Allogenic fetal membrane-derived mesenchymal stem cells contribute to renal repair in experimental glomerulonephritis

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Departments of 1Regenerative Medicine and Tissue Engineering and 6Biochemistry, National Cardiovascular Center Research Institute; Departments of 2Advanced Technology for Transplantation, 3Functional Diagnostic Science, Course of Health Science, and 7Urology, Osaka University Graduate School of Medicine, Suita; 8Department of Perinatology, National Cardiovascular Center, Osaka; 5Second Department of Internal Medicine, Nara Medical University, Nara; and 4Department of Cell Biology, Institute of Nephrology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

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Allogenic fetal membrane-derived mesenchymal stem cells contribute to renal repair in experimental glomerulonephritis. Am J Physiol Renal Physiol 299: F1004–F1013, 2010. First published August 25, 2010; doi:10.1152/ajprenal.00587.2009.—Mesenchymal stem cells (MSC) have been reported to be an attractive therapeutic cell source for the treatment of renal diseases. Recently, we reported that transplantation of allogenic fetal membrane-derived MSC (FM-MSC), which are available noninvasively in large amounts, had a therapeutic effect on a hindlimb ischemia model (Ishikane S, Ohnishi S, Yamahara K, Sada M, Harada K, Mishima K, Iwasaki K, Fujiwara M, Kitamura S, Nagaya N, Ikeda T. Stem Cells 26: 2625–2633, 2008). Here, we investigated whether allogenic FM-MSC administration could ameliorate renal injury in experimental glomerulonephritis. Lewis rats with anti-Thy1 nephritis intravenously received FM-MSC obtained from a human placental tissue. Histopathological examination and immunohistochemical analysis revealed that allogenic FM-MSC transplantation significantly improved renal injury and significantly reduced mesangial matrix accumulation compared with the PBS group (P < 0.05 vs. PBS group). FM-MSC transplantation significantly reduced activated mesangial cell (MC) proliferation, glomerular monocyte/macrophage infiltration, mesangial matrix accumulation, as well as the glomerular expression of inflammatory or extracellular matrix-related genes including TNF-α, monocyte chemotactic protein-1 (MCP-1), type I collagen, TGF-β, type I plasminogen activator inhibitor (PAI-1) (P < 0.05 vs. PBS group). In vitro, FM-MSC-derived conditioned medium significantly attenuated the expression of TNF-α and MCP-1 in rat MC through a prostaglandin E2-dependent mechanism. These data suggest that transplanted FM-MSC contributed to the healing process in injured kidney tissue by producing paracrine factors. Our results indicate that allogenic FM-MSC transplantation is a potent therapeutic strategy for the treatment of acute glomerulonephritis.

prostaglandin E2; cell therapy; anti-Thy-1 nephritis

MESENCHYMAL STEM CELLS (MSC) are multipotent stem cells present in bone marrow (BM), adipose tissue, and many other organs and have the ability to differentiate into a variety of lineages including adipocytes, osteocytes, and chondrocytes (9, 11, 42). Previous reports described that BM-derived stem cells including MSC contributed to the repair of several compartments of the kidney, including the endothelium (47), interstitium (16), epithelium (23), and the mesangium (21). Moreover, several studies have demonstrated that transplanted BM-derived MSC (BM-MSC) contribute to improve renal function in experimental glomerulonephritis induced by anti-Thy1 (31, 58). These features make BM-MSC an attractive therapeutic tool for the treatment of renal diseases.

However, there are limitations in using autologous BM-MSC as a source of regenerative medicine. BM aspiration may be painful and sometimes yields low numbers of MSC on processing (61). Therefore, an alternative source of MSC that can be obtained noninvasively and in large quantities would be advantageous. Fetal membrane (FM), which is generally discarded as medical waste, has been found to be a rich, easily expandable source of MSC (18, 43). Recently, we demonstrated that transplantation of allogenic FM-derived MSC (FM-MSC) as well as autologous BM-MSC induced therapeutic angiogenesis using a rat hindlimb ischemia model (20). These results suggest that allogenic FM-MSC are a potential alternative to autologous BM-MSC as a source of regenerative therapy.

In the present study, we investigated whether systemic administration of allogenic FM-MSC could improve the course of anti-Thy1 nephritis. Anti-Thy1 nephritis is a model of mesangioproliferative glomerulonephritis characterized by mesangiolysis followed by repair via mesangial cell (MC) proliferation, mesangial matrix accumulation, and monocyte/macrophage influx (25). Because we previously reported that MSC possessed paracrine angiogenic and anti-fibrotic effects (20, 39), we also examined the contribution of the paracrine effects to ameliorate anti-Thy1 nephritis after allogenic FM-MSC transplantation.

MATERIALS AND METHODS

Animals. All experimental protocols were approved by the Animal Care Committee of the National Cardiovascular Center Research Institute. Different strains of rats were used according to their major histocompatibility complex (MHC) antigen disparity: Lewis (MHC haplotype: RT-11) and ACI (MHC haplotype: RT-1a) rats (Japan SLC, Hamamatsu, Japan). Green fluorescent protein

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Renoprotective Effect of Fetal Membrane-Derived MSC

Isolation and expansion of FM-MSC and glomerular MC. Isolation and expansion of FM-MSC were performed as previously described (20). In brief, FM was obtained from pregnant rats on day 15 postconception. Minced FM was digested with type II collagenase solution (300 U/ml; Worthington Biochemical, Lakewood, NJ) for 1 h at 37°C. After filtration and centrifugation, FM-derived cells were suspended in α-MEM (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS; Invitrogen) and 1% penicillin/streptomycin (Invitrogen) and cultured in standard plastic dishes. The adherent MSC populations appeared by days 3–7, and these FM-MSC were used for the experiments at passage 3–6.

Glomerular MC were established as described elsewhere (29). MC obtained from Sprague-Dawley rats were cultured in standard medium (DMEM, high glucose, Invitrogen, 10% FBS, and 1% penicillin/streptomycin) and used for experiments at passages 13–15.

Experimental model and design. Mesangial proliferative glomerulonephritis was induced in 6-wk-old male Lewis rats (170–180 g) by intravenous injection of anti-Thy1 monoclonal antibody (mA b1–22-3; 0.5 mg/rat) (24). Because FM-MSC reportedly express high levels of Thy1 (20), we administered FM-MSC on day 2 after anti-Thy1 antibody injection when its antibody in plasma is undetectable (Supplemental Figure and Method; supplemental material for this article is available online at the journal website). On day 2 after mAb injection, rats were randomized to two groups: 1) FM-MSC injection (FM-MSC group; n = 8) and 2) control PBS injection alone (PBS group; n = 8). A total of 5 × 10^6 FM-MSC obtained from MHC mismatched ACI rats or PBS (200 μl each) was injected into the tail vein of Lewis nephritic rats. Sham rats (Sham group; n = 8) received a PBS injection instead of mAb. On days 7 and 14, rats were placed in metabolic cages for collection of urine to determine the excretion of urine protein.

Histological examination. Kidney tissues were fixed with 4% phosphate-buffered formalin solution (Wako Pure Chemical Industries, Osaka, Japan), embedded in paraffin block, and cut into 2-μm sections. To quantify mesangial matrix accumulation, sections were stained with periodic acid-Schiff (PAS), photographed using a digital microscope (BIOREVO BZ-9000; KEYENCE, Osaka, Japan), and evaluated by assessing 30 randomly selected glomeruli/kidney and mean value of 30 randomly selected glomeruli was determined, and the mean value of 30 randomly selected glomeruli was calculated. The number of ED-1-positive monocytes/macrophages was evaluated by counting stained cells per glomerulus in at least 30 randomly selected glomeruli. To evaluate the distribution of GFP-positive administered cells, we counted all the GFP-positive cells in one randomly selected section (n = 4) from each organ and an overall average for all rats was calculated.

Quantitative RT-PCR analysis. Glomerular MC were harvested from rat kidneys using a graded sieving technique (19). Total RNA was extracted from isolated glomeruli using a N appliance mini kit (Qiagen, Hilden, Germany). Obtained RNA was reverse-transcribed into cDNA using a Quantitect Reverse Transcription kit (Qiagen). PCR amplification was performed using Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). β-Actin transcript was used as an internal control. Primers used are listed in Table 1.

Western blot analysis. Western blotting was performed as previously described (36). Briefly, kidney tissues were homogenized in 0.1% Tween 20 with a protease inhibitor, loaded (30 μg) on a 10–20% gradient gel (Bio-Rad, Hercules, CA), and blotted onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA). After blocking for 1 h, membranes were incubated with mouse anti-monoocyte chemoattractant protein (MCP)-1 antibody (1:1,000; BD Biosciences Pharmingen, San Jose, CA), then incubated with peroxidase-labeled secondary antibody (1:1,000; Cell Signaling Technology, Danvers, MA). Positive protein bands were visualized with an ECL kit (GE Healthcare, Piscataway, NJ) and measured by densitometry. A mouse monoclonal antibody against β-actin (Sigma-Aldrich, St. Louis, MO) was used as a control (n = 8 in each group).

Assessment of paracrine effects of FM-MSC on glomerular MC. Conditioned medium was collected from 1 × 10^6 cells of FM-MSC cultured in 8 ml of standard medium (DMEM supplemented with 10% FBS and 1% penicillin/streptomycin) with or without the cyclooxygenase (COX) 2 inhibitor NS-398 (0.1 μM; Wako) for 48 h, and filtered through a 0.22-μm filtration unit (Millipore). MC were plated on six-well plates (2 × 10^5 cells/well) with standard medium for 24 h. The medium was then changed to serum-free DMEM for 24 h, followed by conditioned medium obtained from FM-MSC. After 8 h, total RNA was extracted from MC.

ELISA. The concentration of PGE2 in the conditioned medium of FM-MSC was determined using an ELISA kit, according to the

Table 1. Primers for qRT-PCR

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<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
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<td>5'-GTTCAGACTTTGCTGATGTTG-3'</td>
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<tr>
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<td>5'-ACCTTGCTGCTGCTGATGTTG-3'</td>
</tr>
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<td>5'-ACCTGCTGCTGCTGATGTTG-3'</td>
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<tr>
<td>MMP-2</td>
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<td>5'-CTGAGCTGCTGCTGATGTTG-3'</td>
<td>5'-CTGAGCTGCTGCTGATGTTG-3'</td>
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<tr>
<td>VEGF</td>
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<td>5'-AGGAAAGGCAACTGCTGCTGCTG-3'</td>
</tr>
<tr>
<td>β-Actin</td>
<td>5'-CCCTGAGCTGCTGCTGATGTTG-3'</td>
<td>5'-CCCTGAGCTGCTGCTGATGTTG-3'</td>
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TGF, transforming growth factor; PAI-1, type I plasminogen activator inhibitor; MMP, membrane-type matrix metalloproteinase; TIMP-1, tissue inhibitor of metalloproteinase; PGE2, prostaglandin E2; ELISA, enzyme-linked immunosorbent assay; qRT-PCR, quantitative reverse transcription-polymerase chain reaction.
Reduction of proteinuria by FM-MSC transplantation. In the anti-Thy1 nephritic model, transient renal damage with massive proteinuria developed (25). On day 7, the 24-h urine protein excretion rate was significantly increased in rats with anti-Thy1 nephritis (84.9 ± 7.6 mg/24 h in the PBS group) compared with control (10.7 ± 0.5 mg/24 h in the Sham group), and FM-MSC treatment significantly reduced this increase (60.8 ± 8.0 mg/24 h, \( P < 0.05 \) vs. PBS group) (Fig. 1). On day 14, the 24-h urine protein excretion rate in these three groups fell within the normal range. Creatinine clearance was significantly decreased in nephritic rats (2.14 ± 0.06 ml/min in the PBS group) compared with the Sham group (2.97 ± 0.15 ml/min, \( P < 0.01 \)). However, no significant difference in creatinine clearance was observed between the PBS and FM-MSC groups (2.14 ± 0.09 ml/min in the FM-MSC group).

Inhibition of accumulation of activated MC and mesangial matrix by FM-MSC transplantation. Glomerular expression of α-SMA, a marker of activated MC, was increased after disease induction (Fig. 2, D–F). Expression of α-SMA in nephritic rats increased to a peak level on day 7 and then gradually decreased thereafter (Fig. 2M). On days 7 and 14, α-SMA staining of the glomerular area in FM-MSC-treated rats (30.7 ± 0.8 and 22.4 ± 0.8%, respectively) was significantly decreased compared with the PBS group (37.5 ± 0.6 and 26.6 ± 0.7%, respectively, \( P < 0.01 \) vs. FM-MSC group).

PAS staining in rats with anti-Thy1 nephritis revealed the accumulation of mesangial matrix (Fig. 2, G–I). Similar to α-SMA expression, the glomerular PAS-positive area in nephritic rats reached peak on day 7 (PAS score; 2.72 ± 0.12 in the PBS group vs. 0.20 ± 0.05 in the Sham group, \( P < 0.01 \)) and remained elevated on day 14 (2.97 ± 0.08 in the PBS group and 0.25 ± 0.05 in the Sham group, \( P < 0.01 \)), which was significantly decreased by FM-MSC administration (FM-MSC group; 2.21 ± 0.08 on day 7 and 1.54 ± 0.06 on day 14, \( P < 0.05 \) vs. PBS group) (Fig. 2N). qRT-PCR analysis revealed that the reduction of mesangial matrix accumulation in FM-MSC-treated rats was associated with decreased expression of glomerular type I collagen, transforming growth factor (TGF)-β, type 1 plasminogen activator inhibitor (PAI-1) (\( P < 0.05 \) vs. PBS group) (Fig. 3, A–C). However, FM-MSC treatment did not significantly affect the glomerular expression of membrane-type matrix metalloproteinases (MMPs) and tissue inhibitor of MMP-1 (TIMP-1) in anti-Thy1 nephritic rats (Fig. 3, D–F).

Attenuation of glomerular monocyte/macrophage influx by FM-MSC transplantation. Immunostaining of ED-1 in rats with anti-Thy1 nephritis revealed a significant monocyte/macrophage infiltration into the glomeruli (Fig. 2, J–L). On day 7, the number of infiltrating monocytes/macrophages in the PBS group (7.5 ± 0.2/glomerulus) was significantly higher than in the Sham group (0.4 ± 0.1/glomerulus), which was significantly lower than in the FM-MSC group (6.0 ± 0.2/glomerulus, \( P < 0.01 \) vs. PBS group) (Fig. 2O). A similar result was also observed on day 14 (PBS group 5.1 ± 0.2/glomerulus vs. FM-MSC group 3.9 ± 0.1/glomerulus, \( P < 0.01 \)).

Reduction of renal inflammatory cytokine/chemokine expression by FM-MSC transplantation. We examined the glomerular expression of inflammatory cytokines/chemokines in nephritic rats on day 7. QRT-PCR analysis showed that tumor necrosis factor (TNF)-α expression in glomeruli was significantly increased by 7.70 ± 0.54-fold in the PBS group (\( P < 0.01 \) vs. the Sham group), and this increase was significantly decreased in the FM-MSC group (5.92 ± 0.20-fold, \( P < 0.05 \) vs. PBS group) (Fig. 3G). Glomerular MCP-1 mRNA expression in the PBS group showed a 5.41 ± 0.38-fold increase compared with the Sham group (\( P < 0.01 \)) (Fig. 3H). However, FM-MSC transplantation significantly reduced this increase by >30% (3.51 ± 0.51-fold, \( P < 0.05 \) vs. PBS group). Similarly, Western blot analysis showed that renal MCP-1 protein expression in the PBS group was significantly increased compared with the Sham group (7.65 ± 0.49-fold, \( P < 0.05 \)) (Fig. 4), and FM-MSC administration showed a tendency of decreasing the expression of MCP-1 protein (6.44 ± 0.96-fold vs. the Sham group) (Fig. 4).

Renal expression of VEGF and HGF after FM-MSC transplantation. Previously, we reported that cultured FM-MSC secreted large amounts of angiogenic/antiapoptotic factors including VEGF and HGF (20). Because VEGF and HGF have been reported as renoprotective factors (34, 41, 53, 55), we analyzed glomerular expression of these factors in FM-MSC-transplanted nephritic rats. qRT-PCR analysis revealed that expression of VEGF mRNA in the PBS group was significantly decreased (0.36 ± 0.07-fold vs. Sham group, \( P < 0.05 \)), and no significant upregulation was seen after FM-MSC administration (0.30 ± 0.08-fold vs. Sham group, \( P < 0.05 \)) (Fig. 3I). Glomerular expression of HGF mRNA was significantly increased in the PBS group (2.64 ± 0.38-fold vs. Sham group, \( P < 0.05 \)), but no significant difference was observed between PBS and FM-MSC groups (2.51 ± 0.34-fold vs. Sham group, \( P < 0.05 \)) (Fig. 3J).
Fig. 2. Inhibition of the accumulation of activated MC, mesangial matrix, and glomerular monocyte/macrophages by FM-MSC transplantation in rats with anti-Thy1 nephritis.

A–L: representative micrographs of negative control (A–C), α-smooth muscle actin (SMA; D–F), periodic acid-Schiff (PAS; G–I) and ED-1 (J–L) staining in the Sham (A, D, G, J), PBS (B, E, H, K) and FM-MSC (C, F, I, L) groups on day 7. M: quantitative analysis revealed that the number of α-SMA-positive activated MC was lower in the FM-MSC group compared with the PBS group on days 7 and 14. N: mesangial matrix accumulation was significantly reduced in the FM-MSC group compared with the PBS group on days 7 and 14. O: the number of infiltrated ED-1-positive monocytes/macrophages was significantly reduced in the FM-MSC group compared with the PBS group on days 7 and 14. Scale bars = 20 μm. *P < 0.05 vs. Sham. †P < 0.05 vs. PBS group.
Engraftment of intravenously injected FM-MSC in rats with anti-Thy1 nephritis. To investigate the behavior of intravenously administered FM-MSC in anti-Thy1 nephritic rats, FM-MSC derived from GFP transgenic Lewis rats were intravenously administered into allogenic ACI rats on day 2 after mAb injection (n = 4). Twenty-four hours after FM-MSC transplantation, several GFP-positive cells were detected in the kidney sections (12.7 ± 0.3 cells/cm²) including glomeruli (Fig. 5A), proximal tubule (Fig. 5B), and interstitial area (Fig. 5C). We also detected GFP-positive FM-MSC in sections of lung (Fig. 5D), liver (Fig. 5E), and spleen (Fig. 5F). A significant number of GFP-positive FM-MSC were seen in the

Fig. 3. Quantitative RT-PCR analysis of extracellular matrix, inflammatory-, and angiogenesis-related genes in the kidney of rats with anti-Thy1 nephritis and FM-MSC administration. Expression of mRNA for type I collagen (A), transforming growth factor (TGF)-β (B), type 1 plasminogen activator inhibitor (PAI-1; C), TNF-α (G), and monocyte chemotactant protein (MCP-1; H) in glomeruli was markedly increased in anti-Thy1 nephritic rats on day 7, which was significantly attenuated in FM-MSC-transplanted rats. No significant difference in mRNA expression of membrane-type matrix metalloproteinase 2 (MMP-2; D), MMP-9 (E), tissue inhibitor of MMP-1 (TIMP-1; F), VEGF (I), and HGF (J) was seen between the PBS and FM-MSC groups. (n = 8/group). *P < 0.05 vs. Sham group. †P < 0.05 vs. PBS group.
A. 25kDa 42kDa MCP-1 β-actin

B. MCP-1

Fig. 4. Decreased MCP-1 protein expression in the renal tissue of nephritic rats after FM-MSC administration. A: representative Western blot analysis of MCP-1 in the Sham, PBS, and FM-MSC groups. B: quantitative analysis of immunoreactive bands for MCP-1 demonstrated that MCP-1 protein expression in the PBS group was significantly upregulated compared with the Sham group (P < 0.05, n = 8), and FM-MSC administration showed a tendency toward decreased expression.

lung (119.2 ± 5.1 cells/cm²), and a similar distribution of GFP-positive cells were observed in the liver and spleen (38.4 ± 1.5 and 32.1 ± 2.9 cells/cm², respectively). Although no GFP-positive cell was found in the kidney at 7 days after systemic injection of GFP-positive FM-MSC, several GFP-expressing cells were detected in sections of lung, liver, and spleen (37.6 ± 3.1, 26.1 ± 2.7, and 4.8 ± 0.3 cells/cm², respectively).

**Anti-inflammatory effect of FM-MSC-conditioned medium on cultured MC.** Next, we examined whether FM-MSC possess direct anti-inflammatory effects on MC. When MC were cultured in standard medium, gene expression of TNF-α showed a peak at 8 h and then decreased (n = 3–12, Table 2). FM-MSC-conditioned medium induced a significant (>50%) decrease in TNF-α expression after incubation for 8 h (P < 0.05 vs. standard medium) (Table 2). MCP-1 expression in cultured MC showed a peak at 4 h and then decreased (Table 2). Between FM-MSC-conditioned and standard medium, a significant reduction in MCP-1 expression was seen at 4 (>20%) and 8 (>70%) h (P < 0.05 vs. standard medium).

Because recent reports have shown that PGE₂ is one of the key modulators for the MSC-induced anti-inflammatory response, PGE₂-depleted conditioned medium of FM-MSC was prepared by treatment with NS-398, a selective inhibitor of COX2 activity (38). ELISA revealed that a significant amount of PGE₂ was detected in FM-MSC-conditioned medium (888.1 ± 123.3 pg/ml), and NS-398 treatment significantly suppressed its production (23.2 ± 2.4 pg/ml, P < 0.01). After incubation for 8 h, conditioned medium of NS-389-treated FM-MSC markedly abolished the decreased expression of TNF-α and MCP-1 in rat MC (1.19 ± 0.12- and 0.82 ± 0.06-fold, respectively) (Fig. 6).

**DISCUSSION**

In this study, we demonstrated that 1) intravenous injection of allogenic FM-MSC improved disease manifestations in rats with anti-Thy1 glomerulonephritis; 2) allogenic FM-MSC administration suppressed MC proliferation, glomerular monocyte/macrophage infiltration, mesangial matrix accumulation, and the glomerular expression of inflammatory and extracellular matrix-related molecules in anti-Thy1 nephritis; and 3) FM-MSC-conditioned medium attenuated the expression of these inflammatory cytokines/chemokines in cultured MC through a PGE₂-dependent mechanism. Therefore, our data indicate that allogenic FM-MSC transplantation would be a potent therapeutic strategy for the treatment of acute glomerulonephritis.

MSC are considered to be an attractive cell source for application in regenerative medicine because of their excellent capacities in proliferation and differentiation (8, 33, 35, 62). MSC are present in various tissues, but the most characterized population is BM-MSC (9, 11, 42). Therefore, the potential of MSC for renal repair has been investigated using BM-MSC (17, 30-32, 54, 56). To consider the clinical setting, donor BM is a suitable source of MSC, because MSC are relatively easy to obtain from BM aspirates and autologous donor MSC are unlikely to be immunologically rejected. However, autologous MSC transplantation is difficult to attempt on acute glomerulonephritis patients, because of a cell-preparatory period and cell transplantation timing. Therefore, allogenic MSC transplantation has more practical therapeutic value in clinical medicine. We have previously characterized a population of MSC from FM tissue, which possesses great advantages due to its abundance, easy accessibility, and angiogenic activity (20). In this study, we demonstrated that intravenous injection of allogenic FM-MSC, similar to reported autologous BM-MSC (31, 58), provided significant improvement in rats with anti-Thy1 nephritis, indicating that allogenic FM-MSC have potential as a source for regenerative-based therapy for glomerulonephritis.

In this study, we demonstrated that allogenic ACI-derived FM-MSC have a therapeutic effect in MHC-mismatched Lewis rats with anti-Thy1 nephritis. FM is known to play a role in preventing rejection of the fetus and is thought to have low immunogenicity (2, 3, 59). MSC have been reported to fail to trigger allogenic T cell proliferation and induce immune tolerance (1, 6). Indeed, we previously demonstrated that FM-MSC expressed surface antigens similar to those of BM-MSC. For example, both types of MSC are negative for MHC II (19). These results suggest that FM-MSC as well as BM-MSC could evade T lymphocyte alloreactivity and would be successfully transplantable across MHC barriers.

Several studies have shown beneficial effects of BM-MSC transplantation in renal diseases (17, 30–32, 54, 56). However, the mechanisms underlying the benefit of MSC transplantation remain controversial. One possible mechanism is the differentiation into renal cells of injected MSC. Intravenously administered MSC have been shown to con-
tribute, via differentiation and engraftment, to the cells of many organs, including the kidney (17, 23, 45, 47). In this study, however, we confirmed that these engraftments were low-frequency events that cannot explain the prompt regenerative responses MSC elicit in damaged kidneys. Using the same anti-Thyl nephritis model, Kunter et al. (31) reported that they failed to detect any evidence of transdifferentiation of MSC into renal cells. This evidence suggests that the direct contribution of transplanted MSC to tissue regeneration is minimal.

Another possibility explaining how transplanted MSC mediate the protective and regenerative effects in damaged kidney tissue is paracrine action (31, 56, 58). Our previous studies found that FM- and BM-MSC secreted VEGF and HGF, which are well-known potent angiogenic and anti-apoptotic factors that elicited angiogenesis in a hindlimb ischemia model (20). In experimental ischemic acute kidney injury or glomerulonephritis, VEGF or HGF secreted from MSC exerted beneficial effects (31, 46, 56, 58). Based on these results, we examined the glomerular expression of these regenerative factors in anti-Thyl nephritic rats. Contrary to our expectation, however, no significant induction of VEGF or HGF expression in the kidney was seen after FM-MSC transplantation. Therefore, contribution of these FM-MSC-derived growth factors might be minimal in the repair process of anti-Thyl nephritis.

Table 2. Time course of TNF-α and MCP-1 mRNA levels in MC after incubation with standard or FM-MSC-conditioned medium

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<th>TNF-α</th>
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<td></td>
<td>100.0 ± 22.4%</td>
<td>155.5 ± 28.5%</td>
<td>154.3 ± 23.2%</td>
<td>100.0 ± 14.7%</td>
<td>20.2 ± 1.7%</td>
<td>26.9 ± 2.9%</td>
</tr>
<tr>
<td>FM-MSC-conditioned medium</td>
<td>64.6 ± 19.7%*</td>
<td>83.4 ± 10.6%*</td>
<td>154.4 ± 61.6%</td>
<td>19.9 ± 11.5%*</td>
<td>14.4 ± 1.9%*</td>
<td>31.9 ± 6.7%</td>
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MC, mesangial cells; FM-MSC, fetal membrane-derived mesangial stem cells. *P < 0.05 vs. standard medium.
and declining renal function (10, 44, 48, 60). In glomerulonephritis, these inflammatory cytokines/chemokines participate in the activation and accumulation of macrophages, and their infiltration is strongly associated with proteinuria.

Recent reports demonstrate that MSC-mediated immunosuppression is mediated by direct contact with immunomodulatory cells including T cells, NK cells, and macrophages, followed by paracrine action of secreted PGE$_2$ and TGF-β (13, 27, 38). Because intravenously administered FM-MSC survived in kidney, lung, and reticuloendothelial organs including the spleen and liver, these transplanted FM-MSC might change the activity of immunomodulatory cells in nephritic rats by direct contact and paracrine action, which would reduce the inflammatory state of anti-Thy1 nephritis. Previous studies demonstrate that PGE$_2$ is one of the leading candidates for MSC-induced immune suppression (38). In this study, we confirmed that FM-MSC-conditioned medium contained a significant amount of PGE$_2$, which was completely depleted by treatment with the COX2 inhibitor NS-398. Gene expression of MCP-1 and TNF-α in MC was decreased by FM-MSC-conditioned media, and this decrease was significantly restored by the treatment with NS-398. A previous report demonstrated that PGE$_2$ suppressed cytokine/chemokine expression including TNF-α and MCP-1 in MC, which would relate to its anti-inflammatory activity (50). Therefore, PGE$_2$ would be one of the candidate factors to cause the downregulation of TNF-α and MCP-1 in FM-MSC-treated rats with anti-Thy1 nephritis. Together, previous studies including our own support the hypothesis that paracrine/endocrine actions are of major importance in mediating the protective and regenerative effect of administered MSC after tissue damage.

We have recently reported that MSC transplantation improved cardiac function through an antifibrotic effect in a rat model of dilated cardiomyopathy and acute myocarditis (36, 40) and also demonstrated that the highly expressed genes in cultured MSC included a number of molecules involved in the biogenesis of extracellular matrix (39). These results suggest that transplanted MSC inhibit the fibrogenic process through paracrine actions. In this study, we confirmed that FM-MSC transplantation in anti-Thy1 nephritic rats resulted in reduced mesangial matrix accumulation. In addition, the glomerular expression of several genes involved in fibrogenesis including type I collagen, TGF-β, and PAI-1 was significantly decreased in the FM-MSC group compared with the PBS group. These results support our hypothesis that paracrine/endoctrine actions are of major importance in mediating the protective and regenerative effect of administered MSC after tissue damage.

In conclusion, our observation that FM-MSC transplantation helped recovery from anti-Thy1 nephritis demonstrates the renoprotective effect of FM-MSC. Because FM-MSC is available non-invasively in large amounts, we suggest that cultured, banked FM-MSC could provide a new therapeutic strategy for the treatment of kidney injury.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.
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RENOPROTECTIVE EFFECT OF FETAL MEMBRANE-DERIVED MSC


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