BRIEF REPORT

# Group B *Streptococcus* (GBS) Urinary Tract Infection Involves Binding of GBS to Bladder Uroepithelium and Potent but GBS-Specific Induction of Interleukin 1$\alpha$

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**Group B *Streptococcus* (GBS) causes urinary tract infections, but the pathogenic mechanisms underlying GBS urinary tract infections are unknown. We investigated whether uropathogenic GBS can bind to bladder uroepithelium to initiate urinary tract infection. Uropathogenic GBS isolated from a patient with acute cystitis bound to human T24 bladder uroepithelial cells in close association with F-actin in statistically significantly higher numbers compared with nonuropathogenic GBS. In vivo modeling using transurethrally infected mice revealed superior fitness of uropathogenic GBS for bladder colonization and potent uropathogenic GBS–specific upregulation of interleukin 1$\alpha$ during infection. Thus, binding of uropathogenic GBS to uroepithelium and vigorous induction of interleukin 1$\alpha$ represents the initial stages of GBS urinary tract infection.**

Group B *Streptococcus* (GBS) is a leading cause of infection in newborns, pregnant women, and older persons with chronic medical illness [1]. Cervicovaginal colonization with GBS in pregnant women can result in vertical transmission of GBS to neonates, with a limited number of GBS capsular serotypes being disproportionately associated with colonization and disease; serotypes Ia, III, and V, for example, cause the majority of invasive infections in elderly adults [1]. Multiple serotypes of GBS also cause urinary tract infections (UTIs), which encompass asymptomatic bacteriuria, cystitis, pyelonephritis, urethritis, and urosepsis [1–6]. GBS asymptomatic bacteriuria is particularly common among pregnant women; however, those most at risk for cystitis due to GBS are the elderly and immunocompromised individuals [1]. Predisposing factors for GBS UTI may include diabetes mellitus and chronic renal failure [3]. However, the underlying mechanisms of pathogenesis that lead to acute GBS UTI are unknown. In particular, the interactions between GBS and bladder uroepithelial cells have not to our knowledge been investigated. In this study, we investigated whether uropathogenic GBS (UPGBS) is able to bind to bladder uroepithelial cells as a mechanism of initiating bladder colonization and inflammation that ultimately leads to cystitis and other sequelae of GBS UTI.

***Methods.*** UPGBS was cultured from clean-catch voided urine of a 64-year-old woman without diabetes who presented with dysuria, hematuria, and single-organism bacteriuria level of >100,000 colony-forming units (CFUs)/mL; cystitis was confirmed by urinalysis, which showed urinary leukocyte esterase and pyuria of $\geqslant$10 white blood cells/high-powered field (nonspun). These are generally accepted criteria for the diagnosis of UTI. Non-uropathogenic GBS (non-UPGBS) was isolated from urine of a 39-year-old woman who did not have a UTI but had low-grade asymptomatic genitourinary colonization (urinalysis results were negative for esterase and pyuria; bacteriuria level, <50,000 CFUs/mL), a condition that is common among healthy adult women [7–10]. Isolates were identified by morphology on trypticase soy agar 5% sheep blood agar (Becton Dickinson), tested for catalase, and grouped using a Remel PathoDx latex agglutination kit. Latex agglutination with capsular serotype-specific antiserum (Statens Serum Institut, Denmark) demonstrated that the UPGBS and non-UPGBS isolates were serotypes II and V, respectively. This study was performed in accordance with the ethical standards of the University of Alabama committee on human experimentation and the Helsinki Declaration. The need for informed consent was waived by the Institutional Review Board of the University of Alabama at Birmingham.

To determine whether GBS is able to bind to human bladder uroepithelial cells, we used an in vitro binding assay with T24 (HTB-4) cells (ATCC). Bacteria were grown at 37°C in Todd

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Hewitt broth and on trypticase soy agar. T24 cells were grown in Roswell Park Memorial Institute 1640 medium (Life Technologies) with 25 mmol/L HEPES, 2 mmol/L L-glutamine, 10% heat-inactivated fetal bovine serum, 100 mmol/L nonessential amino acids, 1 mmol/L sodium pyruvate, 100 U/mL penicillin, and 100 mg/mL streptomycin in 5% carbon dioxide at 37°C. Two hundred thousand uroepithelial cells were seeded into 24-well plates and grown until 80%–90% confluence. GBS bacteria were added after washing out the antibiotics (multiplicity of infection, ~5 CFUs/cell); after 2 h, monolayers were rinsed with phosphate-buffered saline, and bound bacteria were enumerated by colony counts. To visualize the interaction between GBS and uroepithelial cells we used confocal microscopy with fluorescein isothiocyanate–stained GBS (0.25 mg/mL in phosphate-buffered saline for 15 min) in poly-D-lysine–coated multiwell chamber slides (BD). Monolayers were rinsed with phosphate-buffered saline after infection and fixed with paraformaldehyde, and F-actin was labeled with phalloidin Alexa-594 (Molecular Probes) to colocalize bacteria and T24 cell surfaces. Cells were counterstained with the nuclear dye Hoechst 33258, and images were acquired on a confocal microscope (Leica Microsystems).

To establish whether binding of GBS to bladder uroepithelial cells facilitates colonization of the bladder in vivo, we used a murine model of GBS cystitis. Female C57BL/6 mice (8–10 weeks old) were purchased from the Animal Resources Center (Australia). Mice were anesthetized by inhalation exposure to isoflurane, and the periurethral area was sterilized by swabbing with 10% povidone-iodine. Mice were catheterized, and 20 $\mu$L of phosphate-buffered saline containing $10^9$ CFUs of GBS was instilled transurethrally. The catheter was removed, and mice were returned to their cages for recovery. After 22–24 h, urine was collected for colony counts, mice were euthanized, and bladders were removed. Some bladders were homogenized for colony counts; other bladders were processed for scanning electron microscopic analysis. Bladders for scanning electron microscopic analysis were fixed in 3% glutaraldehyde in 0.1 mol/L cacodylate buffer (pH, 7.4) and stored at 4°C. After being washed with fresh buffer, bladders were pinned onto wax sheets to prevent curling, postfixed in 1% osmium tetroxide, dehydrated through a graded ethanol series, and critical point dried. Samples were mounted on scanning electron microscopic stubs and sputter coated with platinum. Images were acquired using a JEOL 6300F scanning electron microscope operated at 8 kV. Animal experimentation was approved by the animal ethics committees of the University of Queensland, Griffith University, and the University of Alabama at Birmingham.

To determine whether binding of GBS to bladder tissue triggers inflammation, as occurs in patients with cystitis, we analyzed transcription of interleukin 1$\alpha$ (IL-1$\alpha$), a key cytokine associated with UTI [11, 12]. Amplification of IL-1$\alpha$ complementary DNA from bladders ($n = 5$) was performed using a GeneAmp 7700 sequence detection system (Applied Biosystems). Target genes were amplified in 25-$\mu$L amplification reactions containing 12.5 $\mu$L of TaqMan Universal SYBR Green PCR Master mix (Applied Biosystems), 2.5 $\mu$L of water, 2.5 $\mu$L each of forward and reverse primers, and 5 $\mu$L of a 1/10 dilution of complementary DNA (1 part DNA, 9 parts water). Similar reactions were prepared in duplicate for amplification of glyceraldehyde-3-phosphate dehydrogenase. The thermal cycling conditions were as follows: 10 min at 95 °C, then 45 cycles of 15 s at 95°C and 1 min at 60°C. Dissociation curves were calculated for each amplicon and compared with the melting temperature of the full-length target, which was calculated using Primer Express software (version 2.0; Applied Biosystems), to ensure the fidelity of the polymerase chain reaction. Separate reactions were performed to ensure that the efficiency of amplification of the reference gene was approximately equal to that of the target gene. Relative expression levels were determined by normalizing reaction threshold cycles ($C_T$) to glyceraldehyde-3-phosphate dehydrogenase. The $\Delta C_T$ was used in the formula $2.0^{-}(\Delta C_T)$ for relative messenger RNA expression [13]. Levels of IL-1$\alpha$ protein in bladder homogenates ($n = 8$) and urine were measured by enzyme-linked immunosorbent assay (Endogen).

***Results.*** Initial confocal microscopic analysis of the binding of GBS to human T24 bladder cells in vitro revealed binding of UPGBS to the surface of bladder cells in close association with host-cell F-actin (Figure 1*A*). In some cells, morphological rearrangements in uroepithelial cell actin architecture, to which GBS had bound, indicated that binding of UPGBS to unknown cell surface receptors on bladder uroepithelium may induce intracellular actin assembly (cf Figure 1*A*–1*C*). When we quantitatively measured the binding of UPGBS to T24 cells we found that UPGBS bound in statistically significantly higher numbers compared with non-UPGBS ($P < .001$ [Mann-Whitney $U$ test]) (Figure 1*D*). Similar results were observed when we compared the binding of the bacteria to another human bladder cell line, 5637 cells (Figure 1*E*). The results shown are from 1 experiment (with quadruplicate samples), which are representative of several experiments.

To investigate the binding of UPGBS to bladder tissue in vivo, we developed a murine model of GBS cystitis. Mice challenged transurethrally harbored clusters of UPGBS bound directly to the surface of the bladder uroepithelium after 1 day of infection (Figure 2*A*). Scanning electron microscopic analysis revealed patchy but distinctive patterns of binding of UPGBS to the bladder surface, which were not observed in mice challenged with non-UPGBS (despite extensive scanning electron microscopic survey). Discrete areas of UPGBS enmeshed within the surface of the uroepithelium were observed in flattened bladder tissue (Figure 2*B*–2*C*). In contrast, in tissue that was not flattened, clusters of UPGBS bacteria were observed bound

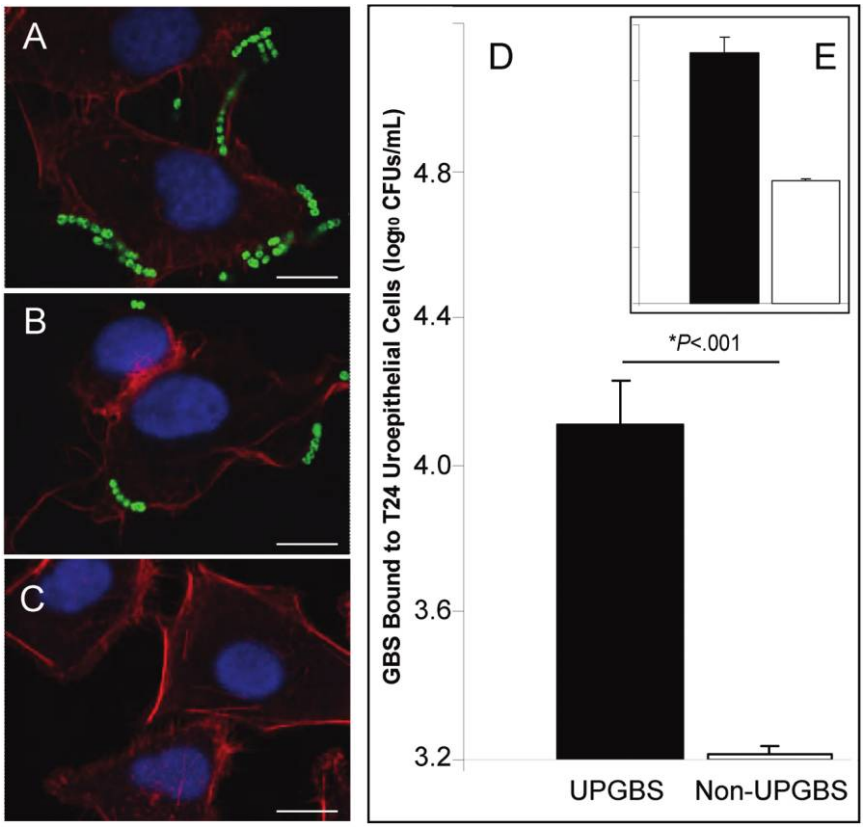


**Figure 1.** Uropathogenic group B *Streptococcus* (UPGBS) bound to the surface of human T24 uroepithelial bladder cells in association with F-actin for a multiplicity of infection of 50 (*A*) and 5 (*B*). Control uninfected cells are also shown (*C*). The scale bar is 8 $\mu$m in each panel. Quantitative measurement of the in vitro binding of UPGBS and non-UPGBS to T24 (*D*) and 5637 (same scale) (*E*) human bladder uroepithelial cells shows higher binding of UPGBS to both bladder cell types. CFU, colony-forming unit; GBS, group B *Streptococcus*.

between the folds of the uroepithelium (Figure 2*D*). Quantitation assays showed that UPGBS bacteria were recovered in statistically significantly higher numbers than were non-UPGBS bacteria ($n = 12$ per group; $P < .001$ [Mann-Whitney $U$ test]) (Figure 2*E*). There were no differences in bacteriuria levels between the mice (Figure 2*F*). To evaluate the binding of GBS to bladder uroepithelium in a broader context of bacterial UTI, we performed experiments with a prototype strain of the most common cause of UTI, uropathogenic *Escherichia coli* (UPEC; strain CFT073). Compared with UPEC, fewer UPGBS bacteria were recovered from bladder tissue after 24 h (Figure 2*E*), and fewer bacteria were shed in urine (Figure 2*F*); however, these differences were not statistically significant.

To analyze whether binding of UPGBS to bladder tissue triggers inflammation, we measured the transcription of IL-1$\alpha$, a key cytokine produced during UTI [11, 12]. Compared with control mice (which received phosphate-buffered saline), animals challenged with UPGBS experienced a 36.9-fold increase in IL-1$\alpha$ messenger RNA after 1 day of infection (mean $C_T$ for control mice, 32.5; mean $C_T$ for treated mice, 28.2; $\Delta C_T$, 4.3 [$P = .001$]). To better gauge the magnitude of this response, we compared the induction of IL-1$\alpha$ triggered by UPGBS to that generated after equivalent infection with UPEC. In mice that received an equivalent challenge of UPEC CFT073, the increase in bladder IL-1$\alpha$ messenger RNA was only 6.0-fold compared with the increase in controls and with the increase in UPGBS-infected mice (mean $C_T$ for control mice, 32.5; mean $C_T$ for UPEC-treated mice, 29.7; $\Delta C_T$, 2.8 [$P = .001$]). Expression of IL-1$\alpha$ protein in bladders and urine of UPGBS-infected mice was confirmed by enzyme-linked immunosorbent assay; mean values ($\pm$ standard error of the mean) for UPGBS-infected vs control mice ($n = 8$) were 155 ($\pm 34$) vs 26 ($\pm 4$) pg/mL for bladders and 25 ($\pm 9$) pg/mL vs undetectable levels for urine ($P = .002$ for both [Mann-Whitney $U$ test]). On the basis of these results, we next decided to determine whether interleukin 1$\beta$ (IL-1$\beta$) was triggered in a similar pathogen-specific manner. However, induction of IL-1$\beta$ was robust after challenge with either uropathogen (fold increase for UPGBS, 29.8; mean $C_T$ for UPGBS control mice, 30.4; mean $C_T$ for UPGBS-treated mice, 25.3; $\Delta C_T$ for UPGBS, 5.1 [$P = .001$]; fold increase for UPEC, 36.9; mean $C_T$ for UPEC control mice, 30.4; mean $C_T$ for UPEC-treated mice, 25.0; $\Delta C_T$ for UPEC,

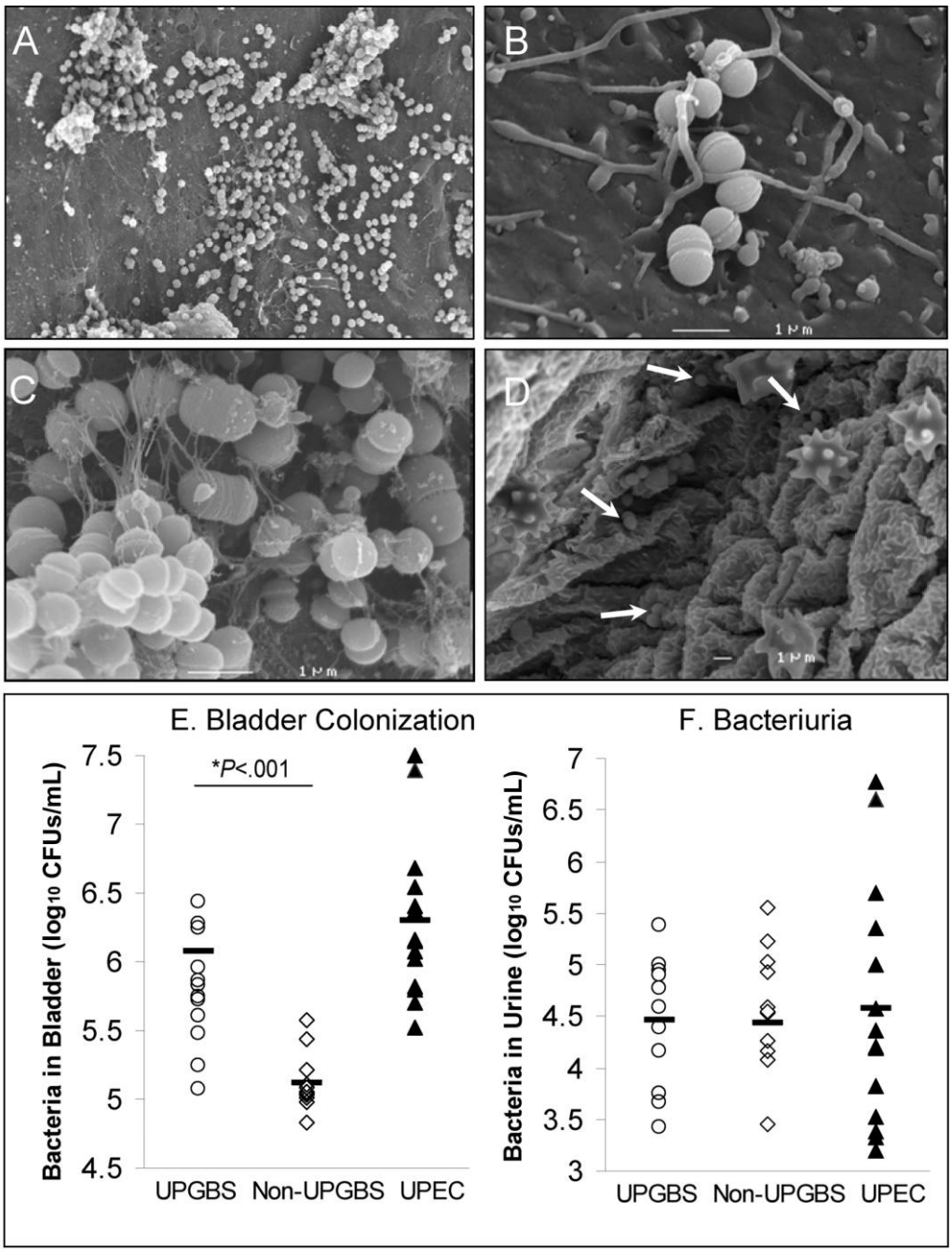


**Figure 2.** Uropathogenic group B *Streptococcus* (UPGBS) bound to bladder uroepithelium in a murine model of group B *Streptococcus* (GBS) cystitis (*A–C*, flattened bladder; *D*, native conformation). The arrows in panel *D* show bound UPGBS between folds of bladder uroepithelium. Statistically significantly more UPGBS bacteria were recovered from bladders 24 h after transurethral challenge than were non-UPGBS bacteria (*E*). Bacteriuria levels were similar between groups (*F*). Comparative assays that measured the binding of uropathogenic *Escherichia coli* (UPEC) strain CFT073 to bladder tissue showed higher binding of UPEC to bladder tissue (*E*) and slightly higher bacteriuria levels (*F*); however, these differences were not statistically significant. CFU, colony-forming unit.

5.4 [$P = .001$]). Non-UPGBS also induced IL-1$\alpha$ and IL-1$\beta$ in the bladder, and the responses were higher than those to UPGBS (compared with control mice that received phosphate-buffered saline, induction by non-UPGBS was 85.0-fold for IL-1$\alpha$ and 107.7-fold for IL-1$\beta$). Expression experiments were performed concurrently (with 1 control group that received phosphate-buffered saline); there were no differences in glyceraldehyde-3-phosphate dehydrogenase expression between infected mice and controls (data not shown).

***Discussion.*** Several studies have highlighted the incidence

of GBS UTIs in nonpregnant adults, and we undertook the current study to investigate the early pathogenic mechanisms underlying GBS UTIs. To this end, we investigated whether UPGBS can bind to human bladder uroepithelium and trigger host responses as a means to colonize and cause inflammation in the bladder. Using an in vitro binding assay with 2 cell lines and a murine model of cystitis, we demonstrated that UPGBS binds directly to human bladder uroepithelial cells, which facilitates colonization of the bladder in vivo. Increased fitness of UPGBS for the bladder compared with non-UPGBS suggests that some GBS may possess virulence factors that aid in colonization or survival in the urinary tract. However, GBS is extremely heterogeneous at the genomic level, and it is uncertain whether this fitness trait is unique to the UPGBS used in this study or reflects unique GBS urovirulence factors. This is a limitation of the current study. Nevertheless, these data demonstrate previously unknown divergent fitness levels among different GBS for the urinary tract, which appears to influence the ability of the organism to cause UTI. Differences in uropathogenic potential in other bacteria depend largely on adhesins that facilitate colonization of the urinary tract. Future analysis of the GBS that cause UTI will help to define what contributes to urovirulence in GBS.

The observation that the UPGBS isolate used in this study triggers more potent induction of IL-1$\alpha$ than does prototypical hypervirulent UPEC CFT073 suggests that the early immune responses triggered by GBS during UTI are unique. This notion is consistent with a recent demonstration of distinct frequencies of host characteristics in UTI patient groups as defined by the causal organism [14]. Our findings also align with the emerging concept that hypervirulent uropathogens (such as UPEC) may act to suppress inflammation perhaps more efficiently than less well adapted counterparts to evade innate immunity [15]. The divergent IL-1$\alpha$ response to UPGBS and non-UPGBS isolates observed in this study also underscores the need for further detailed analysis of the immune responses triggered by UPGBS during cystitis. Recognition of unique characteristics among different UTI patient groups highlights both a diversity of pathogenic mechanisms for bacterial UTIs and a need for further study of these important infections.

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