Recruitment of Bone Marrow Derived Cells to the Bladder After Bladder Outlet Obstruction

Stacy T. Tanaka,* Magaly Martinez-Ferrer, John H. Makari, Marcia L. Wills, John C. Thomas, Mark C. Adams, John W. Brock, III, John C. Pope, IV and Neil A. Bhowmick

From the Division of Pediatric Urology, Monroe Carell, Jr. Vanderbilt Children's Hospital, Nashville, Tennessee

Purpose: Bladder fibrosis is an undesired end point of partial bladder outlet obstruction. In fibrotic disease of the lung, kidney, skin and heart chemokines recruit bone marrow derived cells to injured tissue. Blockade of chemokines like CCL2 results in decreased fibrosis in other organs. To our knowledge we present the first report of bone marrow derived cell recruitment to the bladder in a murine bladder outlet obstruction model.

Materials and Methods: We lethally irradiated WT female mice and reconstituted their bone marrow using fetal liver cells from transgenic mice ubiquitously expressing green fluorescent protein. Periurethral collagen injection was used for bladder outlet obstruction. Obstruction was assessed by urodynamics, and bladder and kidney histological changes. Bladders were harvested 1 to 12 weeks after bladder outlet obstruction and compared to those in nonobstructed controls. The chemokine CCL2 was compared between obstructed and nonobstructed mice with reverse transcriptase-polymerase chain reaction. Green fluorescent protein expressing bone marrow derived cells were identified with immunohistochemistry and fluorescence activated cell sorting.

Results: Bladders showed histological and urodynamic changes consistent with obstruction. CCL2 induction increased after obstruction compared to that in controls. After obstruction bone marrow derived cells were present in the urothelial and stromal layers. Activated epidermal growth factor receptor was found in cells associated with bone marrow derived cells.

Conclusions: Bone marrow derived cells are recruited to the bladder by bladder outlet obstruction and are present in the urothelial and stromal layers. Stromal bone marrow derived cells may have a role in hypertrophy and fibrosis. Further study of the recruitment and function of bone marrow derived cells in the bladder may provide potential targets for antifibrotic therapy.

Key Words: bone marrow cells, chemokines, urinary bladder neck obstruction, fibrosis, animal use alternatives

Abbreviations and Acronyms

BMD = bone marrow derived

BOO = partial bladder outlet obstruction

EGF = epidermal growth factor

FACS = fluorescence activated cell sorting

GFP = green fluorescent protein

PCR = polymerase chain reaction

RT-PCR = reverse transcriptase-PCR

Study received Vanderbilt University Medical Center institutional animal care and use committee approval.

* Correspondence: Division of Pediatric Urology, Monroe Carell Jr. Vanderbilt Children's Hospital, 4102 Doctors' Office Tower, 2200 Children's Way, Nashville, Tennessee 37232-9820 (telephone: 615-936-1060; FAX: 615-936-1061; e-mail: stacy.tanaka@vanderbilt.edu).

BLADDER fibrosis and decreased compliance due to partial BOO are undesired end points of various pediatric urological diseases. Obstruction may be due to anatomical causes such as posterior urethral valves or to functional causes such as the uncoordinated bladder/sphincter complex in spinal dysraphism cases. As the bladder works against increased urethral resistance, smooth muscle hypertrophy and fibrosis can develop. Despite the relief of obstruction bladder dysfunction can persist.

0022-5347/09/1824-1769/0
THE JOURNAL OF UROLOGY®

Vol. 182, 1769-1774, October 2009
Printed in U.S.A.
DOI:10.1016/j.juro.2009.02.081

The association of BMD cells and fibrosis is an active area of research in the heart,[1] lung,[2,3] kidney[4] and skin.[5,6] Virtually all cell types produce chemokines to direct leukocyte trafficking under homeostatic and inflammatory conditions. Chemokines have a role in the recruitment of BMD cells and fibrosis in response to tissue injury. More importantly blockade of chemokine-chemokine receptor interactions decreases fibrosis, suggesting a potential therapeutic target.

We studied BMD cell recruitment to the bladder after BOO. To track BMD cells we used chimeric mice whose bone marrow cells were labeled with GFP. In these experiments we developed a BOO technique that is less invasive than the laparotomy method previously described[7] to minimize any confounding effects of surgical dissection on inflammation. We present our initial results of BMD cell recruitment to the bladder after BOO.

MATERIALS AND METHODS

Animals

Mice with a C57BL/6 background were maintained in a pathogen-free environment and fed a standard diet. Animal studies were approved by the Vanderbilt University Medical Center institutional animal care and use committee.

Chimeric Mice With GFP Labeled Bone Marrow

To track BMD cells chimeric mice with GFP labeled bone marrow were created by reconstituting the bone marrow of WT recipient mice with GFP labeled fetal liver cells.[8] Briefly, timed matings were set up with C57BL/6-Tg(ACTB-EGFP)1Osb/J mice (Jackson Laboratories, Bar Harbor, Maine) whose tissues appear green under ultraviolet light. Fetal livers from embryonic day 14.5 mice expressing GFP were harvested and pooled into single cell suspension.

Recipient WT female mice were prepared for fetal liver cell transplantation. Because estrogen signaling has been shown to decrease bladder inflammation in mice,[9] all recipient animals underwent ovariectomy at age 4 weeks to standardize estrogen. At age 6 weeks recipient ovariectomized mice were lethally irradiated from a ^{137}Cs γ source with a dose of 800 rads, followed 3 hours later by a second dose of 500 rads. After irradiation 2 million donor fetal liver cells were given by tail vein injection to reconstitute the bone marrow. To decrease infection mice were maintained on 100 mg/l neomycin and 10 mg/l polymyxin B in acidified water (pH 2) starting from 2 weeks before transplantation through the duration of the experiment. Using this protocol Everhart et al found greater than 85% reconstitution of bone marrow with GFP labeled cells.[8]

Partial BOO and Urodynamics

Four weeks after transplantation the 21 chimeric mice were obstructed with periurethral collagen injection under inhalational isoflurane anesthesia. Acidified rat tail collagen was neutralized to promote polymerization. After gentle cleansing the skin surrounding the urethral orifice was grasped with atraumatic tissue forceps. A total of 200 μl collagen were injected through a 27 gauge needle at 2 to 3 circumferential periurethral locations (fig. 1, *A*). Seven animals each were sacrificed 1, 4 and 12 weeks after periurethral BOO. An additional 26 control mice underwent ovariectomy and fetal liver cell transplantation with an equal volume of periurethral saline injected.

Urodynamics were performed immediately before harvest to assess bladder changes secondary to obstruction using a modification of a previously described technique.[10] Using general anesthesia the bladder was exposed through a midline abdominal incision and accessed with a 26 gauge needle connected to a pressure transducer and syringe pump. After the bladder was emptied by aspiration saline was infused at a rate of 30 μl per minute, and pressure and infused volume were recorded until urethral leak. Bladder capacity was defined as infused volume before urethral leak. Compliance was defined as bladder capacity divided by end fill pressure. Variables in obstructed vs unobstructed mice were compared by the t test with statistical significance considered at $p \leq 0.05$. Urodynamics data were available on 7 mice each at 1 and 4 weeks, and on 6 at 12 weeks after periurethral collagen

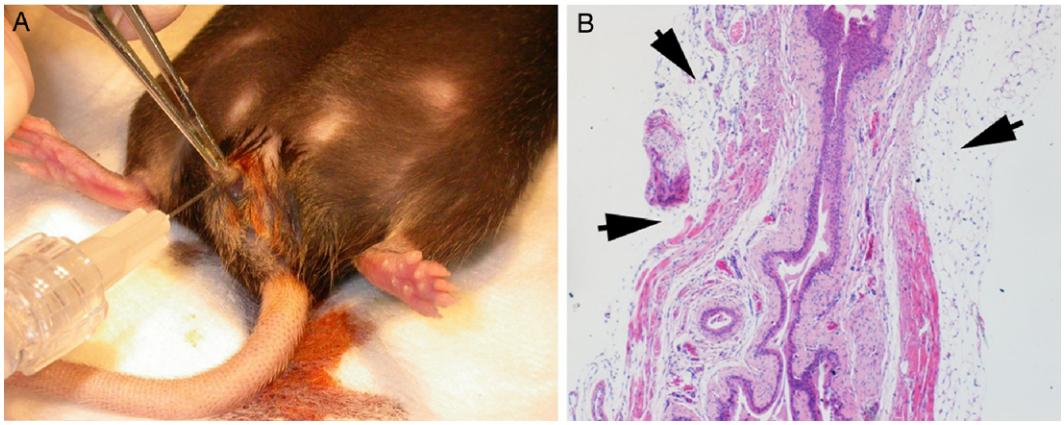

Figure 1. *A*, periurethral collagen injection to cause experimental partial BOO. *B*, longitudinal section of mouse urethra injected with periurethral collagen shows narrowed urethral lumen (arrowheads) and no inflammatory cells at obstruction site. H & E, reduced from ×100.