast cells (cKit<sup>+</sup> FceR<sup>+</sup> I<sup>+</sup>), NK cells (NK1.1<sup>+</sup>), T cells (CD3<sup>+</sup>), and B cells (CD19<sup>+</sup>). All antibodies were from BD Pharmigen, Ebiocience, or Southern Biotech. Activation status was determined using specific MAbs for major histocompatibility complex class II (MHCII). Samples were acquired on a FACSCalibur (BD Biosciences), and data were analyzed using FlowJo software (version 7.6.4). The relative proportion of cellular infiltrates in each bladder was calculated as a percentage of live cells.

**Neutrophil depletion.** Mice were rendered neutropenic as previously described (26). Briefly, an anti-Ly6G MAb (1A8) from Bio X Cell (West Lebanon, NH) was administered intraperitoneally (i.p.) on days 3 and 1 prior to implantation and bacterial challenge. Control mice received IgG isotype control 2A3 (Bio X Cell) in a similar manner.

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**Table 1** List of inhibitors used in this study

<table>
<thead>
<tr>
<th>Name</th>
<th>Dosage/route*</th>
<th>Mode of action</th>
<th>Company/references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone sodium phosphate</td>
<td>10 mg/kg, i.p., 30 min prior to implantation</td>
<td>Glucocorticoid, anti-inflammatory, and immunosuppressant</td>
<td>American Regent, Inc., Shirley, NY (48)</td>
</tr>
<tr>
<td>CP-99,994 dihydrochloride</td>
<td>5–10 mg/kg, i.p., i.v., s.c., 30 min and 3 h postimplantation (when indicated)</td>
<td>High-affinity neurokinin 1 receptor (NK1R) antagonist</td>
<td>Tocris Bioscience, Ellisville, MO (56, 57)</td>
</tr>
<tr>
<td>CP-96,345</td>
<td>5–10 mg/kg, i.p., i.v., s.c., 30 min and 3 h postimplantation (when indicated)</td>
<td>High-affinity NK1R antagonist</td>
<td>Tocris Bioscience (79)</td>
</tr>
<tr>
<td>Aminoguanidine hydrochloride</td>
<td>150 mg/kg at time of infection</td>
<td>Irreversible inducible nitric oxide synthase (iNOS) inhibitor</td>
<td>Tocris Bioscience (33, 67)</td>
</tr>
</tbody>
</table>

* i.v., intravenously; s.c., subcutaneously.
Statistical methods. Comparisons among groups were conducted by the Mann-Whitney U test using GraphPad Prism (GraphPad software, version 5). Values below the limit of detection (LOD) (40 CFU/ml for organs and 20 CFU for implants) were assigned the appropriate LOD value for statistical analyses. All tests were two-tailed, and a P value less than 0.05 was considered significant. Colonization and infection were defined as organs/implants with bacterial titers above LOD at 24 h postinfection (hpi).

RESULTS
Urinary catheterization induces severe edema and release of proinflammatory cytokines. Examination of mouse bladders following implantation of silicone tubing reveals severe edema (18). However, the mechanisms of onset and progression of bladder wall edema following urinary catheterization are poorly defined. Thus, we investigated edema using bladder weight as a readout followed by histological verification. The bladder weights of uninfected mice were determined at 3, 6, 9, 12, 18, and 24 h postimplantation and compared to those of nonimplanted controls. The bladders of implanted mice significantly increased in weight as early as 3 h postimplantation (P = 0.0011 by the Mann-Whitney U test) compared to those of nonimplanted animals (normally 15 to 20 mg) and reached approximately 60 mg by 24 h (Fig. 1A). Histological analysis of bladder tissue from implanted animals at each time point depicts the gradual progression of bladder wall edema over time, corroborating the significant increase in bladder weight (Fig. 1C). The implant-induced edema correlates with plasma protein extravasation (PPE) in the tissue as assessed by the Evans blue extravasation assay performed 6 h postimplantation (Fig. 1D). Significantly higher quantities of Evans blue per gram of bladder tissue were extravasated and recovered from implanted bladders following intravenous injection in the murine tail vein (60 µg), compared to approximately 30 µg/g seen in nonimplanted controls (P = 0.0348). Urinary catheterization is also associated with the upregulation of several inflammatory cytokines (18). Interleukins 1β, 6, 12(p40), and 17 as well as granulocyte colony-stimulating factor (G-CSF) and keratinocyte-derived...
chemokine (KC) are upregulated at least 2-fold in saline-treated implanted animals over nonimplanted mock-infected controls following urinary implantation (Fig. 2A).

**Glucocorticoid treatment partially inhibits implant-induced edema and inflammation.** We used anti-inflammatory and immunosuppressive agents in order to better understand the mechanism by which the severe edema occurs in the bladder as a result of urinary catheterization. We investigated the ability of (i) dexamethasone, a well-characterized glucocorticoid and potent anti-inflammatory agent; (ii) inhibitors of the neurogenic inflammatory pathway (NIP), including the neurokinin 1 receptor (NK1R) antagonists CP-99,994 and CP-96,345 (27–30); or (iii) aminoguanidine (AG), the irreversible inhibitor of inducible nitric oxide synthase (iNOS) (31–33), to immunosuppress catheter-induced edema and proinflammatory cytokines. Mice were intraperitoneally treated with each immunosuppressive agent 30 min prior to implantation. Immunosuppression was evaluated based on reduction in edema as determined by bladder weight, decreased plasma protein extravasation (PPE) by the Evans blue-based assay, and reduced production of proinflammatory cytokines.

Treatment with dexamethasone prevented the development of bladder wall edema at 6 hpi (Fig. 1D) and delayed the increase in bladder weight for up to 9 h postimplantation compared to saline-treated controls (Fig. 2B). However, by 12 h postimplantation, the bladders of dexamethasone-treated animals were as edematous and inflamed as were saline-treated implanted controls (Fig. 2C). Supplemental dosages of dexamethasone administered 30 min prior to implantation and at 9 h postimplantation did not prevent edema at 24 h postimplantation (data not shown). Dexamethasone also significantly decreased interleukins 1β and 12(p40) as well as G-CSF and KC in implanted animals compared to saline-treated implanted controls at 24 h postimplantation (Fig. 2A). In contrast, known inhibitors of the NIP (Fig. 1D), including the specific neurokinin 1 receptor (NK1R) antagonist CP-99,994 or aminoguanidine (AG), the irreversible inhibitor of inducible nitric oxide synthase (iNOS) (31–33), resulted in no reduction in bladder weights, and vascular permeability was observed in im-
planted animals. Similar findings were obtained with CP-96,345 (data not shown). Thus, NK1R-induced neurogenic inflammation does not appear to be a major contributor to the onset of bladder wall edema and vascular permeability following urinary implantation in mice. These data argue that activation of glucocorticoid-sensitive immune pathways contributes to the immediate inflammatory response following urinary implantation but that dexamethasone-insensitive pathways, not related to the NIP, dominate after 6 to 9 h postinfection.

Urinary implantation leads the specific recruitment of myeloid-derived cells in the murine bladder. Very little is known about the cellular constituents of the immune response to urinary catheterization aside from histological analyses of bladder tissues (7, 13, 18). In order to establish the functional role of implant-mediated inflammation, we investigated the cellular nature of immune cells involved in the bladder immunological responses to the silicone implant. We performed flow cytometric analysis of bladder single cell suspensions in nonimplanted and implanted animals at 24 h postimplantation using antibodies raised against specific immune surface markers. These experiments reveal that CD11b+ myeloid cells account for approximately 30% of the live cell population in implanted animals compared to 10% in nonimplanted controls at 24 h postimplantation (P < 0.0001) (Fig. 3A). No significant difference was observed in lymphocyte (CD3+ CD19+) numbers in the presence or absence of implant at 24 hpi (Fig. 3A). Specific markers were used to further specify the myeloid cells. Nonactivated (CD11b+ F4/80+ MHCII−) and activated (CD11b+ F4/80+ MHCII+) macrophages are present with

FIG 3 Neutrophils are important cellular infiltrates during enterococcal CAUTI. Cellular infiltrates from nonimplanted animals (white bars), implanted animals (black bars), or animals implanted and infected with E. faecalis OG1RF (gray bars) at 24 hpi derived from flow cytometry analysis. Nonactivated and activated macrophages (CD11b+ F4/80+ MHCII− and CD11b+ F4/80+ MHCII+, respectively), neutrophils (CD11b+ Gr1hi Ly6Ghi Ly6Clo), eosinophils (SiglecF+), and basophils (cKit+ FcεRI+). Graphs represent the means derived from at least two independent experiments with n = 3 to 5/experiment/condition. Error bars represent standard errors of the means. *, P < 0.05; **, P < 0.005; and ***, P < 0.0005, by the Mann-Whitney U test. ns, not significant (P > 0.05).
implanted animals (imp) on the day of bacterial challenge. (B) H/E staining of bladder sections of 6-h cyclophosphamide (CYP)-treated or implanted mice observed under a light microscope (magnification, ×20). The experiment was performed in duplicate with n = 3 to 5/group. (C) Graph represents OG1RF titers at 6 hpi from retrieved implants and homogenized bladders and kidneys from nonimplanted CYP- or saline-treated and implanted (Imp) animals. For panels A and C, the horizontal bars indicate the median of each data set. Two independent experiments performed with n = 5 to 10/condition/experiment. *, P < 0.05, and **, P < 0.005, by the Mann-Whitney U test. ns, not significant.

FIG 4 Implant-induced inflammation is not sufficient for enterococcal urinary tract colonization. (A) CFU enumeration at 24 hpi of OG1RF from implants and homogenized bladders and kidneys of animals preimplanted (Pre-imp) 24 h before or implanted (imp) 24 h before or implanted (imp) on the day of bacterial challenge. (B) H/E staining of bladder sections of 6-h cyclophosphamide (CYP)-treated or implanted mice observed under a light microscope (magnification, ×20). The experiment was performed in duplicate with n = 3 to 5/group. (C) Graph represents OG1RF titers at 6 hpi from retrieved implants and homogenized bladders and kidneys from nonimplanted CYP- or saline-treated and implanted (Imp) animals. For panels A and C, the horizontal bars indicate the median of each data set. Two independent experiments performed with n = 5 to 10/condition/experiment. *, P < 0.05, and **, P < 0.005, by the Mann-Whitney U test. ns, not significant.

Role of foreign body-induced bladder inflammation in E. faecalis CAUTI. We investigated the role of the implant-mediated edema and inflammation in promoting or altering the outcome of enterococcal infections. In a murine model of cystitis, E. faecalis induces minimal inflammation and is rapidly eliminated from the bladder (35). However, with the introduction of silicone tubing implants into the bladder, E. faecalis becomes adept at causing CAUTI despite the edema and the robust inflammatory response induced by the catheter (36). Thus, we investigated whether E. faecalis virulence was altered in animals preimplanted 24 h before infection to determine the effect of a preexisting catheter-induced inflammatory response on the outcome of infection. Animals that were implanted and simultaneously infected served as a control. Bacterial titers from the implant and organs were then analyzed and compared. As shown in Fig. 4B, E. faecalis colonizes the implants and organs of the two groups to similar levels, indicating that preexisting implant-induced bladder inflammation does not enhance or prevent enterococcal colonization.

To assess whether any inflammation predisposes the bladder to E. faecalis infection in the absence of a foreign body, murine hemorrhagic cystitis was induced via treatment with cyclophosphamide (CYP) (150 mg/kg, intraperitoneally [i.p.]). CYP-induced cystitis is a well-characterized model of bladder inflammation. Even though CYP- and catheter-induced inflammatory responses are not equivalent, they share some important commonalities depicted in Fig. 4B, such as bladder wall edema, mucosal damage, and host immune cell infiltration (31, 32, 37, 38). When introduced in nonimplanted CYP-treated animals, E. faecalis is rapidly cleared from the urinary tract, similarly to saline-treated nonimplanted controls at 6 hpi (Fig. 4C). By 24 hpi, bacteria are recovered at very low levels (102 to 103 CFU/ml in organs) from both experimental groups compared to implanted animals, whose organs remain colonized at very high titers (106 to 109 CFU/ml; data not shown). These findings suggest that the inflammatory state of the bladder is not sufficient to promote E. faecalis infection of the urinary tract in the absence of a foreign body.

Similarly to uninfected implanted animals, CD11b+ myeloid cells comprise the predominant cellular infiltrates at 24 h in implanted animals with E. faecalis infection (Fig. 3A). However, CD11b+ myeloid cells account for approximately 40% of live cell populations in CAUTI animals, significantly higher than in im-
planted controls in the absence of *E. faecalis* (*P* < 0.005). This increase in myeloid cells in the presence of *E. faecalis* is mainly due to a 2-fold increase in the recruitment of neutrophils compared to mock-infected implanted controls (*P* < 0.001) (Fig. 3B). Similar numbers of macrophages, basophils, and eosinophils are present in the bladders of implanted animals whether or not *E. faecalis* is present (Fig. 3B), indicating that recruitment and activation of these immune cells occur specifically in response to the silicone implant. Together, these results indicate that while the silicone implant elicits the specific recruitment of myeloid infiltrates in the bladder, only neutrophil recruitment is enhanced during enterococcal CAUTI.

**Neutrophil recruitment contributes to antienterococcal responses.** Having identified neutrophils as the major immune cells recruited in response to *E. faecalis* infection in the urinary tract of implanted animals (Fig. 3B), we assessed their contribution to the inflammatory response during *E. faecalis* infection. For neutrophil depletion, each mouse was rendered neutropenic following anti-Ly6G (mAb clone 1A8) treatment administered first i.p. 3 days depleted, each mouse was rendered neutropenic following anti-Ly6G (MAB clone 1A8) treatment administered first i.p. 3 days and then 1 day prior to implantation and bacterial challenge (26). At 24 hpi, there were approximately 1- and 1.5-log increases in CFU recovered from implants and bladders of neutrophil-depleted animals, respectively, compared to isotype-control antibody-treated implanted animals (*P* < 0.05 in all cases) (Fig. 5A). There was also a statistically significant ~0.5-log difference in the kidney titers between the two groups. Notably, the bladders from these neutropenic animals were as enlarged as those from their littermate controls in the absence and presence of bacterial infections, indicating that neutrophils are not major contributors to the pathway leading to vascular permeability following urinary catheterization in mice. Together, these findings indicate that neutrophils are dispensable for implant-induced edema but are critical for controlling enteroococcal colonization of the urinary tract during CAUTI.

**Glucocorticoid treatment significantly increases *E. faecalis* urovirulence at 6 hpi.** The effects of dexamethasone-induced immunosuppression on enteroococcal virulence in implanted animals were assessed at 6 hpi and 24 hpi. As shown in Fig. 5B, enteroococcal titers were 10-fold higher (*P* = 0.031) on implants recovered from dexamethasone-treated animals than on those from saline-treated implanted controls at 6 hpi. No significant difference in bacterial titers was observed in the bladder and kidneys between the two groups. By 24 hpi, *E. faecalis* recoveries from implants were similar in dexamethasone- and saline-treated animals (Fig. 5B). Enterococcal infection in dexamethasone-treated animals causes a significant increase in the production of proinflammatory cytokines, including interleukin-6 (IL-6), which was similar to that in saline-treated infected animals, as shown in Fig. 2A. These data suggest that although dexamethasone did not affect the ultimate production of many proinflammatory cytokines in the bladder consequent to CAUTI, other dexamethasone-sensitive pathways, such as immune cell activation and recruitment, contribute to bacterial clearance in this model.

**DISCUSSION**

*E. faecalis* is a major cause of nosocomial infections and an important etiological agent of CAUTI. During CAUTIs, *E. faecalis* takes advantage of the presence of the foreign body within the bladder to produce biofilms and establish persistent infections in the urinary tract. This infection occurs in the face of a robust immune response, which is induced in mice as well as in patients primarily in response to the presence of the foreign body within the bladder. The murine model of foreign body-associated UTI recently optimized for the study of enteroococcal CAUTI and used in the present study helps unravel critical aspects of the interplay between inflammation and enteroococcal colonization, providing new details of the molecular mechanisms leading to the foreign body-mediated inflammatory response and its role in the outcome of *E. faecalis* infection. We specifically showed that while induction of inflammation in the murine bladder either by implantation or chemically in the absence of implants is not sufficient to prevent or promote *E. faecalis* infection, partial immunosuppression with dexamethasone or neutrophil depletion prior to urinary implantation and bacterial challenge enhances enteroococcal colonization of the implants and the urinary tract, suggesting that the immune response in the implanted bladder does not favor enteroococcal infection but is simply ineffective at clearing the infection in our murine model of CAUTI.

The bladder responses to urinary catheterization are character-
ized by severe uroepithelial damage and exfoliation; the onset of bladder wall edema from increased vascular permeability; the production of proinflammatory cytokines IL-6, G-CSF, and KC, as previously reported (18); and the recruitment of myeloid-derived cells, particularly neutrophils. Neutrophils are the primary responders in implanted bladders followed by macrophages, basophils, and some eosinophils. As previously reported (18), infection with \textit{E. faecalis} increases the above cytokines and induces the secretion of IL-1β and IL-12(p40). Here, we also report the induction of IL-17 following \textit{E. faecalis} infection, which was significantly upregulated and showed at least a 2-fold increase over non-implanted uninfected controls. This cytokine was not previously reported because it did not meet the 2-fold-change arbitrary requirement specified above, even though we observed the statistically significant increase in the bladder following infection of implanted animals with \textit{E. faecalis} (data not shown). Furthermore, the neutrophil populations increased approximately 5-fold in the presence of \textit{E. faecalis} in implanted animals compared to implanted animals without bacterial challenge.

All the above immune characteristics, from edema to neutrophilia, are associated with activation of the neurogenic inflammatory pathway in various experimental models of cystitis, including cyclophosphamide-induced hemorrhagic cystitis (39). This is an inflammatory response triggered by the release of proinflammatory neuropeptides and activation of surface receptors, including NK1R, on the surface of sensory neurons (40). However, our data indicate that the NK1R-mediated neurogenic inflammatory response is not a major contributor of implant-induced cystitis in mice since treatment with specific NK1R antagonists and iNOS inhibitors did not prevent plasma protein leakage and edema as was previously shown in cystitis or other experimental models involving activation of the neurogenic pathway (32, 41, 42). Although the contributions of other factors involved in the neurogenic inflammatory response, including mast cells, bradykinins, and NK2 receptors, need to be assessed, identifying the effects of urinary implantation on factors involved in vascular permeability, such as calcium channels, calveolin, RhoGTPases, sphingosine kinases (SphK1), and protein tyrosine phosphatases (SHP2) (43), may shed light on the mechanisms underlying the onset of bladder wall edema following catheterization.

In contrast to studies with specific inhibitors of the neurogenic inflammatory response, glucocorticoid treatment delays the onset of implant-associated edema and vascular permeability and partially decreases cytokine production and cellular recruitment following urinary catheterization at 24 h posttreatment. Given that the effects of dexamethasone on cytokine production persist up to 24 h posttreatment, it is very likely that the cytokines reported here will also be suppressed at early stages (6 h posttreatment). Together, these findings implicate glucocorticoid-responsive inflammatory pathways in the immune response during the early stages of urinary implantation. While previous reports have shown that synthetic glucocorticoids, such as dexamethasone and prednicarbate, inhibit neurogenic vascular permeability in the respiratory tract of rodents and human forearm skin (44–52), the inhibitory effects of glucocorticoids on this pathway remain questionable, as other such compounds, including hydrocortisone and betamethasone, have been shown to have no effects or even to induce this response in airways of rats (53, 54). Synthetic glucocorticoids, including dexamethasone, are among the most effective anti-inflammatory agents used to date for the treatment of chronic inflammatory diseases (55). They are both anti-inflammatory and immunosuppressive molecules whose mechanisms of action involve in part transcriptional regulation via interaction and activation of glucocorticoid receptors (GR) in the host cytoplasm as well as posttranscriptional and -translational regulation of a myriad of genes encoding proteins for cellular and immune processes (55, 56). Glucocorticoids are known inhibitors of inflammatory processes mediated by interleukins (1β, 2, 6, and 8) and other proinflammatory cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor alpha (TNF-α), phospholipase A2, and iNOS. The unresponsiveness to dexamethasone observed in the later stages of urinary implantation has previously been reported in patients suffering from other inflammatory diseases (57), including asthma (58), inclusion body myositis (IBM) (59), and nephrotic syndrome (NS) in children (60). In the case of the implanted bladders, glucocorticoid refractoriness could be attributed to the ongoing exposure to the foreign body (61), the high levels of neutrophils in the bladder which have been previously associated with corticosteroid-resistant asthma (61), a significant reduction in glucocorticoid receptors (62) due to increased urethral exfoliation, or other cellular and immunological pathways that can circumvent the effects of dexamethasone (61). These may include an increased expression of the dominant negative form of the glucocorticoid receptors (GRβ) on immune cells such as neutrophils and macrophages, rendering them insensitive to dexamethasone treatment (63, 64); upregulation of certain cytokines, including IL-2, IL-4, and IL-13; or that from activation of the mitogen-activated protein kinase (MAPK) signaling pathways (61). Notably, the suppression of the early phase of implant-mediated cystitis following glucocorticoid treatment or iNOS inhibition (see Fig. S3 in the supplemental material) led to a significant increase in enteroococcal implant colonization, indicating that by reducing the inflammatory responses, these treatments favor \textit{E. faecalis} survival in the implanted bladder. Remarkably, by 24 h postinfection, enteroococcal infection in dexamethasone-treated animals induces an immune response similar to that elicited in implanted and infected saline-treated controls. At this later time point, no difference in bacterial colonization in glucocorticoid-treated animals relative to untreated controls is observed. Thus, the immune response, while detrimental to \textit{E. faecalis} colonization, fails to completely clear enteroococcal infection. Further, these findings suggest that \textit{E. faecalis} possesses immune evasion mechanisms that allow its survival in the face of this glucocorticoid-resistant immune response and that implant-mediated bladder inflammation, as in the case of preimplanted animals, did not alter the outcome of infection. The acute inflammatory response induced by the implant may even alter or impair the host response to bacteria, as was demonstrated for \textit{Enterococcus faecium} peritonitis following treatment with tetractin or cascin prior to bacterial challenge (65).

\textit{E. faecalis} colonization is also significantly increased in the bladders of neutropenic mice following urinary implantation, corroborating previous reports that neutrophils are important mediators of the antienteroococcal host response in humans and other animal models of infections (66–69). Previous studies demonstrated that \textit{E. faecalis} and \textit{E. faecium} isolated from saliva and root canals are efficiently killed by neutrophils recruited to the site of infection (70) and that TLR-2 is involved in the immune response against \textit{E. faecalis} (71). However, the immune defense during \textit{E. faecalis} infections of the urinary tract at 24 hpi occurs in
a TLR-2- and IL-6-independent manner, as infection of implanted animals deficient in these immune modulators did not alter the outcome of infection (see Fig. 2 in the supplemental material). Further research is required to establish the molecular mechanisms underlying the role of neutrophils during E. faecalis infection. We have recently adapted an in vitro system to grow biofilms on silicone tubing in filtered human urine under fluid flow (72). However, unlike uropathogenic E. coli, E. faecalis failed to produce biofilms under these conditions, even with the addition of extra carbon sources, such as glucose (data not shown). Despite the absence of an in vitro system to study E. faecalis catheter colonization, our in vivo findings strongly support the critical role of neutrophils in delaying and controlling E. faecalis infection. Uncovering the contribution of macrophages and other immune cells, the Toll-like receptors, IL-8, and G-CSF signaling pathways in the host immune response to enterococcal CAUTI will provide more insights into the host response to these infections.

Despite the role of neutrophils in controlling E. faecalis infection, this bacterium is still able to colonize the urinary tract of implanted immunocompetent mice, implying the presence of potential mechanisms to help E. faecalis avoid and/or resist neutrophil killing. Recent studies have demonstrated that the cell wall-anchored pheromone-inducible aggregation substance (AS) and the enterococcal polysaccharide antigen (Epa) in E. faecalis are involved in resistance to neutrophil-mediated killing (73–75). However, the E. faecalis OG1RF strain used in the present study does not bear AS, arguing for alternative mechanisms of immune evasion, such as the downregulation of integrin 4 expression on the surface of neutrophils (76); alteration of the neutrophil properties rendering them nonresponsive to bacterial infections, as is the case during enterococcal sepsis in thermally injured patients and mice (66, 68, 69); and survival within immune cells such as macrophages and biofilm formation, which are well-characterized virulence attributes of enterococci (18, 77–89). Together, these findings indicate that the inflammatory response to the urinary implant is deleterious to E. faecalis but is inefficient at controlling bacterial proliferation and colonization over time. Furthermore, this report is in accord with epidemiological reports of severe enterococcal infections increasingly occurring in immunosuppressed and immunocompromised patients (90–93).

In addition to promoting persistent enterococcal cystitis, the presence of the silicone implants in the bladder allows E. faecalis to gradually and successfully ascend to the kidneys and establish renal colonization in implanted animals. The onset of acute pyelonephritis has been described in postmortem studies of the elderly with indwelling catheters at the time of death (94). In the clinical setting, the removal of infected indwelling urinary catheters is one of the most effective methods used to resolve bacteriuria and CAUTIs (3, 95). However, removing the indwelling medical device, even combined with a long course of antibiotic treatment (95), may not be sufficient for complete resolution of the infection, especially with the rise in antibiotic resistance observed in nosocomial settings (96). In addition, implant removal upon infection in other device-associated infections like prosthetic valve endocarditis is not in itself efficacious (97–99) and thus may not be a suitable therapeutic approach in all instances. Understanding the constituents of the pathophysiology of CAUTIs, namely, biofilm formation and host immune response to urinary catheters, may lead to the development of novel preventative and therapeutic strategies that limit the damage to the uroepithelium while enhancing effective immune responses to bacterial infections.

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