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GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR IS RELEASED BY MOUSE BLADDER UROTHELIAL CELLS IN RESPONSE TO LIPOPOLYSACCHARIDES (LPS)

Hypothesis / aims of study

The role of urothelial cells in host response to bacterial cystitis (UTI) has been poorly studied. We studied in vitro and in vivo response of mouse bladder urothelial cells to Lipopolysaccharides (LPS) exposure as a surrogate of bacterial infection, focusing on granulocyte-macrophage colony-stimulating factor (GM-CSF) signalling as a response to LPS.

Study design, materials and methods

Cultured female C57BL/6 mouse bladder urothelial cells were exposed for 12 hours to different concentrations of LPS (100ng/ml to 10μg/ml). Mouse bladder urothelial cells were also exposed to a single dose of LPS (1 μg/ml) for varying times (3, 6, 12 hours). In vivo experiments were done in which LPS was instilled intravesically, followed by harvest of bladder urothelium 3 to 18 hours later. ELISA measured GM-CSF protein. qPCR quantitated mRNA for GM-CSF, vascular endothelial growth factor-A (VEGF-A), cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), and tumor necrosis factor α (TNF-α). RT-PCR was used to detect mRNA for GM-CSF, GM-CSF receptor α and β in bladder tissues. Immunohistofluorescence and Western blots for GM-CSF receptor α were performed on bladder tissue. Neutralizing GM-CSF antibody (0.1 μg/ml) was used in cell culture experiments to block GM-CSF activity.

Results

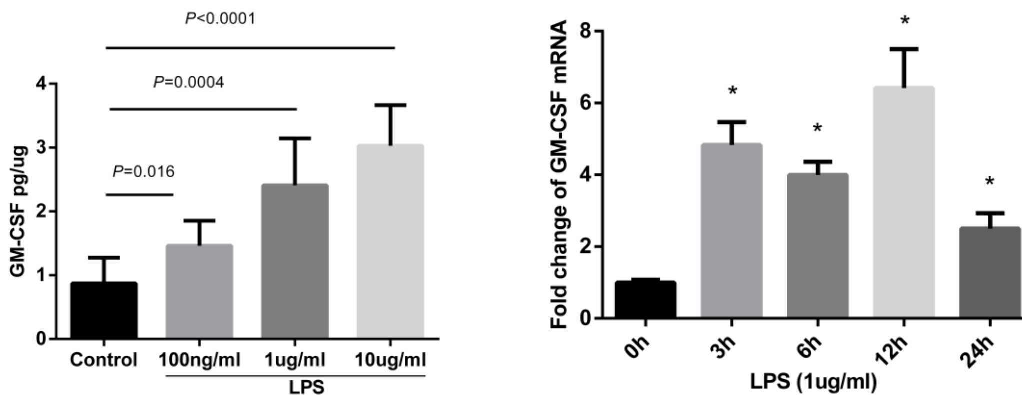
LPS induced a dose-dependent increase of GM-CSF in mouse bladder urothelial cells, with significant increase by 3 hours. In vivo experiments revealed that mouse bladder urothelium did not express GM-CSF mRNA, but 3 hours after LPS exposure, GM-CSF mRNA was expressed. RT-PCR, Immunofluorescence analysis and Western Blot confirmed that GM-CSF Receptor was expressed in bladder urothelium. Neutralizing GM-CSF antibody blocked LPS-induced VEGF and COX-2, but not COX-1 and TNFα mRNA expression in mouse bladder urothelial cells.

Interpretation of results

We demonstrated the novel finding that mouse bladder urothelial cells release GM-CSF in a dose-dependent fashion to LPS. GM-CSF may regulate the urothelial function by autocrine signalling pathway and likely plays a role in early host defence against UTI. GM-CSF may be released by urothelium in response to bacteria/microbiota and mediate bladder afferent signalling. GM-CSF released by bladder urothelial cells may mediate UTI-related bladder pain, and possibly bladder pain syndrome other than UTI. Other urothelial functions such as growth, differentiation and repair may be roles modulated by GM-CSF. Further studies into GM-CSF's effect on urothelial biology seem warranted.

Concluding message

This is the first description of mouse bladder urothelial cells expressing GM-CSF as an early response to LPS. GM-CSF may be a potential regulator for mucosal signalling and bladder function.



A B

Figure 1. Secretion of GM-CSF by mouse bladder urothelial cells in response to LPS. LPS induced a dose-dependent increase of GM-CSF protein in mouse bladder urothelial cells (A, mice number =7). The time course of GM-CSF mRNA expression in response to LPS in mouse bladder urothelial cells (B, mice number = 5).

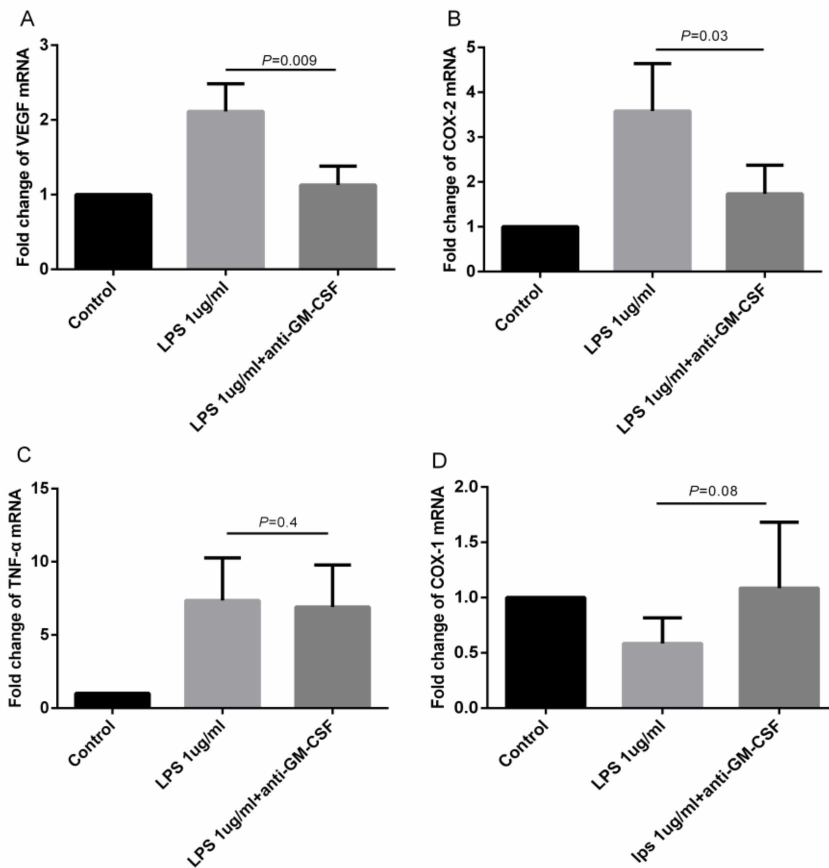


Figure 2. Autocrine effect of GM-CSF on urothelial cells (mice number = 4). GM-CSF is necessary for LPS-induced VEGF and COX-2 mRNA expression (A and B). Blocking GM-CSF didn't have significant effect on LPS-induced TNF-α and COX-1 mRNA expression (C and D).

References

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Disclosures

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