Article

Transplantation of Human Bone Marrow Mesenchymal Stem Cell Ameliorates the Autoimmune Pathogenesis in MRL/lpr Mice

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Recent evidence indicates that mesenchymal stem cells (MSC) possess immunosuppressive properties both *in vitro* and *in vivo*. We previously demonstrated the functional abnormality of bone marrow derived MSC in patients with systemic lupus erythematosus (SLE). In this study, we aimed to investigate whether transplantation of human bone marrow derived MSC affects the autoimmune pathogenesis in MRL/lpr mice. We found that human MSC from healthy donors reduced the proliferation of T lymphocytes from MRL/lpr mice in a dose-dependent fashion. Two weeks after *in vivo* transfer of MSC, we detected significantly reduced serum levels of anti ds-DNA antibodies and 24 hour proteinuria in MRL/lpr mice as compared with control groups without MSC transplantation. Moreover, flow cytometric analysis revealed markedly reduced number of $CD4^+$ T cells while increased Th1 subpopulation in MSC group and MSC + CTX group when compared with controls. Histopathological examination showed significantly reduced renal pathology in MSC-treated mice. Immunohistochemical studies further revealed reduced expression of TGF-β, FN, VEGF and the deposition of complement C3 in renal tissue after MSC and MSC + CTX treatment. Taken together, we have demonstrated that transplantation of human MSC can significantly inhibit the autoimmune progression in MRL/lpr mice. *Cellular & Molecular Immunology*. 2008;5(6):417-424.

Key Words: systemic lupus erythematosus, mesenchymal stem cell, transplantation

Introduction

MRL/lpr mice with the mutation of lpr gene spontaneously develop autoimmune disease that highly resembles human systemic lupus erythematosus (SLE). The lpr mutation results in multiple autoimmune effects including aberrant control of apoptosis, lymphadenopathy, splenomegaly, autoantibody generation and nephritis. Studies have shown that the onset of autoimmune diseases in MRL/lpr mice could be prevented by various treatments such as the injection of monoclonal antibodies (mAbs) against T cells and immunosuppressants. In addition, allogeneic bone marrow transplantation (BMT) or hematopoietic stem cells (HSC) transplantation may also be performed to prolong the life span of MRL/lpr mice (1).

In addition to HSC, mesenchymal stem cells (MSC) are present in bone marrow. Previous studies have revealed that MSC have immunosuppressive properties *in vitro*. For example, MSC inhibit T and B lymphocyte proliferation and antibody production in a dose-dependent fashion (2). The suppression is major histocompatibility complex (MHC) - independent. In human cell cultures, the magnitude of suppression is not reduced when the MSC are separated from the lymphocytes in transwells, indicating that cell-cell contact is not required. It has also been reported that MSC suppress lymphocyte proliferation *in vitro* and prolong skin graft survival *in vivo* (3). Recently, we have shown the functional abnormality of MSC from the bone marrow of SLE patients (4).

MSC can enhance tissue repairing process. Recent findings indicate that MSC could ameliorate ischemic liver

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Abbreviations: MSC, mesenchymal stem cells; MRL/lpr , MRL/Mp-lpr/lpr; HSC, hematopoietic stem cells; SLE, systemic lupus erythematosus; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor; FN, fibronectin; PBS, phosphate-buffered saline; IL, interleukin; IFN, interferon; CTX, cyclophosphamide; mAbs, monoclonal antibodies; PAS, periodic acid-Schiff; L-DMEM, low glucose Dulbecco Modified Eagle Medium; ANA, anti-nuclear antibodies.

and brain in rats. Human bone marrow MSC treatment has also been shown to enhance functional recovery in mice with experimental autoimmune encephalomyelitis (EAE) *via* reducing inflammatory infiltration and demyelinating areas. Tissue protective effects of MSC were also reported in rat kidney model of ischemia/reperfusion injury (5). Also, allogeneic MSCs were successfully used as therapy for collagen-induced arthritis, a mouse model for human rheumatoid arthritis (6). Intracarotid administration of MSC resulted in a significant improvement of renal function, higher proliferation and lower apoptotic indices, as well as lower renal injury with unchanged leukocyte infiltration scores. The significant renoprotection effects of MSC are of considerable therapeutic potential for the cell-based treatment of clinical acute renal failure (ARF). It has been proposed that the beneficial effects of MSC are primarily mediated *via* complex paracrine actions but not by their differentiation into target cells. MSC can markedly accelerate glomerular recovery from mesangiolytic damage possibly related to paracrine growth factor release and not by differentiating into resident glomerular cell types or monocytes/macrophages. MSC treatment has been shown to lead to more rapid recovery from mesangiolysis by increased glomerular cell proliferation and reduced proteinuria (7).

Recent findings indicate that MSC not only support hematopoietic function, but also regulate the immunological responses. However, it remains unknown whether MSC can be used to treat SLE patients. In the present study, we sought to investigate the potential therapeutic effects and possible mechanism of human bone marrow MSC transplantation on ameliorating the autoimmune progression in MRL/lpr mice.

Materials and Methods

Animals and reagents

Female MRL/Mp-lpr/lpr (MRL/lpr) mice were obtained from Shanghai Laboratory Animal Center (Shanghai, China) and maintained until use in our animal facilities under specific pathogen-free conditions at Nanjing University Medical School. The following reagents were used in this study: anti-ds-DNA and anti-nuclear antibodies (ANA) (EUROIMMUN, Germany), PE-Cy5.5-conjugated anti-CD3, Alexa fluor-conjugated anti-CD8, PE-conjugated anti-interleukin (IL)-4 and FITC-conjugated anti-interferon (IFN)-γ (eBioscience, USA), anti-transforming growth factor (TGF)-β (Biovision Inc., USA), anti-vascular endothelial growth factor (VEGF) (Neo Markers Frement CA), anti-complement C3a and anti-fibronectin (FN) (Boster Biological Technology Ltd., China).

T lymphocyte isolation and proliferation assays with human MSC

Bone marrow-derived human MSC (hMSC) were isolated and cultured by standard procedures as described (4). Briefly, five milliliters of heparinized bone marrow were mixed with an equal volume of phosphate-buffered saline (PBS) and the resuspended cells were layered over 1.077 g/ml Ficoll solution (TBD, Tianjin, China) and centrifuged at 600 g for 20 min at room temperature. The mononuclear cells were collected at the interface, then the cells were resuspended in low glucose dulbecco modified eagle medium (L-DMEM, GIBCO) supplemented with 10% heat inactivated fetal bovine serum (FBS, GIBCO). The cells were plated at a density of 2×10^7 cells per 25 cm^2 dish. The cultures were maintained at 37°C in a 5% CO_2 incubator, and the medium was changed after 48 h and then every three days. When the MSC were confluent, the cells were recovered by the addition of 0.25% trypsin-EDTA; they were then replated at a density of 1×10^6 cells per 25 cm^2 dish.

Cell viability was determined by an MTT assay using the Cell Proliferation Kit (Sigma, USA). Isolation of MRL/lpr mouse T lymphocytes from splenocytes was performed by nylon wool columns. hMSC (administered at 1×10^5, 5×10^4, 1×10^4 per well) were cultured in triplicate at 37°C in 5% CO_2 in 96-well round-bottom plate. T lymphocytes from MRL/lpr mice were cocultured with hMSC with different ratio (MSC: T = 1:10, 1:50, 1:100). T lymphocytes without hMSC were used as a control. Cells were activated with PMA at the concentration of 5 μg/ml. After 24 h of incubation at 37°C in humidified 5% CO_2, 25 μl MTT dissolved in PBS was added to cells for 4 h to give a final concentration of 0.1 mg/ml. The blue crystals were dissolved by the addition of 20% SDS in HCl. An automated microplate reader (Bio-Rad Model 550) was used to measure the light absorbance value at 570 nm.

Transplantation protocols

The mice were divided into control group (n = 5), cyclophosphamide (CTX) treated group (n = 4), MSC transplantation group (n = 4), and MSC + CTX group (n = 4). The onset of autoimmune diseases in MRL/lpr mice was monitored by measuring proteinuria (≥ 100 mg/dl). The mice (usually at 4 to 5 months of age) with autoimmune diseases in CTX and MSC + CTX group were received intraperitoneal injections of CTX at a dose of 100 mg/kg body weight for 2 days. One day after the injection, 1×10^6 MSC were injected into the MSC and MSC + CTX groups *via* tail vein. The body weight and 24-hour proteinuria were measured every two weeks.

The institutional research ethics committee gave approval for the study. Written informed consent was obtained from healthy volunteers before bone marrow sampling. All research work with human subjects was in compliance with the Helsinki Declaration.

Laboratory tests

The serum levels of ANA and anti ds-DNA from mice at age of 28 weeks were determined by indirect immunofluorescence assay. Mice at the age of 32 weeks were sacrificed after the serum creatinine was measured.

Cytokine detection in T cells

Peripheral blood samples were diluted with the same volume of RPMI 1640. Phorbol myristate acetate (PMA, 20 ng/ml, Sigma) and ionomycin at 1 μg/ml were added to the diluted

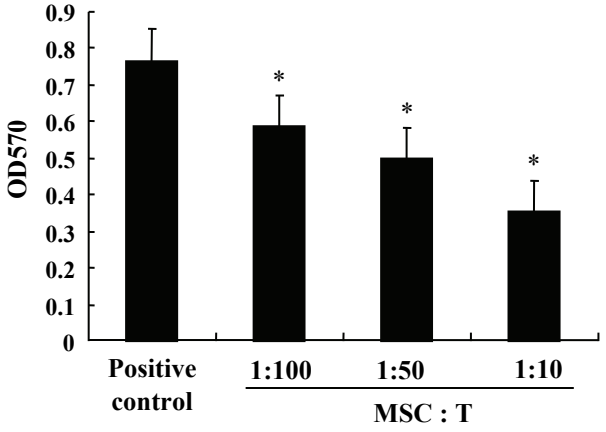

Figure 1. Dose-dependent inhibitory effect of hMSC on PMA-stimulated T lympocytes. T lymphocytes were activated with 5 μg/ml PMA for 24 h. Then they were cocultured with hMSC with different ratios (MSC : T = 1:100, 1:50, 1:10). T lymphocytes without hMSC were used as control. When compared with controls, the proliferation was significantly decreased ($p < 0.05$).

blood samples. Monensin at 3 μg/ml was also added at the beginning of culture to inhibit cytokine secretion. Mixed blood samples were aliquotted into 96-well cell culture plate and incubated for 4 h in 5% CO_2 at 37°C.

After incubation, cells were collected and washed twice with 2 ml staining buffer (Dulbecco's PBS containing 0.1% sodium azide and 0.2% heat-inactivated fetal calf serum) at 500 rpm for 5 minutes. The supernatant was aspirated and surface staining was then performed at room temperature for 30 minutes. The cells were fixed by the addition of 4% paraformaldehyde for 20 minutes, followed by washing the cells in staining buffer. Cells were then permeabilized by the addition of 500 μl permeabilization solution (staining buffer containing 0.1% saponin). Intracellular staining was performed using PE- or FITC-labeled anti-cytokine monoclonal antibodies or isotype-matched control mAb for 30 minutes at room temperature in the dark. Samples were then washed once in 2 ml permeabilization solution before flow cytometric analysis.

Histopathology and immunohistochemistry

The kidneys were dissected through the longitudinal axis, and some were cut into smaller pieces. They were fixed with 10% neutral-buffered formalin, and then embedded in paraffin. Portions of renal cortex were snap frozen in liquid nitrogen, and stored at -80°C for subsequent immunofluorescence analysis.

For histopathological examination, 4-μm-thick paraffin sections were stained with periodic acid-Schiff (PAS). To detect the expression of VEGF, TGF-β and FN, paraffin sections were first incubated with normal serum matched to the species for secondary antibodies. After incubation with the primary antibodies, sections were incubated with HRP conjugated goat anti-rabbit IgG for 30 min at room temperature. After washing in PBS, the reaction products were visualized after incubation with DAB.

Immunofluorescence staining

Immunostaining on frozen sections was performed using the following primary and secondary antibodies for fluorescence microscopy: the sections were immunostained with anti-mouse complement C3a (Boster, China), then the sections were incubated with FITC-conjugated anti-mouse immunoglobulin (Ig) G1 antibody for detection.

Statistical analysis

Results were shown as mean ± SEM and analyzed using one-way ANOVA with statistical analysis software (SPSS11.0). The value of $p < 0.05$ was considered statistically significant.

Results

MSC inhibits T lymphocyte proliferation in a dose-dependent fashion

The effects of MSC on lymphocytes tested by MTT assay were shown in Figure 1. The results showed that incubation of lymphocytes with different concentrations of MSC all decreased lymphocyte viability, in comparison with non-MSC-treated controls. Cell viability of lymphocytes incubation for 24 h after induction at the ratio of 1:10 was significantly decreased ($p < 0.05$), and those of lower concentration (1:50, 1:100) groups also decreased.

MSC transplantation prolongs the survival of MRL/lpr mice

MSC treatment substantially prolonged the survival of MRL/lpr mice. All experimental mice survived up to 32 weeks upon *in vivo* transfer of MSC whereas two mice from the control group died at the age of 32 weeks. The body

Table 1. Changes of body weight of MRL/lpr mice after treatment (g)

Groups	16 week	20 week	24 week	28 week	32 week
Control	29.4 ± 3.6	31.8 ± 3.2	33.1 ± 3.6	33.6 ± 3.3	32.8 ± 3.0
CTX	29.1 ± 2.8	28.2 ± 3.6	32.9 ± 2.9	33.7 ± 3.0	34.2 ± 2.9
MSC	30.5 ± 3.1	32.1 ± 3.2	37.0 ± 3.5	38.1 ± 3.3	38.6 ± 3.3*
CTX + MSC	28.7 ± 2.5	31.8 ± 2.8	34.4 ± 2.6	36.5 ± 3.2	35.8 ± 3.7*

*$p < 0.05$ *vs* control.

Table 2. Changes of serum antibody level after treatment

	Control	CTX	MSC	MSC + CTX
ds-DNA(ln^{-1})	4.6 ± 0.9	3.4 ± 1.3	3.1 ± 1.1	2.7 ± 0.8*
ANA(ln^{-1})	7.7 ± 1.5	7.1 ± 1.4	6.5 ± 1.3	6.9 ± 0.6

*$p < 0.05$ *vs* control.

weight of the CTX group was decreased after the injection of CTX in the first 4 weeks, reaching the lowest level at 20 weeks after the injection. At 32 weeks, the body weight of MSC group (38.6 ± 3.3 g) and MSC + CTX (35.8 ± 3.7 g) group increased significantly as compared with that of the control group (32.8 ± 3.0 g, $p < 0.05$). However, there was no significant difference among the CTX group, MSC group and MSC + CTX group (Table 1).

MSC transplantation reduces serum levels of anti-ds-DNA antibodies in MRL/lpr mice

The serum level of anti-ds-DNA antibodies of the MSC + CTX group (2.7 ± 0.8) was decreased when compared with that of the control group (4.6 ± 0.9, $p < 0.05$). However, there was no significant difference in serum levels of ANA between the treatment and control groups (Table 2).

MSC transplantation reduces 24-h proteinuria in MRL/lpr mice

The proteinuria of the control group was elevated in mice at 20 weeks. The proteinuria of the treated groups was also elevated, but at a much slower rate. Compared with controls, the proteinuria of the CTX group decreased significantly at 24 weeks. However, there was no significant difference between the CTX and control mice at the age of 32 weeks.

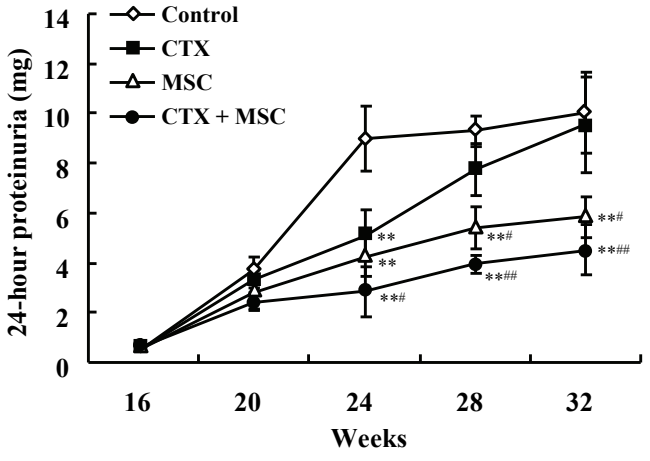

Figure 2. MSC transplantation reduces the 24-h proteinuria of MRL/lpr mice. The urine was collected by metabolic cage and the 24-hour proteinuria was detected with Coomassi brilliant blue method every two weeks. **$p < 0.01$ *vs* control, #$p < 0.05$ *vs* CTX, ##$p < 0.01$ *vs* CTX.

Table 3. Changes of sub-group of T cells after treatment (%)

	IFN-γ	IL-4	$CD4^+$	$CD8^+$
Control	12.2 ± 2.2	64.2 ± 2.8	82.7 ± 2.9	17.3 ± 2.9
CTX	15.4 ± 2.3	62.7 ± 1.5	79.3 ± 2.8	20.7 ± 2.8
MSC	17.9 ± 1.1*	58.4 ± 1.8*	76.4 ± 1.9*	23.6 ± 1.9*
CTX + MSC	18.3 ± 2.1*	57.6 ± 3.2*	73.9 ± 3.2*	26.1 ± 3.3*

*$p < 0.05$ *vs* control.

The proteinuria in the MSC and MSC + CTX groups decreased significantly when compared with the control group at the age of 24 weeks ($p < 0.05$). Moreover, the proteinuria of the MSC and MSC + CTX groups remained continuously decreased when compared with CTX group at 28 weeks onward ($p < 0.05$). Interestingly, there was no significant difference between MSC and MSC + CTX groups (Figure 2).

Effect of MSC transplantation on the levels of serum creatinine in MRL/lpr mice

The serum creatinine decreased significantly in MSC (13.5 ± 2.2) and MSC + CTX (12.7 ± 4.2) groups when compared with the control group (20.8 ± 5.1, $p < 0.05$). However, there was no significant difference in serum creatinine between the CTX group and control group.

Effect of MSC transplantation on the sub-group of T cells

The frequencies of $CD4^+$ T cells significantly decreased in MSC and MSC + CTX group as compared with control group and CTX group. However, the percentage of $CD8^+$ T cells was increased in treatment groups. The IL-4 significantly decreased and IFN-γ significantly increased in MSC and MSC + CTX group (Table 3).

MSC transplantation ameliorates renal pathology of MRL/lpr mice

Histopathologic examination showed that glomerular sclerosis, mesangial cell proliferation, increased matrix and lymphocyte infiltration into tubulointerstitial space were evident in the control group. However, the degree of glomerular sclerosis and interstitial fibrosis was markedly decreased in MSC and MSC + CTX groups (Figure 3). Moreover, immunofluorescent staining revealed markedly reduced deposition of complement C3 in MSC-treated group as compared with the control and CTX groups (Figure 4).

MSC transplantation modulates gene expression in renal tissue of MRL/lpr mice

The VEGF was detected in the glomerular epithelial cells and showed weak positive in the cytoplasm and brush-border of proximal tubules in kidneys of control mice by immuno-staining.

The expression of VEGF in glomeruli was decreased in the MSC-treated group (Figure 5). Meanwhile, the MSC-treated mice exhibited significant reduced TGF-β1 expression

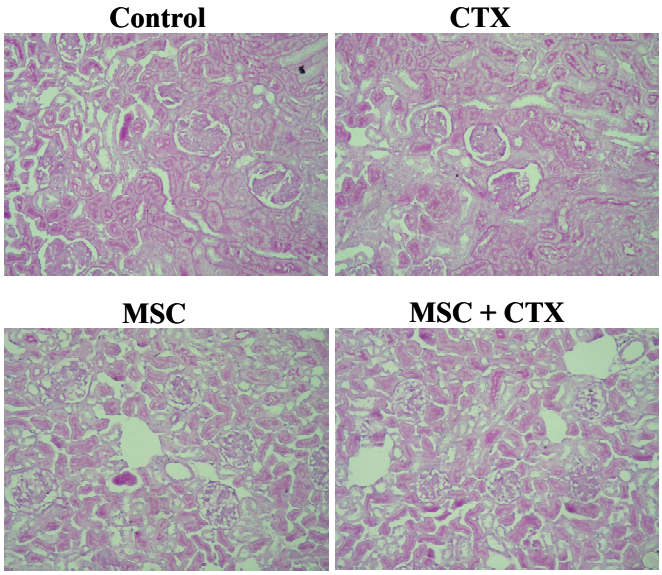

Figure 3. MSC transplantation ameliorates renal pathology of MRL/lpr mice. MRL/lpr mice were killed at the age of 32 weeks after treatment with CTX, MSC, or MSC + CTX. The kidneys were dissected through the longitudinal axis and fixed with 10% neutral-buffered formalin, and then embedded in paraffin. The sections were stained with PAS (original magnification: × 200).

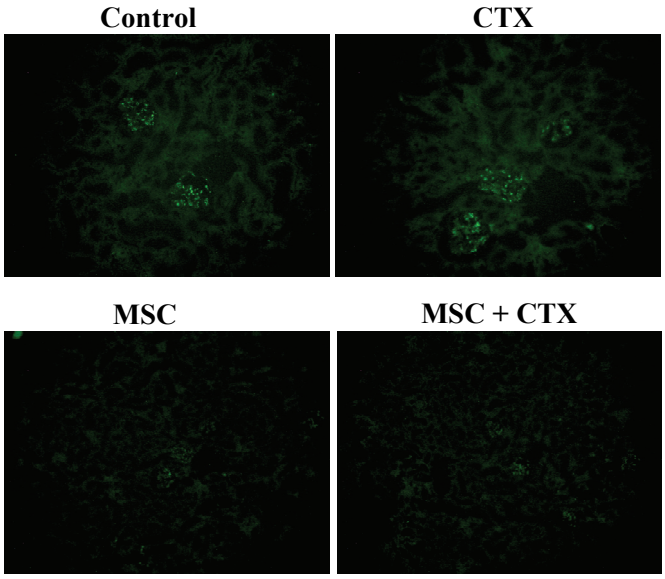

Figure 4. Deposition of C3 in the kidney of MRL/lpr mice. Mice were treated with CTX, MSC, or MSC + CTX to detect complement C3 by immunofluorescent staining of kidney tissue sections (original magnification: × 100).

in glomeruli, whereas the control group and CTX- treated group showed more TGF-β1 expression in glomeruli (Figure 5). The deposition of FN was increased in glomeruli of control and CTX group. MSC treatment greatly attenuated FN deposition in glomeruli (Figure 5).

Discussion

In the present study, human MSC were isolated by density gradient centrifugation and adherence methods. Consistent with our previous studies, the cultured cells can be expanded *in vitro* for many passages. These MSC express CD29, CD44 and CD105, but do not express hematopoietic and endothelial markers such as CD34, CD45, CD14 and HLA-DR (4).

Our results showed that human MSC inhibited T lymphocyte proliferation in a dose-dependent manner. MSC transplantation was effective in inhibiting autoimmune pathogenesis in MRL/lpr mice. Upon treatment with MSC, the 24-hour proteinuria, serum creatinine, anti-ds-DNA antibodies and $CD4^+$ T cells were decreased in MRL/lpr mice. Th2 cell subpopulation was also found to be down-regulated. The body weight decreased to the lowest level at 4 weeks after the treatment in CTX group. The body weight of MSC + CTX group did not decrease. At 24 weeks, the 24-hour proteinuria significantly decreased in CTX group, MSC group and CTX+MSC group compared with control group. However, the values in CTX + MSC group decreased more significantly. After 28 weeks, the 24-hour proteinuria was significantly decreased in MSC group and CTX + MSC group compared with that of CTX group. These findings indicated that single MSC injection was effective and kept the therapeutic effect much longer than CTX group.

After MSC transplantation, the $CD4^+$ T cells and the ratio of CD4/CD8 significantly decreased in MSC and MSC + CTX group than those in control and CTX groups. However, there was no significant difference between MSC and MSC + CTX groups. The mutation of lpr gene in MRL/lpr mice leads to the defective function of Fas molecule, resulting in apoptosis dysfunction. The $CD4^+$ T cells play a critical role in the pathogenesis of MRL/lpr mice. Lpr gene promotes the proliferation of $CD4^+$ cells, which participate in the development of auto-antibodies, vasculitis, arthritis and nephritis. The antibodies against CD4 can notably reduce the glomerular nephritis and renal vasculitis as well as decrease the levels of ANA, anti-ds-DNA and total IgG (8). Several studies have shown that MSC could inhibit the proliferation of T, B lymphocytes in particular, by inhibiting the differentiation of B cells to antibody-secreting plasma cells. MSC seem to be capable of reducing the ratio of CD4/CD8 and the production of auto-antibodies through the inhibition of T and B lymphocytes, which subsequently improve the renal function of MRL/lpr mice. After the transplantation of human MSC, the reduced levels of anti-ds-DNA antibodies were observed. The levels of the ANA showed no significant difference between the control and treated groups, which may be related with the small sample.

Some studies indicated that the MSC exerted their function by repairing the damaged renal tissue *via* the secretion of cytokines such as IL-10, β-fibroblast growth factor and TGF-α (9). IFN-γ and IL-4 were the typical

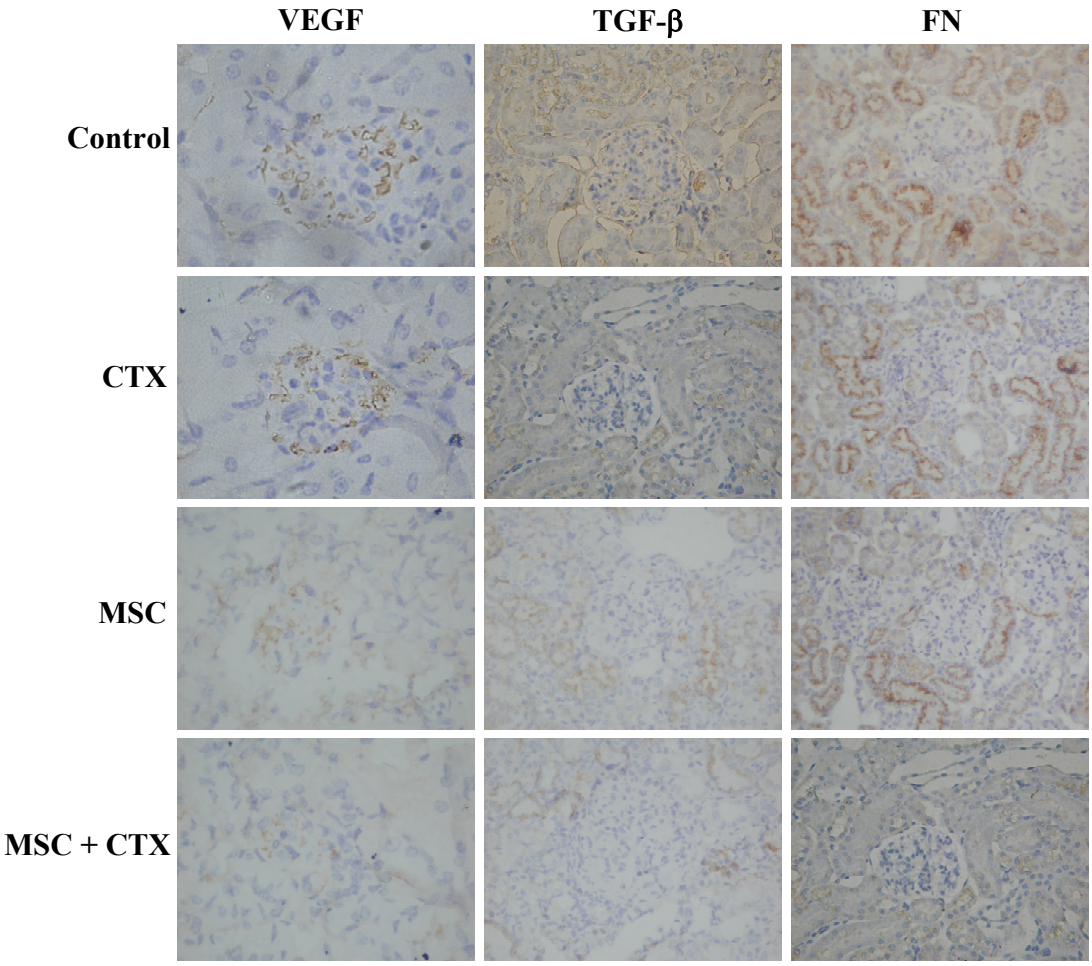

Figure 5. MSC transplantation modulates gene expression in renal tissue of MRL/lpr mice. Expressions of VEGF (left panel), TGF-β (middle panel), and FN (right panel) in the kidney of MRL/lpr mice untreated or treated with CTX, MSC, or MSC + CTX were detected by immunohistochemistry (original magnification: × 400).

cytokines representing Th1 and Th2 cytokines, respectively. We found the Th2 subpopulation decreased and Th1 subpopulation increased after MSC transplantation. Th2 cells are mainly involved in the development of humoral immunity. The regulation of Th1/Th2 balance and inhibition of autoantibody production by human MSC transplantation might be the underlying mechanisms for the improvement of renal tissue damage. Several studies have reported that the increased level of IFN-γ might induce tissue damage in MRL/lpr mice. Prud'homme et al. (10) found the mRNA expressions of IL-6, IL-10, IFN-γ in lymph nodes were increased in MRL/lpr mice. The glomerular nephritis was improved in the IFN-γ gene knockout MRL/lpr mice. These results supported that the Th1/Th2 balance was deviated to Th1 polarization. On the other hand, the disease course was not improved by using the monoclonal antibody of IFN-γ. Findings by Schwarting (11) confirmed that the elevated IFN-γ could inhibit the proliferation of macrophages through a negative feedback mechanism. The renal inflammation alleviated. These results indicated that IFN-γ modulated disease development in the early stage and had protective effect on the advanced stage of the disease progression. There is also compelling evidence that the balance of Th1/Th2 is deviated to Th2. Jabs (12) found severe Th2 cells infiltration in the lachrymal gland of MRL/lpr mice. Shimizu (13) found the secretion of IFN-γ significantly decreased and IL-4 decreased in WSX-1 gene knockout mice, resulting in glomerular nephritis. The serum levels of IgG1 and IgE significantly decreased, whereas the severity of lymphadenopathy and nephritis was significantly reduced. The antagonist of IL-4 can decrease the titer of anti-DNA antibody with improved nephritis. These data demonstrated that both Th1 and Th2 were involved in the autoimmune pathogenesis in MRL/lpr mice. There is no conclusion whether the Th1 or Th2 was preponderance in the pathogenesis of MRL/lpr mice. In the present study, Th2 decreased after MSC transplantation. Our finding supported that Th2 might play a preponderant role in the pathogenesis of MRL/lpr mice.

The immunohistochemical studies showed that the expression of VEGF, TGF-β and FN in glomerular decreased,

and the renal pathology was ameliorated after the MSC transplantation. It is well established that TGF-β, a major fibrogenic cytokine driving tissue fibrosis, could stimulate the growth of fibroblasts and induced formation of extracellular matrix (ECM) by increasing expression of ECM proteins and decreasing proteolytic activity (14). TGF-β up-regulates plasminogen activator inhibitor type 1 (PAI-1), which lowers laminin (LM) and FN degradation (15). FN is an important component of ECM, which is located in the glomerular mesangium and glomerular basement membranes. In the early stage of glomerular inflammation, FN is increased with the cell and matrix proliferation, and promotes macrophages to phagocytose and clears the foreign substance, or attracts macrophages/monocytes to localized infiltrating. At the middle and later stages, FN and other ECM increased constantly with cell proliferation, which was the pathological base of glomerulosclerosis. The significantly increased synthesis of FN represents the excessive accumulation of ECM. Locally accumulated FN and collagen promote the proliferation of fibroblasts, mesangial cells, renal tubular epithelial cells, and, consequently, the development of renal fibrosis. Our study suggested that MSC transplantation decreased the expression of FN, inhibited the proliferation of ECM, and reduced renal fibrosis.

VEGF is one of the key cytokines in the development of renal glomeruli through regulating angiogenesis. The overexpression of VEGF may act as a compensatory response to vascular endothelial injury, decrease of capillary and glomcrulus remodeling (16). The accumulation of VEGF can mediate the secondary vascular endothelial proliferation, repair capillary following injury, alter the function of glomerular capillary endothelium, and increase the permeability of microvascular (17). VEGF also can promote the proliferation of intraglomerular mesangial cell and enhance the synthesis and secretion of ECM such as hyaluronic acid and collagen. In conclusion, VEGF is involved in the development of renal fibrosis by regulating the mesangial matrix synthesis and degradation. VEGF can increase the permeability of glomerular capillary endothelium *in vitro*, decrease the negative ion number of glomerular basement membrane. Thus VEGF involved in the generation of proteinuric through the regulation of endothelial permeability (18). Therefore, MSC transplantation can effectively delay and reduce the development of glomerular sclerosis by regulating the synthesis and degeneration of ECM, reducing proteinuria by modulating microvascular permeability.

Our current findings have demonstrated that human MSC transplantation proves to be an effective treatment for MRL/lpr mice. The regulation of Th1/Th2 balance and inhibition of autoantibody production by B cells might be the underlying mechanisms for the effective treatment with MSC transplantation. The improvement of glomerulosclerosis and amelioration of renal histological injury by MSC probably are likely due to the suppressive effect of MSC on the expression of local TGF-β, FN, and VEGF. Thus, this novel approach may provide a new therapeutic possibility for SLE patients.

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