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Infection and Immunity

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Immune Modulation by Group B *Streptococcus* Influences Host Susceptibility to Urinary Tract Infection by Uropathogenic *Escherichia coli*

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Urinary tract infection (UTI) is most often caused by uropathogenic *Escherichia coli* (UPEC). UPEC inoculation into the female urinary tract (UT) can occur through physical activities that expose the UT to an inherently polymicrobial periurethral, vaginal, or gastrointestinal flora. We report that a common urogenital inhabitant and opportunistic pathogen, group B *Streptococcus* (GBS), when present at the time of UPEC exposure, undergoes rapid UPEC-dependent exclusion from the murine urinary tract, yet it influences acute UPEC-host interactions and alters host susceptibility to persistent outcomes of bladder and kidney infection. GBS presence results in increased UPEC titers in the bladder lumen during acute infection and reduced inflammatory responses of murine macrophages to live UPEC or purified lipopolysaccharide (LPS), phenotypes that require GBS mimicry of host sialic acid residues. Taken together, these studies suggest that despite low titers, the presence of GBS at the time of polymicrobial UT exposure may be an overlooked risk factor for chronic pyelonephritis and recurrent UTI in susceptible groups, even if it is outcompeted and thus absent by the time of diagnosis.

Urinary tract infection (UTI) is one of the most common bacterial infections in humans, occurs mostly in women, and is most often caused by uropathogenic *Escherichia coli* (UPEC). Approximately 8 million outpatient visits occur each year due to UTI, causing an estimated $2 billion in annual health care costs in the United States (15). It is commonly held that the urinary tract (UT) and its contents are sterile in the absence of overt UTI. However, many reports strongly suggest that the female UT is routinely exposed to a diversity of microbes, translocated by physical activity such as sexual intercourse from the nearby periurethral, vaginal, or rectal flora (8, 21, 61). We reason that the diversity and abundance of microbes present in rectovaginal and periurethral "staging areas" (2, 10, 14, 49) likely result in bacterial exposures of the UT that are polymicrobial in nature. Despite this, experimental studies have not been performed to examine whether common host carrier states (commensals or potential pathogens of the female urogenital tract) alter the host-pathogen equation when present together in a polymicrobial UT exposure.

UPEC is the most common cause of UTI and has been extensively studied using murine models of transurethral inoculation. Colonization of the urinary tract triggers early innate host responses involving both hematopoietic and stromal cells that function in mediating the clearance of acute UPEC infection (19, 53, 54). These acute responses to UPEC rely on the induction of local and systemic chemokines and cytokines, exfoliation of epithelial barriers, and infiltration of innate immune cells such as neutrophils and monocytes (18, 43). UPEC employs a number of virulence mechanisms in order to subvert these innate defenses, which enables the pathogen to persist and cause disease (18).

Adhesive pili assembled by the chaperone/usher pathway, such as type 1 pili, contain adhesins at their tips that are thought to play an important role in host-pathogen interactions. The mannose-binding type I pilus tip adhesin FimH mediates UPEC attachment and invasion of bladder epithelial cells (30, 31, 66), and thus, UPEC can be isolated from both luminal and gentamicin-protected intracellular compartments as early as 1 h postinfection (hpi) (29). Within superficial bladder epithelial cells, UPEC can replicate to form biofilm-like intracellular bacterial communities (IBCs) (1). Escape of UPEC into the cytosol and subsequent IBC formation may be a mechanism to subvert Toll-like receptor 4 (TLR4)-dependent expulsion of UPEC via an exocytic vesicle (3, 60). During IBC maturation, UPEC organisms undergo morphological changes, disperse from the IBC biomass, and flux out of the epithelium into the extracellular milieu, where they are capable of invading new epithelial cells to begin the cycle anew (28).

Reflective of different disease manifestations in humans, experimental UPEC infection can result in various long-term sequelae. One distinct outcome is the formation of antibiotic-tolerant, quiescent intracellular reservoirs (QIRs) that form in the bladder within 7 to 10 days during the resolution of bacteriuria in acute infection. QIRs are distinct from IBCs that form during the first 24 hpi, and they are comprised of fewer than 15 bacteria persisting in a membrane-bound dormant state within transitional epithelial cells. Resolution of bacteriuria and bladder titers of less than 10^3 are reflective of QIR formation and have been studied mainly in C57BL/6 mice, in which they can persist in the bladder and are later able to seed new rounds of infection (40, 52). An alternative outcome is the development of long-lasting,

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chronic cystitis characterized by persistent, high-titer bacteriuria ($>10^4$ CFU/ml), as well as high-titer bacterial bladder burdens at sacrifice >4 weeks postinfection (wpi), chronic inflammation, and urothelial necrosis. Severe pyuria and elevated levels of interleukin 6 (IL-6), granulocyte colony-stimulating factor (G-CSF), keritinocyte-derived chemokine (KC), and IL-5 in serum within the first 24 hpi precede chronic infection in C3H/HeN mice and are greater than 95% predictive of future development of chronic UTI (17).

Despite a strong acute inflammatory response directed toward UPEC, C3H/HeN mice not only are susceptible to acute cystitis but also often develop high-titer chronic cystitis and chronic ascending pyelonephritis (17, 23). Recent studies show that elevated systemic inflammatory cytokine responses are prognostic indicators of chronic cystitis development by UPEC (17). In contrast, the related C3H/HeJ mouse strain, which lacks functional TLR4 responses to Gram-negative lipopolysaccharide (LPS) (24, 47), exhibits higher-titer cystitis and pyelonephritis than C3H/HeN mice, without evidence of significant histological inflammation (16, 19, 23, 54, 56). Taken together, these data provide a detailed understanding of monomicrobial UPEC infection, particularly in the C3H/HeN and C3H/HeJ mouse backgrounds, although UPEC pathogenesis and IBC formation have also been studied in C57BL/6J, CBA/J, and FVB/NJ mice (13). We employed the C3H/HeN and C3H/HeJ models to investigate whether the presence of a common urogenital inhabitant and potential pathogen, group B streptococcus (GBS), at the time of UPEC exposure can fundamentally alter the cellular or molecular dynamics of UPEC-host interactions.

GBS is a Gram-positive bacterium that asymptomatically colonizes the lower gastrointestinal, vaginal, and/or urinary tract in up to 65% of healthy women (38). While the bacterium has evolved to coexist peacefully in most immunocompetent adults, certain susceptible individuals, such as newborns, pregnant women, and the elderly, are at higher risks of developing invasive GBS infections (59, 70). GBS is best known as a leading cause of pneumonia, bacteremia, sepsis, and meningitis in newborns (5, 70). A less recognized focus of infection for GBS is the UT. GBS causes about 1% of all monomicrobial UTIs (approximately 80,000 annually), which occur largely in immunocompromised, diabetic, elderly, and pregnant populations (11, 65). We recently demonstrated that GBS mediates both immune suppression and activation in a murine model of UTI (32). GBS uses sialic acid residues of its capsular polysaccharide to suppress oxidative burst responses of murine polymorphonuclear cells (PMNs), and unnaturally high levels of sialic acid O-acetylation (OAc^{hi} strain) block the ability of GBS to suppress PMNs compared to an isogenic OAc^{lo} strain (32, 68). We further showed that GBS OAc^{lo} organisms, which were capable of suppressing PMN oxidative burst, survived better in the urinary tract of C3H/HeN mice than the isogenic OAc^{hi} strain (32, 68). In this study, we investigated the hypothesis that GBS presence within a polymicrobial UT exposure may shape inflammatory processes that influence host-UPEC interactions. We show that GBS can influence long-term outcomes of UPEC UTI despite its own rapid elimination in the presence of UPEC. Further, we present evidence that prior to its demise, GBS employs sialic acid residues of its capsular polysaccharide to fundamentally alter the cellular dynamics of early UPEC-host interactions.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Wild-type (WT) uropathogenic *E. coli* strain UTI89 (39), UTI89 att_{HK022}::*COM-GFP* (kanamycin resistant [Kan^r]) (69), UTI89 FimH Q133K (SLC2-35-1) (6), and UTI89 FimH wild-type control strain (SLC2-33-1) (6) were inoculated from single colonies grown on LB agar into LB broth, containing kanamycin at 25 μg/ml where appropriate, and grown statically overnight (18 to 24 h) at 37°C. In the wild-type UPEC strains under investigation, these standardly used growth conditions result in optimal expression of type 1 pili (6, 28), necessary for establishing a urinary tract infection. *Streptococcus agalactiae* (also called group B streptococcus [GBS]) wild-type strain COH1, a well-characterized strain that expresses low levels of the β-hemolysin and high levels of the capsular polysaccharide, or isogenic mutants of GBS COH1 expressing sialic acids with minimal O-acetylation (OAc^{lo}) or hyper-O-acetylation of sialic acids (OAc^{hi}) (34, 35, 67, 68) were used in these studies. As previously described, the WT COH1 strain has an intermediate level of O-acetylation (67). For all experiments, GBS was inoculated from single colonies grown on Todd-Hewitt (TH) agar into TH broth with antibiotics where appropriate, grown statically overnight, and then diluted 1:10 in fresh TH broth for an additional 1 to 2 h at 37°C to an optical density at 600 nm (OD_{600}) of approximately 0.4 (logarithmic phase) as previously described for GBS UTI and other *in vivo* virulence studies (9, 32, 68).

Murine infections. Bacterial cultures, grown as described above, were collected by centrifugation and resuspended in phosphate-buffered saline (PBS). Female wild-type mice, 7 to 10 weeks of age, were obtained from Harlan (C3H/HeN) or the Jackson Laboratories (C3H/HeJ). Mice were anesthetized by inhalation of 3% isoflurane. Mice were then voided prior to transurethral bacterial inoculation with 1×10^7 to 2×10^7 CFU in 50 μl (26). For mixed infections, GBS and UPEC were mixed to obtain a 50-μl bacterial suspension of 1×10^7 to 2×10^7 CFU of each organism. At various time points, mice were euthanized and bladders and kidneys were aseptically removed. The number of bacteria present in the tissues was determined by homogenization of bladders or kidney pairs in PBS and plating of serial dilutions on LB or TH agar. For coinfection studies, samples were plated on MacConkey agar and Columbia CNA agar plus 5% sheep blood, supplemented with antibiotics when appropriate, to differentiate between Gram-negative and Gram-positive bacteria, respectively. Alternately, *ex vivo* gentamicin protection assays were performed on excised bladders to assess the extracellular and intracellular populations in the bladder (29). Briefly, bladders were removed at 3 hpi, quadrisected to assist in thorough washing of the tissue, and washed 3 times in 500 μl fresh PBS with gentle mixing. Enumeration of total CFU from the 3 pooled washes indicates luminal or loosely attached extracellular bacteria. Gentamicin treatment (100 μg/ml) proceeded at 37°C for 90 min, followed by thorough washing, homogenization, and enumeration of gentamicin-protected bacteria. The supernatant from the gentamicin treatment was plated separately on nonselective media to ensure that all extracellular UPEC and/or GBS organisms were killed by the gentamicin during the incubation. Statistical analyses were performed using the Mann-Whitney test with GraphPad Prism software (version 5.00 for Windows; GraphPad Software). Recovered titers of 0 are graphed at the limit of detection of the assay, and median titers are indicated in all figures. All animal studies were performed in accordance with the Committee for Animal Studies at Washington University School of Medicine.

Cytokine measurement. UPEC, GBS, and UPEC plus GBS were inoculated into mouse bladders as described above, and venous blood was collected at 24 hpi by submandibular puncture into 400-μl Microtainer serum separation tubes (BD). After coagulation, Microtainer tubes were subjected to centrifugation at 15,000 × *g* for 5 min at 4°C and stored at −20°C. Cytokine expression was measured using the Bio-Plex multiplex cytokine bead kit (Bio-Rad), which measures 23 different proinflammatory cytokines. Statistical analyses were performed in GraphPad Prism using the Mann-Whitney U test.

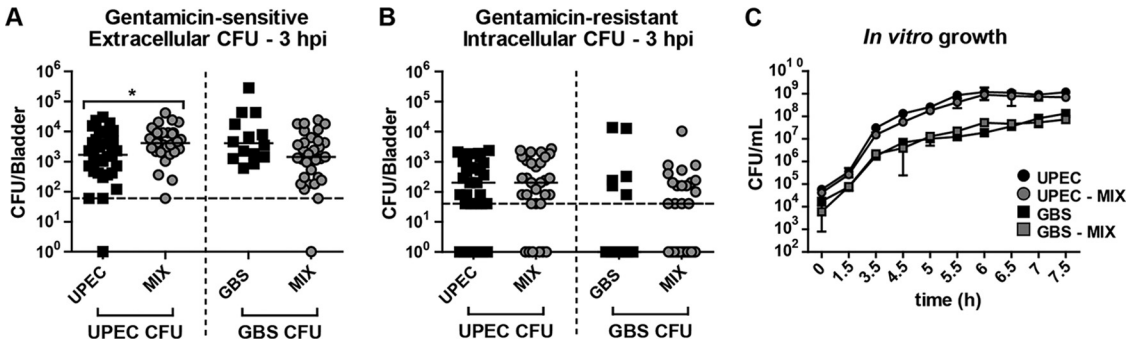

FIG 1 GBS alters the cellular dynamics of UPEC-host interactions in the bladder. Gentamicin protection assays were performed on excised bladders of mono- or coinoculated animals at 3 hpi. (A) Lumen titers were determined by bacterial enumeration of pooled washes of quadrisected bladder tissue and may include bacteria loosely attached to the bladder tissue. (B) Gentamicin-resistant CFU associated with bladder tissue. $n = 5$ or 6, at least 5 mice per experiment. The horizontal bar represents the median value for each group of mice. Statistical significance was determined by the Mann-Whitney test. *, $P < 0.05$. The dashed horizontal line indicates the limit of detection of the assay. Titers below the limit of detection were assigned a value of 1 for visualization on the log scale and 0 for statistical analyses. (C) Bacteria were grown in mono- or coculture *in vitro*, indicated by black or gray shapes, respectively. Error bars represent the standard deviations of 3 technical replicates sampled at each time point.

NF-κB assays. RAW-Blue cells (murine RAW 264.7 macrophages with chromosomal integration of a secreted embryonic alkaline phosphatase [SEAP] reporter construct inducible by NF-κB; Invivogen) were maintained in Dulbecco modified Eagle medium (DMEM), 4.5 g/liter glucose, 2 to 4 mM L-glutamine, 10% (vol/vol) fetal bovine serum, 50 U/ml penicillin, 50 μg/ml streptomycin, 100 μg/ml Normocin, and 200 mg/ml Zeocin at 37°C in 5% CO_2. For infection experiments, cells were seeded into a 96-well plate (Falcon Microtest tissue culture plate, flat bottom, low evaporation lid, catalog no. 35307; BD) at ~100,000 cells/well in 200 μl antibiotic-free medium, according to the supplier's instructions. After overnight incubation, cells were washed with PBS, and 180 μl fresh medium was added. Wells (triplicate for each condition) were treated with 20 μl LPS from *E. coli* O26:B6 at 10 ng/ml (catalog no. L2654; Sigma), empirically determined for optimal SEAP activity in LPS dilution series experiments. Cells were also infected with GBS, UPEC, or GBS plus UPEC (at a 20:1, 10:1, or 1:1 ratio of bacteria to macrophage) in PBS or PBS only as a mock-infected control and incubated at 37°C in 5% CO_2. At 6 hpi, 20 μl supernatant was removed and added to 180 μl QUANTI-Blue (Invivogen), the mixture was incubated at 37°C overnight, and SEAP levels were determined at 640 nm. Results shown depict one representative experiment of at least three biologically independent experiments performed. Lactate dehydrogenase assays (Clontech) were performed according to the manufacturer's instructions on macrophage supernatants after exposure to WT GBS for 6 h.

RESULTS

Bladder coexposure to GBS and UPEC alters host interactions with both organisms. To investigate the impact of bacterial coinoculation on the early cellular dynamics of host-microbe interactions in the bladder, we measured lumen- and tissue-associated titers of GBS and UPEC following mono- and coinoculations. In order to evaluate the number of bacteria that had invaded the bladder tissue 3 h after transurethral exposure to bacteria (GBS, UPEC, or coinoculated GBS and UPEC), an *ex vivo* gentamicin protection assay was performed on each murine bladder (39). Extracellular bacteria surviving in the bladder lumen were measured from tissue washes prior to gentamicin treatment, followed by recovery and enumeration of gentamicin-protected, tissue-associated bacteria. In these experiments, extracellular UPEC CFU were significantly elevated in the lumen at 3 hpi in the presence of GBS compared to UPEC alone (Fig. 1A). The ability of UPEC to invade the urothelium and enter gentamicin-protected intracellular compartments was not altered by the presence of GBS (Fig. 1B). While UPEC presence led to an apparent reduction of GBS fitness in the acutely coinfected bladder lumen, UPEC did not affect the ability of GBS to occupy a gentamicin-protected niche (Fig. 1B). This is, to our knowledge, the first report that GBS, like UPEC, can inhabit gentamicin-protected bladder tissue compartments *in vivo*. We obtained identical growth curves for GBS and UPEC during mono- and coculture of the organisms *in vitro*, strongly suggesting that host factors contribute to the observed population dynamics in the bladder (Fig. 1C). These data show that GBS accesses multiple bladder niche environments within the first few hours of coinfection and leads to increased fitness of UPEC in the bladder lumen during acute infection.

GBS capsule sialic acids promote UPEC survival in the bladder lumen. Previous studies have shown that bladder epithelial cells are capable of expelling invaded UPEC (60). Thus, increased luminal titers of UPEC in the presence of GBS may involve intracellular transit of UPEC followed by their expulsion into the lumen. The FimH::Q133K mutation, which abolishes the primary mannose-dependent adhesive interaction of UPEC with bladder epithelial cells (25), was used to investigate whether FimH-mediated binding and invasion of the bladder tissue were required for the enhanced early survival of UPEC in the presence of GBS. Mice were mono- or coinfected with GBS and the FimH::Q133K mutant of UPEC strain UTI89 (6). In monoinfected mice, UPEC bearing the Q133K FimH mutation was unable to bind or invade the bladder epithelium and was cleared from the bladders of most animals by 3 hpi (Fig. 2A) (6). However, we discovered that in the presence of GBS, UPEC bearing the Q133K FimH mutation survived significantly better in the acutely infected bladder lumen (Fig. 2A). In contrast, the invasion of Q133K UPEC remained defective even in the presence of GBS, as determined by gentamicin protection (Fig. 2A). GBS-mediated enhancement of UPEC bearing FimH::Q133K was limited to acute infection and was not observed at 24 hpi (data not shown). These results demonstrate that FimH-mediated attachment and invasion by UPEC are not required for GBS augmentation of UPEC survival in the bladder lumen.

We recently showed that GBS capsular sialic acid residues sup-

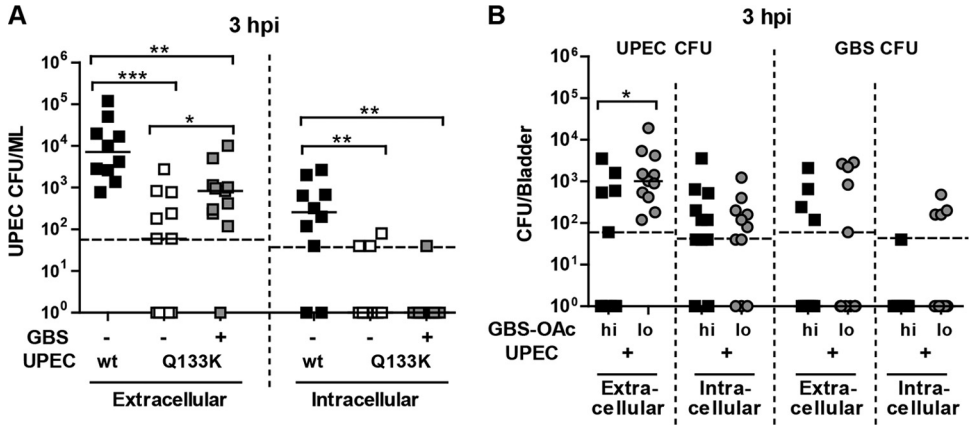

FIG 2 GBS capsule sialic acids enhance UPEC survival in the bladder lumen during acute infection. Gentamicin protection assays were performed on excised bladders (3 hpi) of mono- or coinoculated animals. (A) Lumen bladder survival assays using the bladder binding-defective UPEC FimH::Q133K mutant in the presence or absence of WT GBS. (B) Lumen bladder survival assays after coinoculation of WT UPEC and GBS capsule mutants. $n = 2$, at least 5 mice per experiment. The horizontal bar represents the median value for each group of mice. Statistical significance was determined by the two-tailed Mann-Whitney test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. The dashed horizontal line indicates the limit of detection of the assay. Titers below the limit of detection were assigned a value of 1 for visualization on the log scale and 0 for statistical analyses.

press PMN oxidative burst responses and enhance GBS survival in the urinary tract of C3H/HeN mice (32, 68). To determine whether GBS capsular sialic acids also play a role in the observed GBS augmentation of UPEC survival, we analyzed intracellular and extracellular UPEC survival in the presence of isogenic GBS strains bearing high or low levels of sialic acid O-acetylation. Bladders were removed 3 h after transurethral coinoculation, followed by enumeration of luminal and intracellular GBS and UPEC organisms. While the lumen titers of GBS OAc^{hi} and OAc^{lo} strains did not differ significantly at this early time point, wild-type luminal UPEC CFU were elevated in the presence of GBS OAc^{lo} compared to GBS OAc^{hi} (Fig. 2B). There were no differences observed in the intracellular UPEC or GBS populations under any of these conditions. These results establish that GBS acts through a sialic acid-mediated mechanism to enhance UPEC titers in the bladder lumen.

GBS augmentation of UPEC in the bladder lumen occurs in C3H/HeN but not C3H/HeJ mice. To further investigate the hypothesis that GBS may suppress immune responses during acute infection, we examined the fate of UPEC in mono- or coinfected C3H/HeJ mice, which lack a functional TLR4 receptor and thus are unable to mount a robust inflammatory response to UPEC (16, 22, 53, 54). We reasoned that if GBS suppresses TLR4-mediated inflammatory responses, UPEC should derive no significant benefit from GBS in the bladder of C3H/HeJ coinfected mice, since immune responses to UPEC in this host background are already blunted. UPEC CFU in the bladder lumens of C3H/HeJ and C3H/HeN mice were compared after coinfection with GBS. Consistent with our hypothesis, C3H/HeJ mice (in contrast to their C3H/HeN counterparts) did not exhibit a GBS-dependent augmentation of UPEC titers in the bladder (Fig. 3A). Enumeration of bacterial titers in the kidneys revealed a significant GBS-dependent reduction of UPEC titers in C3H/HeJ mice compared to C3H/HeN mice (Fig. 3B). These data further support the hypothesis that GBS modulation of TLR4-dependent innate processes is likely responsible for the observed augmentation of UPEC in the acute bladder. Moreover, the data suggest that GBS may have distinct effects on UPEC-host interactions in the bladder and kidneys for reasons that are currently unknown. However, recent studies have shown that there is at least one additional

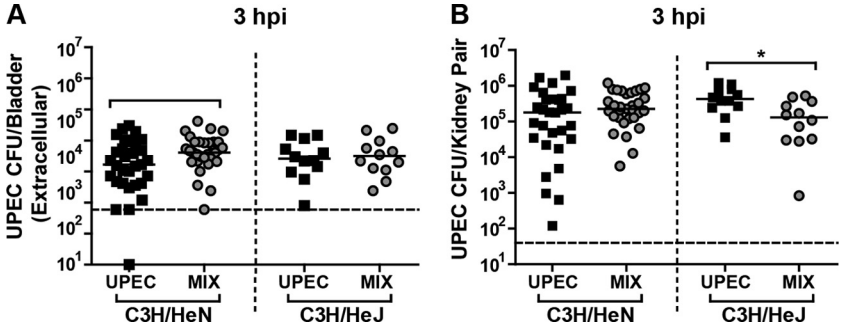

FIG 3 UPEC augmentation by GBS in the bladder lumen does not occur in C3H/HeJ mice. Gentamicin protection assays were performed on excised bladders (A) and kidney CFU were determined (B) in mono- or coinoculated C3H/HeN and C3H/HeJ mice at 3 hpi. UPEC bladder CFU from C3H/HeN mouse data shown here are replotted from Fig. 1A for ease of comparison with data obtained from C3H/HeJ mice. Metadata from 6 experiments are shown for C3H/HeN mice; $n = 2$ for C3H/HeJ mice, with at least 5 mice per experiment. The horizontal bar represents the median value for each group of mice. Statistical significance was determined by the two-tailed Mann-Whitney test. *, $P < 0.05$. The dashed horizontal line indicates the limit of detection of the assay. Titers below the limit of detection were assigned a value of 1 for visualization on the log scale and 0 for statistical analyses.

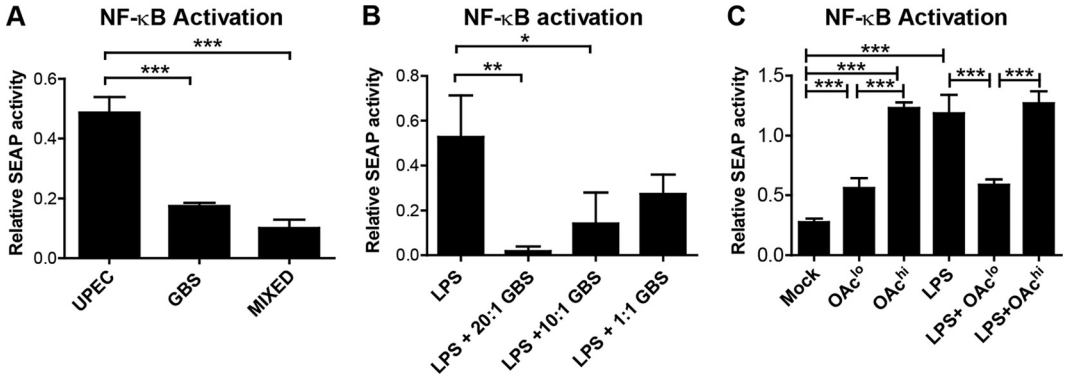

FIG 4 GBS suppresses macrophage NF-κB-driven inflammation in response to LPS and UPEC. Murine macrophage RAW267.4 cell line expressing a stable chromosomally integrated secreted embryonic alkaline phosphatase (SEAP) reporter downstream of 6 repeated NF-κB-responsive elements was stimulated with UPEC, GBS, or UPEC plus GBS (mixed bacterial inoculation) (A); LPS in the presence or absence of GBS at MOIs of 1:1, 10:1, and 20:1 (B); or LPS in the presence of GBS capsule mutants (MOI, 10:1) that are augmented (OAc^{lo}) or attenuated (OAc^{hi}) in GBS murine cystitis (C). Following 6 h of stimulation, NF-κB-dependent transcriptional activity was measured in cell supernatants (SEAP activity). Data shown reflect one representative experiment of at least 3 biologically independent experiments performed. Each bar reflects data from 3 technical replicates. Statistical significance was determined by the unpaired two-tailed *t* test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

genetic locus (in comparison to C3H/HeN mice) that contributes to the well-known susceptibility of the C3H/HeJ strain to UTI (62), and thus, further studies were carried out to investigate the effect of GBS on TLR4-mediated processes.

NF-κB-driven transcription by murine macrophages in response to UPEC and LPS is suppressed by GBS in a sialic acid-dependent manner. The hypothesis that GBS may suppress immune responses specifically resulting from LPS-TLR4 interactions was investigated by measuring the ability of GBS to affect LPS- or UPEC-driven activation of the transcription factor NF-κB in murine macrophages. Macrophages encoding a stable secreted alkaline phosphatase reporter downstream of NF-κB-responsive promoter sequences were exposed to different combinations and doses of UPEC, LPS, and/or GBS. As expected, strong induction of NF-κB-dependent transcription was observed in response to LPS alone or UPEC alone at 6 hpi in control experiments (Fig. 4A and B). In contrast, when GBS was added to macrophages together with UPEC (Fig. 4A) or LPS (Fig. 4B), macrophages no longer responded by upregulating NF-κB-dependent transcription. Although GBS has previously been shown to induce macrophage apoptosis at a relatively high multiplicity of infection (MOI) (100:1 ratio of bacteria to macrophage) following 24 h of exposure (12, 64), the lower MOI (1:1, 1:10, or 1:20) and shorter duration (6 h) of these experiments with WT GBS did not result in macrophage death, as evidenced by the absence of lactate dehydrogenase (27) in supernatants of macrophages exposed to WT GBS (data not shown). These data demonstrate that the Gram-positive GBS engages in suppression of NF-κB-driven macrophage responses to Gram-negative LPS.

To determine if GBS capsule sialic acids participate in the modulation of macrophage NF-κB-driven responses, similar to their contribution to suppression of PMN activity (32), we investigated macrophage activation with OAc^{hi} and OAc^{lo} strains of GBS in the presence or absence of simultaneous LPS stimulation. In the absence of LPS stimulation, OAc^{lo} GBS suppressed NF-κB-driven proinflammatory responses in murine macrophages, while high levels of sialic acid O-acetylation on the isogenic OAc^{hi} strain blocked the ability of GBS to suppress macrophage activation (Fig. 4C). A similar phenotype was observed in the presence of LPS, where OAc^{lo} GBS suppressed LPS-induced NF-κB-driven inflammation, while OAc^{hi} GBS was unable to limit NF-κB-driven macrophage responses in the presence of LPS. This discovery adds GBS to a growing number of pathogens that can inhibit NF-κB signaling as a pathogenic strategy (33, 42). Taken together with evidence that capsule sialic acids also contribute to UPEC survival in the bladder lumen (Fig. 2A), these data strongly implicate direct immune modulation of TLR4-dependent processes by the GBS capsule in alteration of UPEC-host interactions following polymicrobial UT exposure.

GBS presence during acute UTI reduces the risk of chronic high-titer UPEC cystitis but increases the risk of latent bacterial reservoirs and the severity of ascending kidney infection. The ramifications of GBS exposure on long-term UTI outcomes were also investigated. Analysis of serum cytokines at 24 hpi was performed to examine the possibility that GBS may shift the previously described checkpoint of host acute inflammatory responses linked with the development of chronic cystitis (17). Mice were inoculated with UPEC alone or coinoculated with GBS, followed by analysis of serum cytokines at 24 hpi, urine CFU over the course of infection, and bladder and kidney titers at 4 wpi. We found that the inflammatory cytokine signature previously shown to predict the development of chronic UPEC cystitis in monoinfected mice (IL-6, KC, and G-CSF) (17) also correlated with the development of chronic infection following mixed inoculation regardless of GBS dose (Fig. 5A to C); however, the proportion of mice succumbing to chronic high-titer cystitis was affected (see below). UPEC CFU in the bladder at 24 hpi, the time point at which cytokines were analyzed, did not differ between mono- and coinfected animals. In contrast, GBS had been preferentially eliminated in the presence of UPEC from most animals by 24 hpi (Fig. 5D).

Examining the consequence of coinoculation on long-term infections, we observed a unique dose-dependent effect of GBS on the development of UPEC chronic high-titer cystitis ($>10^4$ CFU/ml with bacteriuria), the presence of latent UPEC reservoirs ($<10^4$ CFU/ml without bacteriuria), and the ability of UPEC to cause high-titer infections of the kidney. When high-dose GBS and UPEC ($\sim 10^7$ each) were coinoculated, significantly fewer

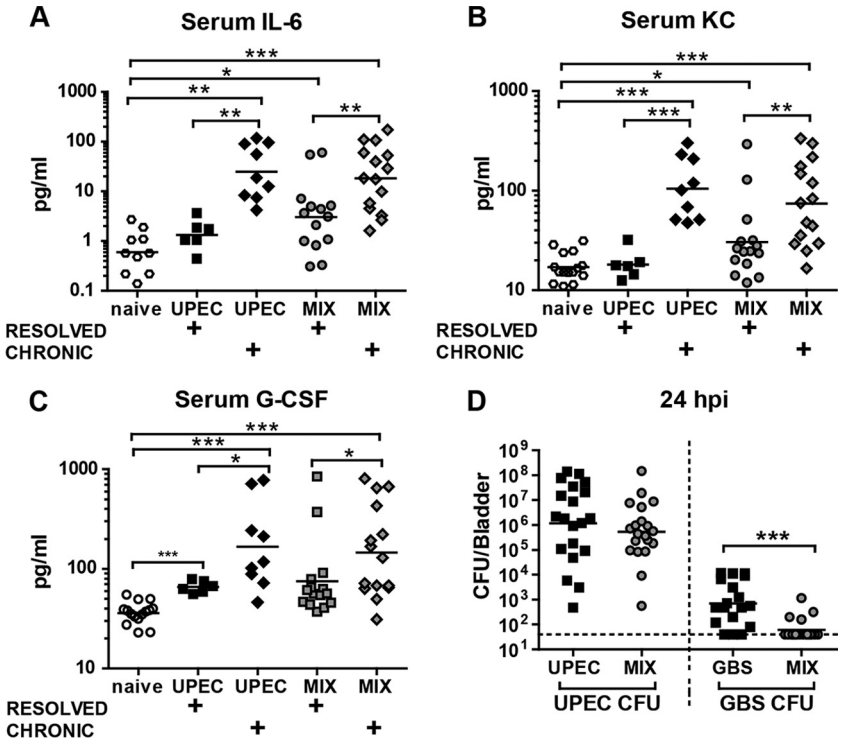

FIG 5 GBS coinoculation induces cytokines associated with UPEC chronic cystitis in C3H/HeN mice. (A to C) Serum cytokine analysis at 24 hpi of C3H/HeN mice mono- and coinoculated with ~10^7 UPEC UTI89 alone or coinoculated with 1×10^7 to 2×10^7 GBS COH1 CFU (high dose) or 2×10^6 to 9×10^6 GBS COH1 CFU (low dose), stratified by animals that went on to develop chronic cystitis or that resolved infection (see Fig. 6). $n = 3$, at least 5 mice per experiment. (D) UPEC and GBS CFU in the bladder at 24 h after transurethral inoculation with ~10^7 GBS COH1 organisms alone or coinoculated with ~10^7 UPEC UTI89 organisms. $n = 4$ experiments, with at least 5 mice per experiment. The horizontal bar represents the median value for each group of mice. The dashed horizontal line indicates the limit of detection of the assay. Titers below the limit of detection were assigned a value of 1 for visualization on the log scale and 0 for statistical analyses. For both serum cytokine levels and CFU, statistical significance was determined by the two-tailed Mann-Whitney test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

mice developed persistent bacteriuria (data not shown) and chronic cystitis ($>10^4$ CFU/ml) at 4 wpi compared to animals that received monomicrobial inoculation of UPEC (Fig. 6A). Instead, high-dose GBS shifted UPEC outcomes toward a lower-titer ($<10^4$ CFU/ml) latent reservoir as previously described (22–24). Coinfection with a slightly lower dose of GBS (approximately 2:1 ratio of UPEC to GBS) eliminated the GBS-dependent shift in UPEC outcomes toward latent reservoirs, instead rendering the animals more susceptible to high-titer chronic cystitis than with high-dose GBS (Fig. 6A). Consistent with this finding, mice coinfected with the lower dose of GBS had significantly higher UPEC titers in the bladder and kidneys at 4 wpi than with UPEC alone or high-dose GBS (Fig. 6B and C). Together, these data demonstrate that GBS presence within the first 24 h after mixed microbial ex-

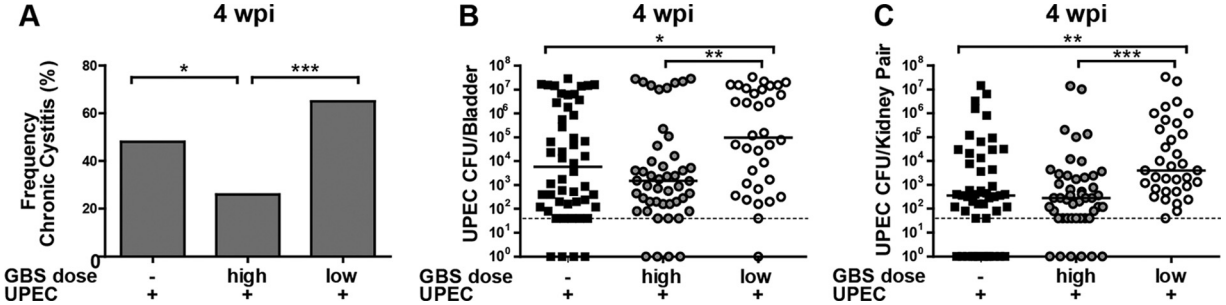

FIG 6 GBS coinoculation has dose-dependent effects on the development of chronic UPEC cystitis and pyelonephritis C3H/HeN mice. (A) The percentage of mice displaying $>10^4$ CFU/bladder at 4 wpi, indicative of chronic cystitis following infection of C3H/HeN mice with ~10^7 UPEC UTI89 organisms alone, or coinoculated with high-dose GBS (1×10^7 to 2×10^7 GBS COH1 CFU, ~1:1 ratio of UPEC to GBS) or low-dose GBS (2×10^6 to 9×10^6 GBS COH1 CFU, ~2:1 ratio of UPEC to GBS). Fisher's exact test was used for statistical analysis. According to previous work, animals with persistent infection with $<10^4$ CFU/bladder are characterized by the presence of latent quiescent reservoirs. (B) UPEC CFU in the bladder at 4 wpi. (C) UPEC CFU in the kidneys at 4 weeks following monoinoculation with UPEC or coinoculation. For CFU analysis, $n = 3$ to 8, at least 5 mice per experiment. The horizontal bar represents the median value for each group of mice. Statistical significance was determined by the two-tailed Mann-Whitney test. *, $P < 0.05$; ***, $P < 0.001$. The dashed horizontal line indicates the limit of detection of the assay. Titers below the limit of detection were assigned a value of 1 for visualization on the log scale and 0 for statistical analyses.

posure of the urinary tract strongly influences the outcome of persistent UPEC UTI.

DISCUSSION

Urinary tract infections are highly prevalent and afflict otherwise healthy individuals across their life span, from infancy to old age. Significant efforts have been made to understand the molecular mechanisms underlying UTI, focusing largely on UPEC since it is the uropathogen most frequently isolated from infected individuals. The "gold standard" laboratory model in the field takes advantage of 7- to 8-week-old female mice transurethrally infected with a single uropathogen. Studies using this model have revealed novel aspects and mechanisms for UTI pathogenesis. However, our understanding of the complexities associated with UTI susceptibility in at-risk groups and under more realistic infection conditions is limited. The results presented here demonstrate that GBS may be an underappreciated uropathogen, as it can shift complex immunological responses in the urinary tract during acute infection, altering UPEC-host interactions in ways that change the long-term consequences of infection. These findings call for further experimental and clinical studies to examine GBS, as well as other common vaginal and gut bacteria, as potential risk factors for UTI in susceptible groups, including pregnant, elderly, and immunocompromised populations.

Our findings are consistent with coinfection studies at other mucosal sites, bolstering the concept that bacterial community members within the same niche are not blind to one another but can interact in mutualistic and/or antagonistic ways (4, 45, 50). In humans, clinical UTI is associated with increased sexual intercourse frequency in the 14 days prior to diagnosis (8). It is likely that polymicrobial UT exposure occurs prior to, or as a result of, these events. When polymicrobial urine cultures are found in clinically diagnosed UTI, the general clinical paradigm in healthy, nonpregnant adults is that titers of Gram-positive bacteria of $<10^5$ CFU/ml of urine are etiologically and clinically insignificant in the background of a dominant UPEC infection. However, the data presented here suggest that bacteria present at the time of initial exposure can influence the outcome of UTI and still be at low or undetectable levels by the time of UTI diagnosis. For these reasons, we argue that the contribution of polymicrobial inoculation to host UTI susceptibility has likely been underestimated. Our findings call into question some long-held assumptions in the field of clinical urology and urinary tract pathogenesis, showing that the composition of a UT exposure can indeed influence the cellular dynamics of host-uropathogen interactions and the outcome of UTI.

Remarkably, GBS exerts effects on UPEC UTI despite its own rapid clearance. While GBS did not measurably alter UPEC invasion, exfoliation of epithelial cells (data not shown), or recruitment of inflammatory cells (data not shown), its presence augmented early survival of UPEC in the bladder lumen. This GBS-mediated augmentation of UPEC did not occur in C3H/HeJ mice, which lack TLR4-driven responses, suggesting that the influence of GBS on UTI outcomes may occur via GBS modulation of host innate immune responses. Indeed, *in vivo* experiments showed that GBS uses a capsule-mediated mechanism to augment early titers of UPEC in the bladder lumen following coexposure. *In vitro* experiments with murine macrophages further demonstrated that the GBS capsule contributes to a state of LPS tolerance via suppression of NF-κB-driven responses. In this way, GBS likely promotes the survival of UPEC, and possibly other uropathogens, including otherwise unfit bacteria (such as UPEC FimH mutants), in the acutely infected bladder. GBS suppression of innate immune responses during acute UTI may, at optimal doses, create a more hospitable environment for UPEC, leading to the transcendence of early infection bottlenecks and alteration of long-term infection (55).

Few bacterial species exist as monocultures in their native environments, and yet, the vast majority of microbiological studies to date have been monomicrobial in nature. Recently, there has been a growing appreciation for the significance of polymicrobial infections as well as the importance of a healthy flora for resistance to infection. Synergistic polymicrobial behavior has been implicated in a variety of disease states, including sepsis (46), cystic fibrosis lung infection (20, 51, 57, 58), community-acquired respiratory infections (36, 37, 41), periodontal disease and other oral pathogen infection models (44, 48), and UTI (7, 63). Our increasing understanding of the complexity of infectious disease states and the contribution of multiple etiologic agents underscores the need for modern genetic and molecular diagnostics along with treatment tailored accordingly, a need especially important for chronic and recurrent diseases, such as UTI, that are increasingly recalcitrant to treatment.

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REFERENCES

1. **Anderson GG, et al.** 2003. Intracellular bacterial biofilm-like pods in urinary tract infections. Science **301**:105–107.
2. **Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI.** 2005. Host-bacterial mutualism in the human intestine. Science **307**:1915–1920.
3. **Bishop BL, et al.** 2007. Cyclic AMP-regulated exocytosis of Escherichia coli from infected bladder epithelial cells. Nat. Med. **13**:625–630.
4. **Brogden KA, Guthmiller JM, Taylor CE.** 2005. Human polymicrobial infections. Lancet **365**:253–255.
5. **CDC.** 2009. Trends in perinatal group B streptococcal disease—United States, 2000–2006. MMWR Morb. Mortal. Wkly. Rep. **58**:109–112.
6. **Chen SL, et al.** 2009. Positive selection identifies an in vivo role for FimH during urinary tract infection in addition to mannose binding. Proc. Natl. Acad. Sci. U. S. A. **106**:22439–22444.
7. **Croxall G, et al.** 2011. Increased human pathogenic potential of Escherichia coli from polymicrobial urinary tract infections in comparison to isolates from monomicrobial culture samples. J. Med. Microbiol. **60**:102–109.
8. **Czaja CA, et al.** 2009. Prospective cohort study of microbial and inflammatory events immediately preceding Escherichia coli recurrent urinary tract infection in women. J. Infect. Dis. **200**:528–536.
9. **Doran KS, Liu GY, Nizet V.** 2003. Group B streptococcal beta-hemolysin/cytolysin activates neutrophil signaling pathways in brain endothelium and contributes to development of meningitis. J. Clin. Invest. **112**:736–744.
10. **Eckburg PB, et al.** 2005. Diversity of the human intestinal microbial flora. Science **308**:1635–1638.
11. **Edwards MS, Baker CJ.** 2005. Group B streptococcal infections in elderly adults. Clin. Infect. Dis. **41**:839–847.
12. **Fettucciari K, et al.** 2006. Group B *Streptococcus* induces macrophage apoptosis by calpain activation. J. Immunol. **176**:7542–7556.
13. **Garofalo CK, et al.** 2007. Escherichia coli from urine of female patients with urinary tract infections is competent for intracellular bacterial community formation. Infect. Immun. **75**:52–60.
14. **Gill SR, et al.** 2006. Metagenomic analysis of the human distal gut microbiome. Science **312**:1355–1359.

15. **Griebling Tl.** 2007. Urinary tract infection in women. U.S. Government Printing Office, Washington, DC.
16. **Hagberg L, et al.** 1984. Difference in susceptibility to gram-negative urinary tract infection between C3H/HeJ and C3H/HeN mice. Infect. Immun. **46**:839–844.
17. **Hannan TJ, Mysorekar IU, Hung CS, Isaacson-Schmid ML, Hultgren SJ.** 2010. Early severe inflammatory responses to uropathogenic *E. coli* predispose to chronic and recurrent urinary tract infection. PLoS Pathog. **6**:e1001042. doi:10.1371/journal.ppat.1001042.
18. **Hannan TJ, et al.** 2012. Host-pathogen checkpoints and population bottlenecks in persistent and intracellular uropathogenic Escherichia coli bladder infection. FEMS Microbiol. Rev. **36**:616–648.
19. **Haraoka M, et al.** 1999. Neutrophil recruitment and resistance to urinary tract infection. J. Infect. Dis. **180**:1220–1229.
20. **Hoffman LR, et al.** 2006. Selection for Staphylococcus aureus small-colony variants due to growth in the presence of Pseudomonas aeruginosa. Proc. Natl. Acad. Sci. U. S. A. **103**:19890–19895.
21. **Hooton TM, et al.** 1999. Perineal anatomy and urine-voiding characteristics of young women with and without recurrent urinary tract infections. Clin. Infect. Dis. **29**:1600–1601.
22. **Hopkins W, Gendron-Fitzpatrick A, McCarthy DO, Haine JE, Uehling DT.** 1996. Lipopolysaccharide-responder and nonresponder C3H mouse strains are equally susceptible to an induced Escherichia coli urinary tract infection. Infect. Immun. **64**:1369–1372.
23. **Hopkins WJ, Gendron-Fitzpatrick A, Balish E, Uehling DT.** 1998. Time course and host responses to Escherichia coli urinary tract infection in genetically distinct mouse strains. Infect. Immun. **66**:2798–2802.
24. **Hoshino K, et al.** 1999. Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. J. Immunol. **162**:3749–3752.
25. **Hung CS, et al.** 2002. Structural basis of tropism of Escherichia coli to the bladder during urinary tract infection. Mol. Microbiol. **44**:903–915.
26. **Hung CS, Dodson KW, Hultgren SJ.** 2009. A murine model of urinary tract infection. Nat. Protoc. **4**:1230–1243.
27. **Jiang W, Bell CW, Pisetsky DS.** 2007. The relationship between apoptosis and high-mobility group protein 1 release from murine macrophages stimulated with lipopolysaccharide or polyinosinic-polycytidylic acid. J. Immunol. **178**:6495–6503.
28. **Justice SS, et al.** 2004. Differentiation and developmental pathways of uropathogenic Escherichia coli in urinary tract pathogenesis. Proc. Natl. Acad. Sci. U. S. A. **101**:1333–1338.
29. **Justice SS, Lauer SR, Hultgren SJ, Hunstad DA.** 2006. Maturation of intracellular Escherichia coli communities requires SurA. Infect. Immun. **74**:4793–4800.
30. **Kline KA, Dodson KW, Caparon MG, Hultgren SJ.** 2010. A tale of two pili: assembly and function of pili in bacteria. Trends Microbiol. **18**:224–232.
31. **Kline KA, Falker S, Dahlberg S, Normark S, Henriques-Normark B.** 2009. Bacterial adhesins in host-microbe interactions. Cell Host Microbe **5**:580–592.
32. **Kline KA, Schwartz DJ, Lewis WG, Hultgren SJ, Lewis AL.** 2011. Immune activation and suppression by group B streptococcus in a murine model of urinary tract infection. Infect. Immun. **79**:3588–3595.
33. **Le Negrate G.** 2012. Subversion of innate immune responses by bacterial hindrance of NF-kappaB pathway. Cell. Microbiol. **14**:155–167.
34. **Lewis AL, et al.** 2007. NeuA sialic acid O-acetylesterase activity modulates O-acetylation of capsular polysaccharide in group B Streptococcus. J. Biol. Chem. **282**:27562–27571.
35. **Lewis AL, Nizet V, Varki A.** 2004. Discovery and characterization of sialic acid O-acetylation in group B Streptococcus. Proc. Natl. Acad. Sci. U. S. A. **101**:11123–11128.
36. **Lysenko ES, Lijek RS, Brown SP, Weiser JN.** 2010. Within-host competition drives selection for the capsule virulence determinant of Streptococcus pneumoniae. Curr. Biol. **20**:1222–1226.
37. **McCullers JA.** 2006. Insights into the interaction between influenza virus and pneumococcus. Clin. Microbiol. Rev. **19**:571–582.
38. **Meyn LA, Krohn MA, Hillier SL.** 2009. Rectal colonization by group B Streptococcus as a predictor of vaginal colonization. Am. J. Obstet. Gynecol. **201**:76.e71–76.e7.
39. **Mulvey MA, et al.** 1998. Induction and evasion of host defenses by type 1-piliated uropathogenic Escherichia coli. Science **282**:1494–1497.
40. **Mysorekar IU, Hultgren SJ.** 2006. Mechanisms of uropathogenic Escherichia coli persistence and eradication from the urinary tract. Proc. Natl. Acad. Sci. U. S. A. **103**:14170–14175.
41. **Nakamura S, Davis KM, Weiser JN.** 2011. Synergistic stimulation of type I interferons during influenza virus coinfection promotes Streptococcus pneumoniae colonization in mice. J. Clin. Invest. **121**:3657–3665.
42. **Neish AS, Naumann M.** 2011. Microbial-induced immunomodulation by targeting the NF-kappaB system. Trends Microbiol. **19**:596–605.
43. **Nielubowicz GR, Mobley HL.** 2010. Host-pathogen interactions in urinary tract infection. Nat. Rev. Urol. **7**:430–441.
44. **Orth RK, O'Brien-Simpson NM, Dashper SG, Reynolds EC.** 2011. Synergistic virulence of Porphyromonas gingivalis and Treponema denticola in a murine periodontitis model. Mol. Oral Microbiol. **26**:229–240.
45. **Peters BM, Jabra-Rizk MA, O'May GA, Costerton JW, Shirtliff ME.** 2012. Polymicrobial interactions: impact on pathogenesis and human disease. Clin. Microbiol. Rev. **25**:193–213.
46. **Peters BM, et al.** 2010. Microbial interactions and differential protein expression in Staphylococcus aureus-Candida albicans dual-species biofilms. FEMS Immunol. Med. Microbiol. **59**:493–503.
47. **Poltorak A, et al.** 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. Science **282**:2085–2088.
48. **Ramsey MM, Rumbaugh KP, Whiteley M.** 2011. Metabolite cross-feeding enhances virulence in a model polymicrobial infection. PLoS Pathog. **7**:e1002012. doi:10.1371/journal.ppat.1002012.
49. **Ravel J, et al.** 2011. Vaginal microbiome of reproductive-age women. Proc. Natl. Acad. Sci. U. S. A. **108**(Suppl 1):4680–4687.
50. **Rogers GB, et al.** 2010. Revealing the dynamics of polymicrobial infections: implications for antibiotic therapy. Trends Microbiol. **18**:357–364.
51. **Sagel SD, et al.** 2009. Impact of Pseudomonas and Staphylococcus infection on inflammation and clinical status in young children with cystic fibrosis. J. Pediatr. **154**:183–188.
52. **Schilling JD, Lorenz RG, Hultgren SJ.** 2002. Effect of trimethoprim-sulfamethoxazole on recurrent bacteriuria and bacterial persistence in mice infected with uropathogenic Escherichia coli. Infect. Immun. **70**:7042–7049.
53. **Schilling JD, Martin SM, Hung CS, Lorenz RG, Hultgren SJ.** 2003. Toll-like receptor 4 on stromal and hematopoietic cells mediates innate resistance to uropathogenic Escherichia coli. Proc. Natl. Acad. Sci. U. S. A. **100**:4203–4208.
54. **Schilling JD, Mulvey MA, Vincent CD, Lorenz RG, Hultgren SJ.** 2001. Bacterial invasion augments epithelial cytokine responses to Escherichia coli through a lipopolysaccharide-dependent mechanism. J. Immunol. **166**:1148–1155.
55. **Schwartz DJ, Chen SL, Hultgren SJ, Seed PC.** 2011. Population dynamics and niche distribution of uropathogenic Escherichia coli during acute and chronic urinary tract infection. Infect. Immun. **79**:4250–4259.
56. **Shahin RD, Engberg I, Hagberg L, Svanborg Eden C.** 1987. Neutrophil recruitment and bacterial clearance correlated with LPS responsiveness in local gram-negative infection. J. Immunol. **138**:3475–3480.
57. **Sibley CD, et al.** 2008. A polymicrobial perspective of pulmonary infections exposes an enigmatic pathogen in cystic fibrosis patients. Proc. Natl. Acad. Sci. U. S. A. **105**:15070–15075.
58. **Sibley CD, Surette MG.** 2011. The polymicrobial nature of airway infections in cystic fibrosis: Cangene Gold Medal Lecture. Can. J. Microbiol. **57**:69–77.
59. **Skoff TH, et al.** 2009. Increasing burden of invasive group B streptococcal disease in nonpregnant adults, 1990–2007. Clin. Infect. Dis. **49**:85–92.
60. **Song J, et al.** 2009. TLR4-mediated expulsion of bacteria from infected bladder epithelial cells. Proc. Natl. Acad. Sci. U. S. A. **106**:14966–14971.
61. **Stamey TA, Timothy MM.** 1975. Studies of introital colonization in women with recurrent urinary infections. I. The role of vaginal pH. J. Urol. **114**:261–263.
62. **Suhs KA, Marthaler BR, Welch RA, Hopkins WJ.** 2011. Lack of association between the *Tlr4* (Lps^d/Lps^d) genotype and increased susceptibility to Escherichia coli bladder infections in female C3H/HeJ mice. mBio **2**:e00094-11. doi:10.1128/mBio.00094-11.
63. **Tsuchimori N, Hayashi R, Shino A, Yamazaki T, Okonogi K.** 1994. Enterococcus faecalis aggravates pyelonephritis caused by Pseudomonas aeruginosa in experimental ascending mixed urinary tract infection in mice. Infect. Immun. **62**:4534–4541.
64. **Ulett GC, Maclean KH, Nekkalapu S, Cleveland JL, Adderson EE.** 2005. Mechanisms of group B streptococcal-induced apoptosis of murine macrophages. J. Immunol. **175**:2555–2562.

65. **Ulett KB, et al.** 2009. Diversity of group B streptococcus serotypes causing urinary tract infection in adults. J. Clin. Microbiol. **47**:2055–2060.
66. **Waksman G, Hultgren SJ.** 2009. Structural biology of the chaperone-usher pathway of pilus biogenesis. Nat. Rev. Microbiol. **7**:765–774.
67. **Weiman S, et al.** 2009. Genetic and biochemical modulation of sialic acid O-acetylation on group B Streptococcus: phenotypic and functional impact. Glycobiology **19**:1204–1213.
68. **Weiman S, et al.** 2010. O-acetylation of sialic acid on group B Streptococcus inhibits neutrophil suppression and virulence. Biochem. J. **428**: 163–168.
69. **Wright KJ, Seed PC, Hultgren SJ.** 2005. Uropathogenic Escherichia coli flagella aid in efficient urinary tract colonization. Infect. Immun. **73**:7657–7668.
70. **Zaleznik DF, et al.** 2000. Invasive disease due to group B Streptococcus in pregnant women and neonates from diverse population groups. Clin. Infect. Dis. **30**:276–281.