

IMPAIRED NEUTROPHIL RECRUITMENT DRIVEN BY DECREASED AND INTERRUPTED EXPRESSION LEVEL OF MIP-2, KC, IL-6 AND MCP-1 PROLONGS UTI IN DIABETES

Hypothesis / aims of study

Diabetic population is very vulnerable to infections, and Lower Urinary Tract Infections (LUTI), mainly referring to the infection of bladder (cystitis), is the most common infection type they suffer [1]. LUTI both affect quality of life of patients and cause high financial and economic burden. Therefore, to learn the local immune functions resulting in the propensity to UTI in diabetics is very important to improve preventive and therapeutic strategies. We hypothesize that diabetic condition causes the high propensity of LUTI by affecting the expression level of chemoattractants along with eventual reduced neutrophil recruitment to the bladder. Our aim is to investigate the bacterial clearance efficiency in diabetic mice upon acute bacterial cystitis *in vivo*, and evaluate neutrophil recruitment to bladder with respect to the expression of MIP-2 (Macrophage inflammatory protein-2), KC (keratinocyte-derived chemokine), and MCP-1 (Monocyte chemotactic protein-1), which are neutrophil-chemoattractant chemokines, and IL-6 (interleukin-6), which is an infection-related cytokine.

Study design, materials and methods

Streptozotocin (STZ)-induced diabetic mice was used as the model system to study LUTI. STZ-injected 8-week-old C57BL/6J female mice were installed with Type-1 pilated Uropathogenic Eschericia Coli (UPEC) to induce cystitis. Bacterial clearance was tested on a time dependent manner at urine and tissue level after UPEC installation. Mice urine was collected, serially diluted and bacterial counts were determined as colony forming units (CFU) on agar. Neutrophils in urine were counted on hemacytometer. Harvested bladder homogenates were used to test cytokine/chemokine expression by ELISA and Quantitative real time PCR (qRT/PCR). Tissue neutrophils were also confirmed by using MPO (myeloperoxidase) assay in homogenates.

Results

Diabetic mice showed prolonged bacterial clearance, significantly reduced and delayed MIP-2, IL-6 and MCP-1 expression and decreased KC expression on a time dependent manner following UPEC infection. Neutrophil accumulation reached its peak in urine at 6th hour post-infection in controls, but at 12th hour in diabetics with significantly lower numbers ($p < 0.001$). qRT-PCR analysis (in bladder) and ELISA results (in bladder and urine) showed the lower and interrupted gene expression of MIP-2, KC, IL-6 and MCP-1 upon UPEC installation in diabetic mice. MPO-assay results additionally confirmed the ineffective NR in diabetic bladder and urine ($p < 0.005$, $p < 0.01$, respectively).

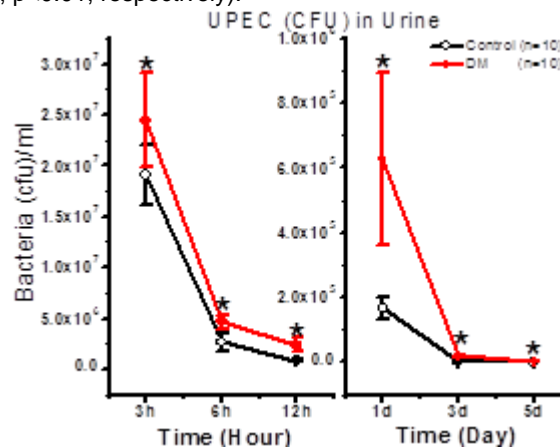


Figure 1. *In vivo* bacterial clearance assay. UPEC colony forming units (CFU) in urine at specific time points following instillation of UPEC. LB agar plates were incubated at 37°C overnight with serially diluted urine samples of bladder tissue harvested from 10 mice/ group at the indicated time points. Numbers of CFU were calculated as per ml of urine after multiplying by the dilution factor. Asterisks indicate statistical significance of $p < 0.05$ between DM and control, calculated for each time point by Student 't' Test.

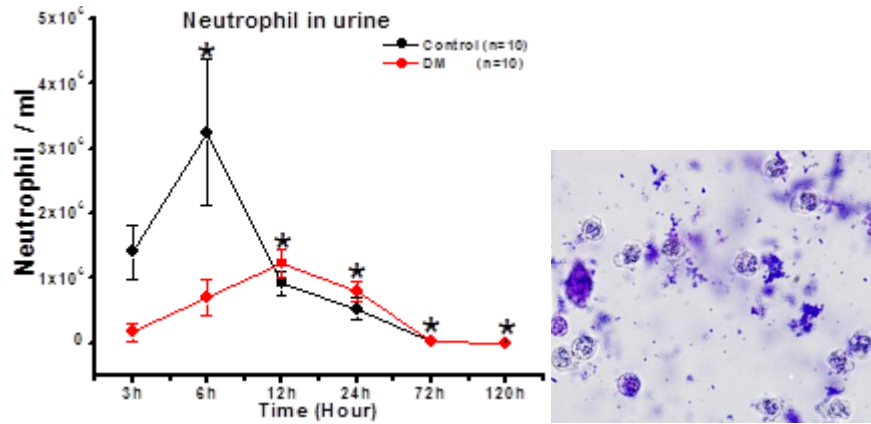


Figure 2. Neutrophil counts in the urine of diabetic (DM) and control mice at the indicated time points after 1h of UPEC challenge. Image: Microscopic view of TURK's-stained neutrophils in urine from a mouse 6 h after UPEC infection, 100x oil-objective, bright field microscope, Zeiss.

Interpretation of results

In the present study, we showed that bacterial clearance is impaired in diabetic mice due to the lower and delayed expression levels of MIP-2, KC, IL-6 and MCP-1 resulting in lower neutrophil recruitment to the infection site.

Concluding message

Locally secreted chemokine and cytokine levels have crucial effects on regulating proper immune response and directing neutrophils to the infected bladder within the early hours of infection. Vigorous neutrophil recruitment to the infection site is the main determinant of successful resolution of the UPEC infection [2]. Further characterization of bacterial clearance defect in UTI in diabetic setting should include studies for neutrophil functions, urothelial cell layer damage and epigenetic control of expression of the reported genes.

References

1. Geerlings SE. Urinary tract infections in patients with diabetes mellitus: epidemiology, pathogenesis and treatment. *Int J Antimicrob Agents.* Feb 2008;31 Suppl 1:S54-57.
2. Agace WW, Hedges SR, Ceska M, Svanborg C. Interleukin-8 and the neutrophil response to mucosal gram-negative infection. *The Journal of clinical investigation.* 1993;92(2):780-785.

Disclosures

Funding: NIH-NIDDK: P20 DK090871 (Principal Investigator; Firouz Daneshgari, MD) **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Mouse C57BL/6J Female **Ethics Committee:** The institutional animal care and use committee of Case Western Reserve University in compliance with the Public Health Service policy on humane care and use of laboratory animals. Animal protocols has been approved by IACUC.