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BLADDER UROTHELIAL BIG POTASSIUM (BK) CHANNEL REGULATES IN VIVO UROTHELIAL CYTOKINE RELEASE INDUCED BY LIPOPOLYSACCHARIDE (LPS) – NEW INSIGHT INTO UROTHELIAL INNATE IMMUNE RESPONSE IN PATHOGENESIS OF URINARY TRACT INFECTION

Hypothesis / aims of study

Bladder urothelium serves as the first line of host defense against uropathogens and drives subsequent innate immune response. We have previously reported: (1) Presence of large conductance voltage-gated calcium-activated potassium (BK) channel in bladder urothelial cells *in vitro* [1]; (2) Significant increase in BK activity *in vitro* with exposure to lipopolysaccharide (LPS), surrogate for uropathogenic gram-negative bacteria [1]; (3) Release of granulocyte-macrophage colony-stimulating factor (GM-CSF) *in vitro* in response to LPS [2]. Based on these findings, we hypothesize that LPS-induced increase in urothelial BK channel activity regulates downstream urinary cytokine release. In this study, we test this hypothesis *in vivo* using pretreatment of mice with intravesical iberiotoxin (IBTX), a BK-specific blocker, to determine (1) whether IBTX blocks LPS-induced urinary cytokine release and (2) whether induction and inhibition of urinary cytokine release occur at the level of urothelium.

Study design, materials and methods

Using 12-week-old female C57BL/6 mice in our murine model, treatment (Figure 1) consisted of transurethral instillation of 150 μL of LPS (1 mg/mL in phosphate buffered saline, PBS) with or without IBTX (1 μM), following priming exposure to PBS or IBTX (1 μM in PBS). Instillation lasted 30 minutes under anesthesia, followed by activity ad lib in cage and urine collection within two hours. Prior to priming exposure and LPS treatment, mice underwent instillation with PBS or IBTX and urine collection within two hours to establish basal cytokine release for comparison with subsequent LPS treatment. Urine specimens were analyzed for levels of 32 cytokines using multiplex enzyme-linked immunosorbent assay (ELISA). Bladders were harvested from mice in each treatment group within two hours of LPS instillation, and urothelium was isolated from bladder mucosa using our unique dissection technique [3], followed by extraction of urothelial ribonucleic acid (RNA), reverse transcription (RT), and quantitative polymerase chain reaction (qPCR) using primers for cytokines of interest.

Results

Of the 32 cytokines surveyed, LPS significantly increased urinary concentrations of 19 cytokines in our murine model: Eotaxin, G-CSF, GM-CSF, IL-1α, IL-4, IL-5, IL-6, IL-15, IP-10, KC, LIF, MCP-1, M-CSF, MIG, MIP-1α, MIP-1β, MIP-2, RANTES, TNFα. Of these 19 cytokines, 15 showed significant reduction in urinary concentrations with pre-treatment with IBTX (Figure 2). Urothelial RNA expression of four select cytokines showed that LPS-induced increases in expression were significantly inhibited by pre-treatment with IBTX (Figure 3).

Interpretation of results

Blockage of BK channel significantly inhibited LPS-induced increases in urinary concentrations of 15 of 19 cytokines surveyed. Transcript analyses of expression by dissected bladder urothelium for a sample of four cytokines demonstrated that urothelium was the source of these urinary cytokines induced by LPS and inhibited by IBTX.

Concluding message

This is the first *in vivo* proof-of-concept demonstration that urothelial BK channel regulates urothelial innate immune response to uropathogens. Future treatment strategies for urinary tract infection may leverage this newly evidenced mechanism.

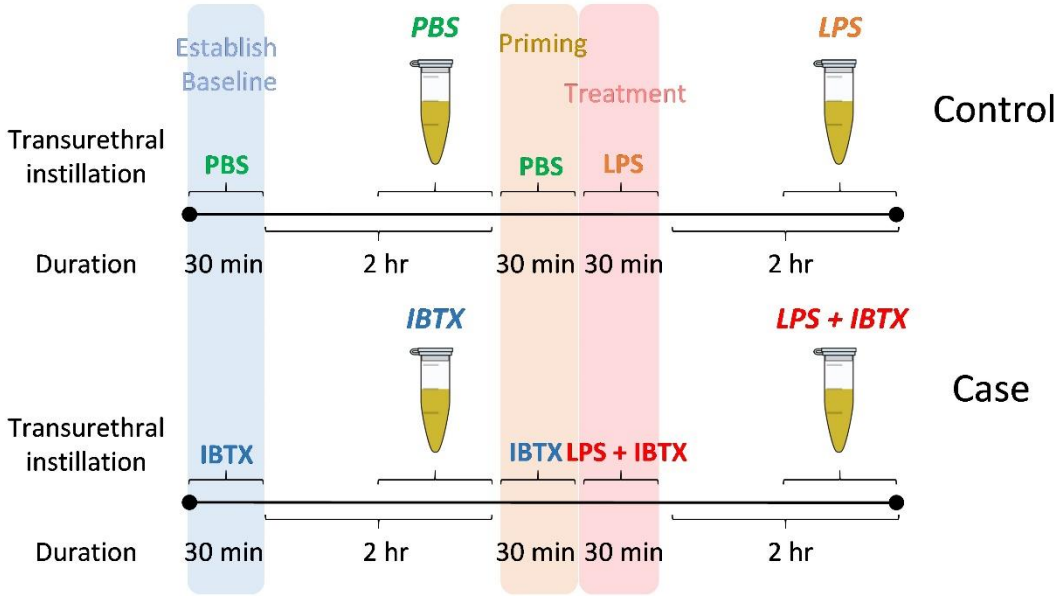

Figure 1. Schematic for experimental protocol for transurethral instillation and urine collection. Treatment (pink) consists of transurethral instillation with lipopolysaccharide (LPS) with or without iberiotoxin (IBTX), a BK-specific blocker, followed by urine collection within two hours. Treatment with LPS is preceded by priming (orange) exposure to phosphate buffered saline (PBS) or IBTX. Prior to priming exposure and LPS treatment, baseline (blue) is established using instillation with PBS or IBTX followed by urine collection with two hours.

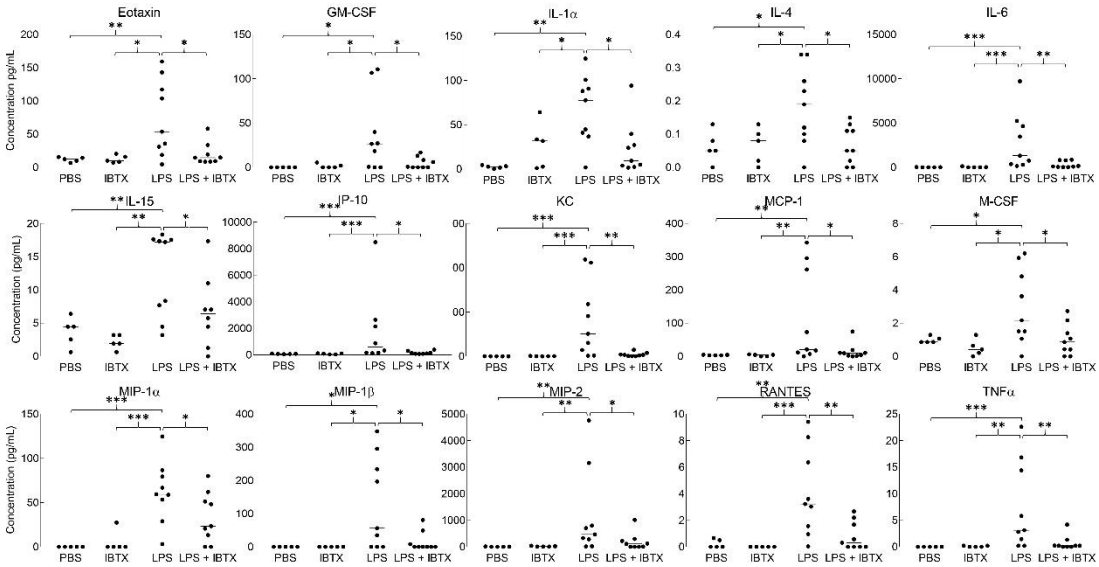

Figure 2. Cytokines with differential urinary release with LPS induction and IBTX inhibition. Scatter plot with median cytokine concentration for urine specimens collected from mice (n = 5-9) in each treatment group. Each data point corresponds to an individual mouse. Asterisks indicate statistical significance (* for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$) on one-tailed Mann-Whitney test.

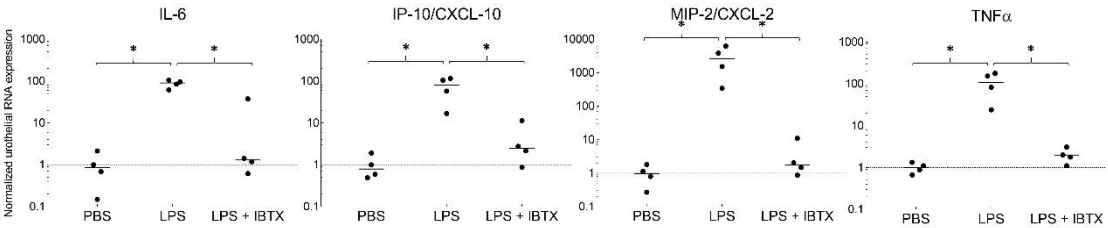

Figure 3. Select cytokines with differential urothelial RNA expression with LPS induction and IBTX inhibition. Scatter plot with median fold change of urothelial cytokine expression for select cytokines on RT-qPCR for bladder urothelium from mice (n = 4) in each treatment group normalized to PBS group. Asterisks indicate statistical significance (* for $p < 0.05$) on one-tailed Mann-Whitney test.

References
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3. Lu M, Chai TC. A method to study bladder urothelial cellular function in situ. Bladder 2014;1(1):e3.

Disclosures
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