INFLAMMATORY PRECONDITIONING OF MULTIPOTENT STROMAL CELLS IMPROVES THEIR IMMUNOMODULATORY POTENCY UNDER ACUTE PYELONEPHRITIS IN RATS

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INTRODUCTION AND AIMS

Acute pyelonephritis is the one of the most frequent infectious diseases of urinary tract and a leading cause of kidney failure worldwide. It is more likely that a key role in kidney damage under pyelonephritis belongs to inflammatory process, rather than bacterial colonization. Preventing or limiting the inflammation and oxidative renal damage during the acute phase should be considered as a major goal in the treatment of pyelonephritis. One strategy for modulating excessive inflammatory responses under pyelonephritis is administration of the mesenchymal multipotent stromal cells (MMSCs). In the present study the putative protective effect of MMSCs against experimental acute pyelonephritis was examined.

METHODS

Acute pyelonephritis in rats was induced by intrauretheral infection of autofecal bacterial composition. Three days after intravenous injection of MMSCs into jugular vein was done. On the 7th day after pyelonephritis induction blood samples and kidneys were taken for further analysis. Blood leukocytes were counted using the veterinary hematology analyzer. The kidneys were homogenized in ice-cold PBS for determination of malonyldialdehyde (MDA), myeloperoxidase (MPO) activity, TNFα, western blotting and zymography. Formalin-fixed kidney tissue, embedded in paraffin was used for histopathological examination.

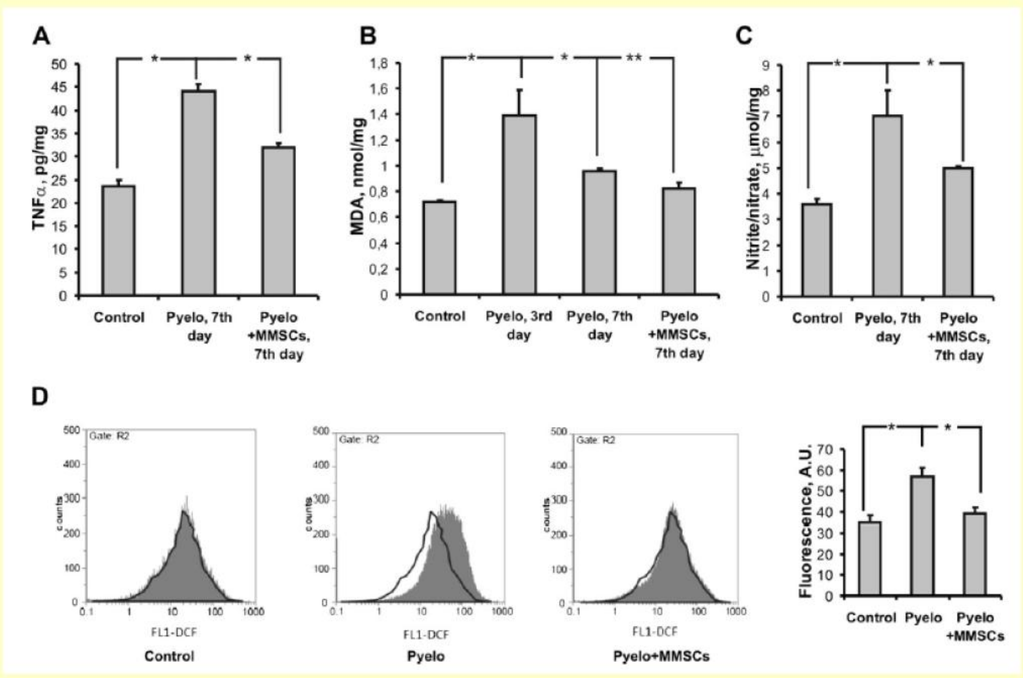

Figure 1. Effects of MMSC injection in rats on oxidative stress and inflammation in the renal tissue in acute experimental pyelonephritis. (A) Production of TNF-α significantly increased in pyelonephritic kidneys and decreased after treatment with MMSCs. (B) Inflammation in the kidney was accompanied by oxidative stress as measured by MDA accumulation in the tissue. (C) Nitrite/nitrate concentration reflecting a total NO level is shown. (D) Generation of ROS in blood leukocytes as measured by DCF fluorescence is shown with mean intensities on the diagram belonging to healthy leukocytes (open histogram) and pyelonephritic leukocytes (shaded histogram).*P < 0.05; **P < 0.1. A.U., arbitrary units.

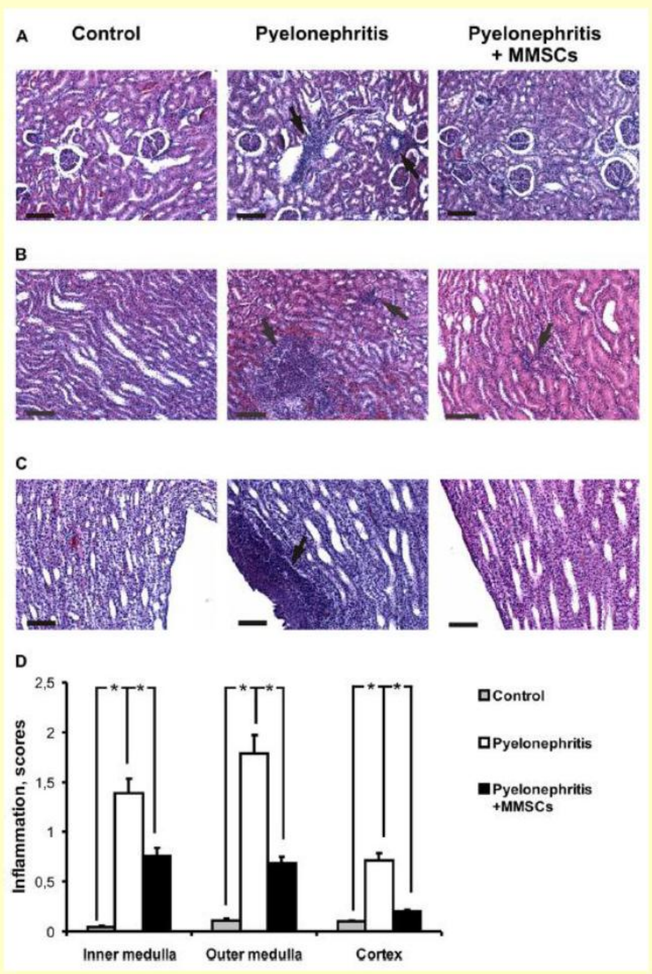

Figure 2. Effects of MMSCs on pyelonephritis-induced pathologic features in renal morphology. Histologic sections of renal cortex (A), outer medulla (B) and inner medulla (C) stained with hematoxylin and eosin. In pyelonephritic kidney, apparent infiltration with leukocytes is observed (shown by arrows) in cortex and medulla, which was alleviated by MMSC treatment. (D) Histologic scores of inflammation in different kidney layers. (Scale = 100 mm.) *P < 0.05.

RESULTS

We found obvious signs of oxidative stress and inflammation in the kidney under acute pyelonephritis in rats. Particularly, pro-inflammatory cytokine TNFα levels, malonyldialdehyde, nitrite and myeloperoxidase activity were considerably increased (**Fig. 2, 4**). Histological evaluation revealed a number of attributes of inflammation and tissue damage in kidney (**Fig. 2**). MMSCs treatment caused a remarkable decrease of all of these pathological signs in renal tissue. Also we showed that activated leukocytes induced preconditioning-like signaling in MMSCs. We showed alterations of expression or activity of iNOS, TGFα, MMP-2 and GSK-3β (**Fig. 3**), which could mediate immunomodulation and protective effects of MMSCs, and these signaling could be characterized as the inflammatory preconditioning. The beneficial capacity of MMSCs to alleviate renal inflammation was more pronounced when preconditioned MMSCs were used (**Fig. 4**).

CONCLUSIONS

We summarize that MMSCs provide immunomodulatory effects under experimental pyelonephritis by paracrine regulation that depends on the inflammatory microenvironment. This approach could be used to prime MMSCs with different inflammatory modulators to enhance their engraftment and function in an immunoprotected fashion.

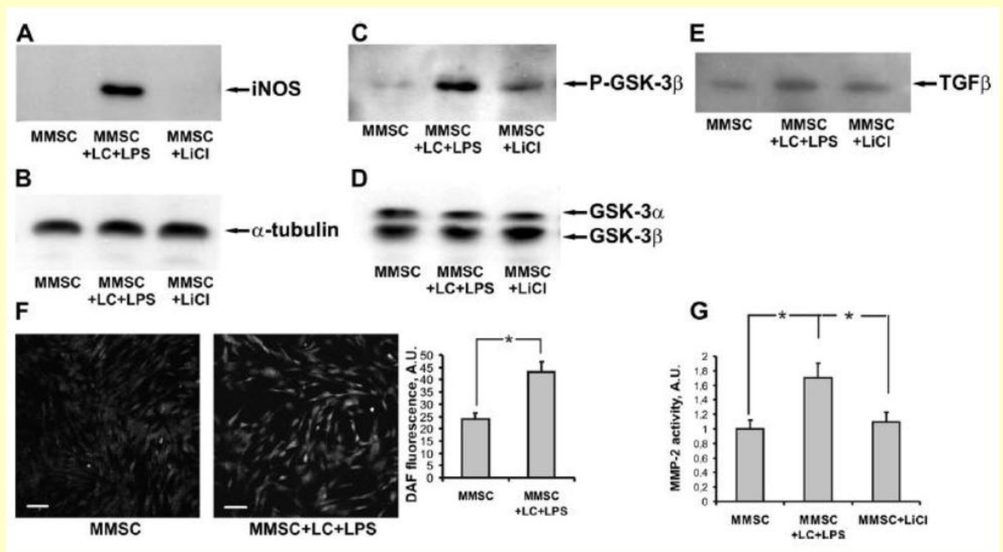

Figure 3. Pre-conditioning-like signaling in MMSCs, induced by co-cultivation with leukocytes (LC) or LiCl treatment. Immunoblotting revealed increase of iNOS (A), phospho-GSK-3β (B) and TGF-β (E) after co-cultivation with LPS-activated LC. Referent proteins include α-tubulin (B) and total GSK-3 (D). Co-cultivation of MMSCs with leukocytes induced an increase of NO production measured by DAF-2FM fluorescence (F) and activation of MMP-2 as estimated by zymography (G). *P < 0.05.

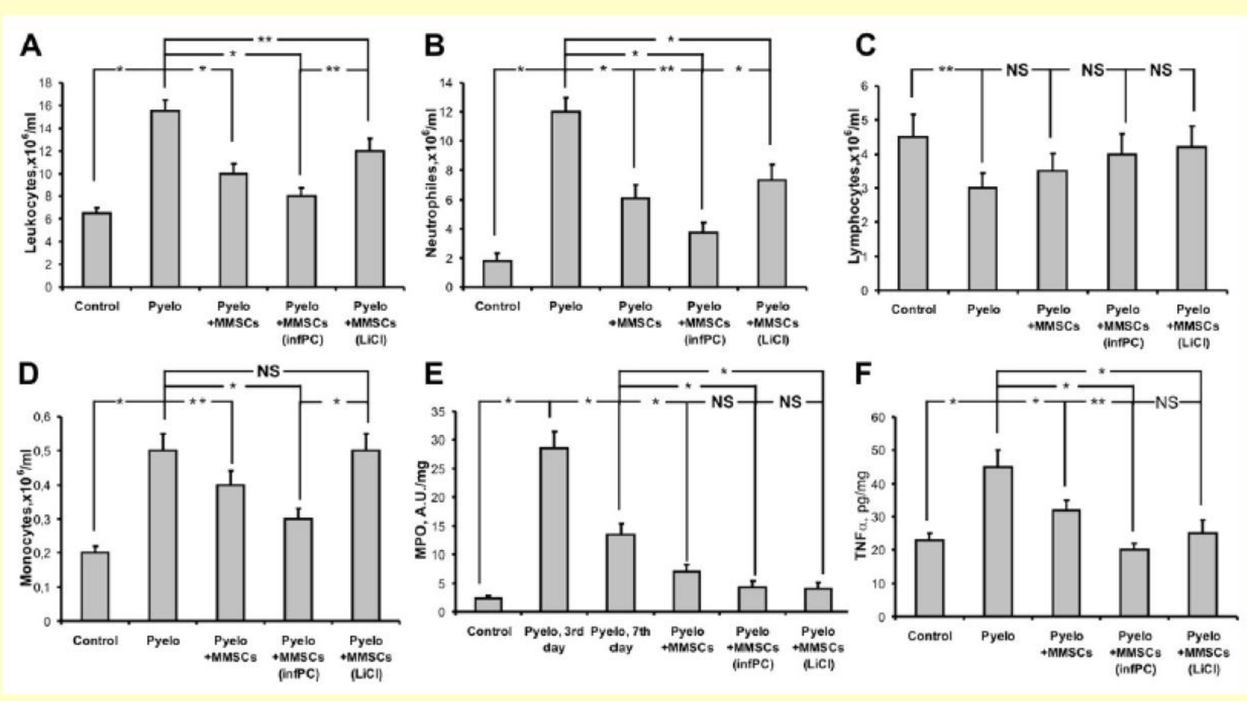

Figure 4. Alterations in blood leukocytes of rats with pyelonephritis are restored by pre-conditioned MMSCs. Increase of leukocytes (A), especially neutrophils (B) and monocytes (D) content, in the blood of pyelonephritic rats with invariable lymphocytes (C). All of these inflammatory changes were alleviated by MMSC treatment and were more pronounced after treatment with inflammation-treated but not LiCl-primed MMSCs. The positive effects of native, inflammation-treated and LiCl-primed MMSCs were monitored by MPO activity (E) and TNF-α levels (F) in kidney tissue. *P < 0.05; NS, not significant.

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