Restoration of bladder contraction by bone marrow transplantation in rats with underactive bladder

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ABSTRACT
We attempted to increase bladder contraction by bone marrow cell transplantation in rats with underactive bladder due to bladder outlet obstruction (BOO). Twelve female rats were anesthetized with halothane to create BOO. After 1 month, the urethral obstruction was removed and they were divided into a transplant group and a sham-operated group (n = 6 each). Bone marrow cells (1 × 10^7 / 0.2 mL) isolated from green fluorescent protein transgenic rats were injected into the bladder wall of the transplant group. Rats from the sham-operated group received injection of culture medium alone. One month after transplantation, isovolumetric cystometry parameters and histological features of bladder were observed as well as intact control rats (n = 6). The amplitude of bladder contractions was larger and the interval between contractions was shorter in the transplant group than the sham-operated group, and there were no differences in these parameters between the transplant group and the control group. Some green fluorescent muscle layers were found in the bladder wall of the transplant group, and these layers were also labeled by anti alpha-smooth muscle actin antibody. These results suggest that transplanted bone marrow cells may improve bladder contractility by differentiating into smooth muscle-like cells.

Lower urinary tract symptoms (LUTS) have a significant impact on a person’s psychosocial well-being and quality of life. Benign prostatic hyperplasia is the most common cause of LUTS in older men (8), and these patients often suffer from storage and/or voiding disorders. Storage disorders are mainly treated with medications such as anticholinergic agents, sometimes in combination with an α-1 blocker (13). However, prolonged bladder outlet obstruction (BOO) may cause an underactive bladder in which bladder contraction is impaired by changes of the detrusor muscle. An underactive bladder leads to difficulty in voiding that causes incomplete emptying of the bladder. Cholinergic agents or anticholinesterase agents are suggested to enhance bladder contractility and promote bladder emptying in patients with underactive bladder (2), but it is often difficult to manage their voiding disorders by medications alone. Clean intermittent catheterization is also employed in patients with an underactive bladder, but it can increase the risk of urinary tract infection and some patients refuse catheterization because of pain (20). Therefore, a new method of increasing bladder contractility in patients with underactive bladder is desired.

Recently, it has been shown that bone marrow stem cells have the potential to differentiate into bone, cartilage, tendon, muscle, and fat (12). Some studies have revealed that these cells can also give rise to myocardial cells, ovary cells, and nerve cells.
that constitute the vital organs under specific conditions (3, 12). Clinically, bone marrow transplantation has already been used to restore the function of various organs and/or blood components (3, 11, 16, 17). In the present study, therefore, we attempted to restore bladder contraction by bone marrow cell transplantation in rats with underactive bladder due to BOO.

MATERIALS AND METHODS

Rats. Two green fluorescent protein (GFP) transgenic female Sprague–Dawley (SD) rats and 18 female SD rats weighing 230–250 g were used in this study. Transgenic SD rats carrying the GFP transgene were supplied by Dr. Masaru Okabe, Genome Information Research Center, Osaka University, and were established by use of the same construct and technique to produce GFP transgenic mice (14).

Preparation of bone marrow cells from donor. Two GFP transgenic rats were anaesthetized with halothane (2%), and bone marrow cells were harvested from the femur by flushing with ice-cold phosphate-buffered saline (PBS: Gibco) using a syringe. Collected bone marrow cells were centrifuged at 1,600 rpm for 7 min, and were resuspended in Dulbecco’s modified eagle’s medium (DMEM: Gibco) containing 10% fetal bovine serum (FBS: Gibco) (FBS/DMEM) at 37°C in an incubator. After 24 h, non-adherent cells were removed and the medium was replaced. After 5 days, the supernatant was removed and adherent cells were collected by flushing with PBS. Then these cells were used as GFP-positive bone marrow cells for transplantation.

Animal model as recipient. Twelve rats were anaesthetized with halothane, and the bladder and proximal urethra were exposed through a lower abdominal incision. Then a polyethylene catheter (Intramedic PE50 with an outer diameter of 0.96 mm; Clay Adams, NJ, USA) was placed under the urethra, and a 4-0 silk ligature was tied around both the urethra and catheter to create partial BOO (18). After the ligature had been secured, the catheter was pulled out. Each animal received subcutaneous injection of an antibacterial agent (ampicillin at 100 mg/day) for 2 days after surgery to prevent infection. One month after creating BOO, natural voiding was checked and the urethral obstruction was removed under halothane anesthesia. Then a polyethylene catheter (PE50) was inserted into the bladder through the urethra, and the residual urine volume was measured to confirm that the bladder had expanded due to BOO. We used rats with a residual urine volume over 4 mL as the underactive bladder recipients in the transplant experiment, and they were divided into a transplant group (n = 6) and a sham-operated group (n = 6). We also used 6 normal untreated rats as a control group, and residual urine volume was measured by same procedure.

Transplantation of bone marrow cells into the bladder wall with underactive bladder recipients. Rats in the transplant group and sham-operated group were anesthetized with halothane and the bladder was exposed. GFP-positive bone marrow cells suspended in FBS/DMEM (1 × 10^7 / 0.2 mL) were injected directly at several sites on the bladder wall (between the space between mucosa layer and muscle layer) in the transplant group using a 27G needle. Rats in the sham-operated group were injected with culture medium (0.2 mL) by same procedure. Each animal received subcutaneous injection of an antibacterial agent (ampicillin at 100 mg/day) for 2 days after surgery to prevent infection.

Isovolumetric cystometry. One month after transplantation, isovolumetric cystometry was performed in the intact control group (n = 6), the transplant group, and the sham-operated group. Immediately after checking the natural voiding, all rats were anaesthetized with urethane (0.8 g/kg subcutaneously and 0.4 g/kg intraperitoneally). Then a polyethylene catheter (PE-50) was inserted into the bladder through the urethra, and the residual urine volume was measured. The urethra was ligated over the catheter near the external urethral meatus to produce isovolumetric conditions in the bladder, while the ureters were transected and the proximal cut ends were left open. The catheter was connected via polyethylene tubing to an infusion pump and a pressure transducer via three-way stopcock, and bladder pressure was displayed on a chart recorder. The bladder was filled with physiological saline to the residual urine volume plus 0.5–1.5 mL through the catheter, thus inducing isovolumetric rhythmic bladder contractions. Cystometry was continued for at least 120 min, and some parameters (the interval between bladder contractions, the intravesical baseline pressure, the amplitude of bladder contraction pressure, and the duration of bladder contraction) were measured after stable conditions were achieved for 60 min.

Histological experiment. After isovolumetric cyst-
RESULTS

The residual urine volume at 1 month after creating BOO (and before bone marrow cell transplantation) was significantly larger in the transplant group (in which the bone marrow transplant was scheduled) (5.9 ± 2.6 mL, p = 0.004) and the sham-operated group (6.0 ± 2.9 mL, p = 0.002) than in the control group (0.1 ± 0.1 mL) (Fig. 1A). The residual urine volume at 1 month after bone marrow cell transplantation was significantly smaller in the transplant group than in the sham-operated group, although it was still larger than in the control group (B). ∗: p < 0.05, ∗∗: p < 0.01.

When the isovolumetric cystometry was performed at 1 month after bone marrow cell transplantation, the amplitude of bladder contraction pressure was significantly larger and the interval between bladder contractions was significantly shorter in the transplant group than in the sham-operated group, while there were no significant differences between the control and transplant groups. ∗: p < 0.05, ∗∗: p < 0.01.

Statistical analysis: Results were reported as the mean ± standard deviation (SD). Student’s t-test (paired or unpaired) was used for analysis, and p < 0.05 was considered to indicate statistical significance.
These green fluorescent muscle layers were also labeled by anti-alpha-SMA antibody (Fig. 4).
DISCUSSION

We carried out bone marrow transplantation into the bladder wall in rats with underactive bladder due to BOO in order to restore bladder contraction. As the result, residual urine volume was smaller, the amplitude of bladder contractions was larger, and the interval between contractions was shorter in the transplant group than in the sham-operated group. Histologically, some green fluorescent muscle layers were found in the bladder wall of the transplant group, and these layers were also labeled by anti-alpha-SMA antibody. Therefore, these results suggest that bone marrow cell transplantation can improve bladder contractility in rats with underactive bladder because the donor cells differentiate into smooth muscle-like cells in recipients.

There have already been various transplantation studies using bone marrow cells or embryonic stem cells, but it was difficult to follow the differentiation of transplanted cells using specific antibodies to detect surface antigens because the expression of some antigens changes during differentiation. However, it has now become easy to observe the fate of transplanted cells by using green fluorescent protein (GFP) transgenic animals as donors. GFP is a one of the proteins responsible for green bioluminescence of the jellyfish, *Aequorea Victoria*, and many GFP mutants have been developed that display modified fluorescence spectra and an increased extinction coefficient. Some transgenic animals were produced with ‘enhanced’ GFP (EGFP) cDNA under the control of chicken beta-actin promoter and a cytomegalovirus enhancer (14). All of the cells and tissues from these transgenic animals, with the exception of erythrocytes and hair, show green fluorescence after excitation (14). Therefore, using bone marrow cells from such animals for transplant experiments made it possible to distinguish between cells derived from the recipient or donor and observe the differentiation of donor cells under fluorescence microscope. In the present study, we used bone marrow cells from GFP rats and could easily identify transplanted marrow cells in the bladder tissue that had differentiated into alpha-SMA positive smooth muscle cells.

The bone marrow is a complex tissue containing hemopoietic stem cells in close contact with stromal cells that constitute the marrow microenvironment. Recently, mesenchymal stem cells (MSCs) have been conventionally isolated from the stromal cells of the bone marrow (15), and the extensive capability of MSCs for self-renewal and multilineage differentiation under appropriate environmental conditions have been reported (1). MSCs also locate on several parts of body, and those populations originating from the brain, spleen, liver, kidney, renal glomeruli, lung, bone marrow, muscle, thymus, and pancreas have a similar morphology and a reasonably similar surface marker profile (5). On the other hand, differentiation assays have shown some variation with respect to the percentage of cells actually differentiating into an osteogenic or adipogenic phenotype, as well as the extent of differentiation in relation to the origin of MSCs (4). This might be due to the influence of the local environment from which these cells originate, reflecting the importance of the microenvironment in establishing the phenotype of stem cells (7).

In the present study, the residual urine volume was significantly increased and the amplitude of bladder contraction was significantly decreased by BOO, suggesting that decreased bladder contractility in these rats was caused by overdistension. Decreased bladder contractility due to BOO was improved by bone marrow cell transplantation, and the transplanted cells in the bladder wall were positive for anti-alpha-SMA antibody. Although we did not purify the bone marrow cells and confirm the existence of MSCs, these results suggest that there is a possibility of the existence of the cells which can differentiate into smooth muscle-like cells and the decreased bladder contractility is improved by bone marrow cell transplantation. Moreover, we transplanted bone marrow cells into the space under the mucosa layer of the bladder in the transplant group after a short period of culture without any growth factors in order to observe the properties of the native marrow cells. As the results, bone marrow cells grew in the bladder tissue and differentiated into smooth muscle-like cells especially between the smooth muscle of the recipient and serosa of the bladder at 1 month after transplantation. It is reported that bone marrow cells can acquire a smooth muscle cell-like phenotype in the environment created by cultured smooth muscle cells (19). Therefore, the required cells may be able to migrate into the required place and that differentiation into smooth muscle-like cells from bone marrow cells in vivo may be contributed to environment condition on acellular matrix grafts of the bladder.

There are some reports about the transplantation of bone marrow cells (including MSCs) for treatment of disease or injury. It is reported that intravenously transplanted MSCs improved cardiac performance and promoted the regeneration of blood vessels and cardiomyocytes in patients (9). MSCs
also enhance recovery from ischemia/reperfusion-induced acute renal failure in rats (10). Our findings suggest that restoring bladder function by bone marrow cell transplantation may be useful to improve micturition (voiding) disorders in patients with underactive bladder after removing BOO. The immunosuppressive effect of human MSCs is much stronger (1000-fold) than that of animal MSCs since expansion of purified human MSCs is much more rapid than that of murine MSCs (6). Therefore, MSCs should be more usable in humans than in animals.

In conclusion, we attempted to increase bladder contraction by bone marrow cell transplantation in rats with underactive bladder due to BOO. The results we obtained suggested that transplanted bone marrow cells may improve bladder contractility by differentiating into smooth muscle-like cells. This method may also be useful clinically to improve micturition disorders in patients with underactive bladder after removing BOO.

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