Transient Increase in Circulating Myeloid-Derived Suppressor Cells after Partial Bladder Outlet Obstruction

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Purpose: Partial bladder outlet obstruction causes a significant increase in tissue and systemic oxidative stress markers and tissue inflammatory cytokine levels. Myeloid-derived suppressor cells, IFN-γ, IL-10 and aldosterone are believed to be associated with oxidative stress and inflammation. We investigated alterations in plasma myeloid-derived suppressor cells, IFN-γ, IL-10 and aldosterone levels in partial bladder outlet obstruction and after its reversal.

Materials and Methods: Rats with surgically induced partial bladder outlet obstruction were divided into 4 groups of 4 each, including sham treated, 4-week obstruction, and 4 and 8-week obstruction with relief. Plasma levels of circulating myeloid-derived suppressor cells, IFN-γ, IL-10 and aldosterone were assessed by flow cytometry or enzyme-linked immunosorbent assay.

Results: The circulating myeloid-derived suppressor cell level was markedly increased in the obstruction group compared to the sham treated group and it returned to normal in the 4 and 8-week obstruction with relief groups. Plasma IFN-γ, IL-10 and aldosterone were similarly increased in the obstruction group and returned to normal in the 4 and 8-week obstruction with relief groups.

Conclusions: Levels of circulating myeloid-derived suppressor cells, IFN-γ, IL-10 and aldosterone were increased in rats with partial bladder outlet obstruction but returned to normal after reversal. This suggests that an increase in these parameters may be a good predictive indicator of patients at increased risk for urinary symptoms.

Key Words: urinary bladder neck obstruction, cell differentiation, inflammation, oxidative stress, cytokines

Abbreviations and Acronyms

IL-10 = interleukin-10

INF-γ = interferon-γ

MDSC = myeloid-derived suppressor cell

PBOO = partial bladder outlet obstruction

RAS = renin-angiotensin system

ROS = reactive oxygen species

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PARTIAL bladder outlet obstruction is a common urological problem in adult and pediatric patients that may have profound consequences, including urinary tract infection, bladder stones and renal insufficiency.[1] Previous studies showed that patients with benign prostatic hyperplasia and PBOO have lower urinary tract syndromes as well as increased serum C-reactive protein and urinary nerve growth factor levels. These findings imply that an inflammatory response may exist after PBOO. Animal models of PBOO have successfully demonstrated most of the clinical features of PBOO.[2,3] Various animal studies revealed that PBOO is

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intimately associated with inflammation and oxidative stress, indicating a role for a systemic immune response in PBOO pathogenesis.[3–6]

MDSCs have been studied extensively in cancer and other diseases of many major organ systems, including the lungs and gastrointestinal tract.[7–11] MDSCs are a heterogeneous population of cells consisting of precursors of granulocytes, macrophages, dendritic cells and myeloid cells at earlier stages of differentiation.[8,9] They are implicated in the regulation of chronic inflammation and can trigger production of IL-10, an anti-inflammatory and immunosuppressive cytokine.[10,12] Moreover, MDSCs can exert immune responses via induction of ROS production.[13]

However, their role in lower urinary tract diseases has rarely been explored. We examined the dynamic change in the profiles of MDSCs and IL-10 in a rat model of PBOO and its reversal. For a more comprehensive view of the immune response after PBOO we also studied IFN-γ, a major proinflammatory cytokine secreted from the helper T cells that activate macrophages. In addition, we evaluated changes in plasma aldosterone, a well recognized regulator of systemic inflammation and the oxidative stress response. Our data provide interesting insights into the relationship between PBOO and systemic immunological responses.

MATERIALS AND METHODS

PBOO Animal Model

All animals were housed and treated according to guidelines in the National Science Council of Taiwan Handbook of the Laboratory Animal Breeding and Research Centre. The Chang Gung Medical Foundation animal ethics committee approved all experimental protocols used in this study. We divided 16, 8 to 12-week-old male Sprague Dawley® rats (Laboratory Animal Centre, Chang Gung Medical Foundation, Chia-Yi, Taiwan) into 4 groups of 4 per group, including controls, 4-week obstruction, and obstruction reversal with 4 and 8-week recovery, respectively.

For surgery induced PBOO each rat was anesthetized by 1% to 5% isoflurane inhalation. Controls underwent sham treatment. In the 3 treatment groups PBOO was induced using 2-zero silk as previously described.[3,4] At 4 weeks rats in the 4-week obstruction group were sacrificed. For reversal studies the silk was removed from all obstructed rats in the remaining treated groups, which were kept another 4 and 8 weeks, respectively, before sacrifice. Blood samples were collected from all rats before sacrifice.

Flow Cytometry and Blood MDSCs

Cell surface staining and flow cytometry analysis of MDSCs were performed. Briefly, 1×10^6 cells were stained in 20 µl MDSC blood samples from the PBOO and control groups. After lysis of red blood cells in lysis buffer for 6 minutes the samples were washed twice in phosphate buffered saline solution. Separated sets of cells were stained with fluorescence labeled antibodies, including APC-CD11b/c (OX42) (BioLegend®), fluorescein isothiocyanate-His48 (anti-granulocytes) and labeled IgG2b isotype control (eBioscience, San Diego, California) antibodies, respectively, for 30 minutes at 4C in the dark.

Stained cells were examined with a BD FACSCanto™ II Flow Cytometer. For each analysis the fluorescence intensity of at least 10,000 cells was determined and analyzed with BD CellQuest™. Cells that stained doubly positive for $His48^{high}$ and $CD11b/c^{+}$ were identified as MDSCs and gated for subsequent phenotype analysis.[14]

IFN-γ and IL-10 Assays

IFN-γ and IL-10 levels were analyzed by a cytometric bead array method. This measures soluble analytes in a particle based immunoassay by detecting amplified fluorescence of the immune reaction product on a flow cytometer.[15]

IFN-γ and IL-10 were quantified simultaneously using a rat cytokine cytometric bead array kit (BD Pharmingen™). Cytokine concentrations were analyzed and calculated according to the manufacturer protocol. Briefly, microparticles were prepared and coupled to the desired antibodies. The array kit provided a mixture of 2 microbeads. Populations with a distinct fluorescence intensity (FL-3) were precoated with antibody specific to each cytokine. An aliquot of 50 µl supernatant of pleural fluid and standard cytokines were added to the premixed microbeads in 12 × 75 mm Falcon tubes (BD™ Biosciences®). After adding 50 µl of a mixture of phycoerythrin conjugated antibodies against cytokines the mixture was incubated for 3 hours in the dark at room temperature, washed and centrifuged at 500 × gravity for 5 minutes. The pellet was resuspended in 300 µl wash buffer. The BD FACSCanto II was calibrated with setup beads and 10,000 events were detected per sample.

Individual cytokine concentrations were calculated from fluorescence intensity.[15] Experimental data were analyzed using FCAP Array software provided by the manufacturer.

Plasma Aldosterone

Plasma aldosterone levels in rats were determined using commercially available kits (Cayman Chemical, Ann Arbor, Michigan). All samples were run in duplicate and the average of 2 determinations was obtained. Samples were diluted 1:2 according to manufacturer instructions. Plasma specimens collected from rats were centrifuged at 600 × gravity at 4C for 10 minutes and supernatant was stored at −70C before use. Standard curves were constructed using pure aldosterone. The absorbance of each sample was used to calculate the corresponding aldosterone level based on these curves. In this study all measured values were within the calibration curve range.

RESULTS

There was no significant difference in the body weight of rats in the 4 groups (see table). Bladder weight increased significantly (about threefold)

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