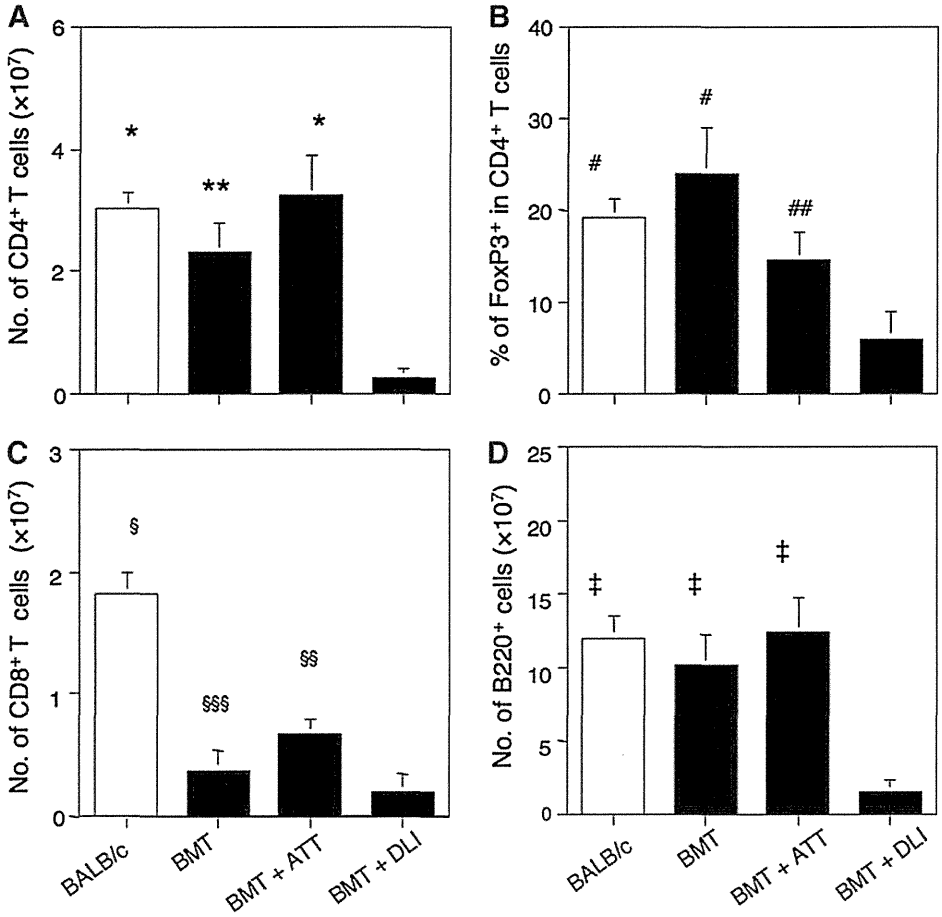


FIG. 3. Numbers of lymphocytes in the spleen from leukemia-bearing mice treated with BMT+ATT. Numbers of $CD4^+$ T cells **(A)**, percentage of $FoxP3^+$ cells in $CD4^+$ T cells **(B)**, numbers of $CD8^+$ T cells **(C)**, and $B220^+$ B cells **(D)** in the spleen were evaluated in normal BALB/c mice, leukemia-bearing mice treated with BMT alone, BMT+ATT, or BMT+DLI. The experiments were performed 5 weeks after BMT. $^*P<0.05$ compared with BMT alone and BMT+DLI. $^{**}P<0.01$ compared with BMT+DLI. $^{\#}P<0.05$ compared with BMT+ATT and BMT+DLI. $^{\#\#}P<0.05$ compared with BMT+DLI. $^{\S}P<0.01$ compared with BMT, BMT+ATT, and BMT+DLI. $^{\S\S}P<0.05$ compared with BMT and BMT+DLI. $^{\S\S\S}P<0.05$ compared with BMT+DLI. $^{\ddagger}P<0.01$ compared with BMT+DLI. Data are shown as means±SD. Normal BALB/c ($n=5$), BMT ($n=5$), BMT+ATT ($n=5$), and BMT+DLI ($n=5$).

than those treated with BMT alone, whereas they showed a higher percentage of regulatory T cells than BMT+DLI. Since regulatory T cells suppressed GVHD, preserving GVT and GVL [21,22] the intermediate percentage of these cells also played an important role in the mechanism underlying our results. The elevation of regulatory T-cell numbers compared with BMT alone suggested that some regulatory T cells in BMT+ATT may be produced from the TT [13].

The percentages of EM T cells were highest in the mice treated with BMT+DLI, followed by those treated with BMT+ATT, and lowest in those treated with BMT alone. In contrast, CM and naïve T cells were lowest in the BMT+DLI group, followed by the BMT+ATT group, and highest in those treated with BMT alone. Since freshly isolated CM and naïve T cells in DLI induce GVHD and GVL [23,24], the observation of the lowest numbers of these cells in BMT+DLI suggested that these cells in DLI had differentiated into EM T cells with activation and/or were consumed [25].

The mice treated with BMT+ATT showed sufficient mitogen responses to both T and B cells, whereas those treated with BMT alone showed low T-cell response, and those treated with BMT+DLI showed low responses to both T and B cells. Such immunodeficiency in BMT+DLI may be induced by GVHD [20]. These findings indicated that BMT+TT is the best method compared with BMT alone or BMT+DLI. Although the mechanism underlying the long-term survival in the BMT+DLI group is unclear, the SPF condition may have prevented severe infection. Alternatively, the IBM–BMT method itself may suppress lethal GVHD by DLI [6].

Analysis of cytokines indicated significantly elevated IL-2 production in the mice treated with BMT+ATT compared with those treated with BMT alone or with BMT+DLI, whereas IFN-γ production was significantly higher in those treated with BMT+DLI. Although several cytokines play a role in GVHD, IL-2 is effective in inducing strong GVL while avoiding GVHD [26]. In addition, the highest degree of elevation of IFN-γ production may contribute to GVHD with lymphoid hypoplasia (immunodeficiency) in BMT+DLI [27]. Therefore, the cytokine patterns may also be at least partly associated with the pathogenesis.

We thus found that donor-derived T cells play an important role in the treatment of leukemia. However, further gene analyses are needed for a more comprehensive understanding. For example, cytokine profiles at the transcriptional level should be analyzed by using DNA microarry in T cells [28]. In addition, differences in T-cell clones may become evident between GVL and GVH, or between host and donor-thymus derived by next-generation sequencing and/or other methods [29]. These findings would help detail the mechanism of BMT+TT.

We examined means of treating several intractable diseases and/or serious complications by BMT+TT [8–14]. In most studies, BMT+TT showed better results than BMT alone or BMT+DLI. The results of the current study were also compatible with these previous findings. The thymus is

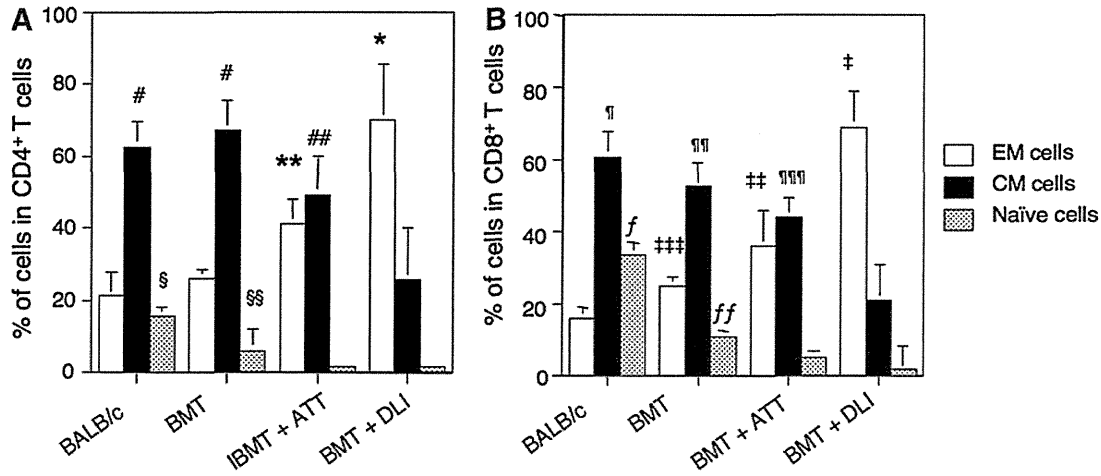


FIG. 4. Proportions of EM, CM, and naïve T cells from leukemia-bearing mice treated with BMT+ATT. Percentages of $CD62L^-CD44^+$ EM, $CD62L^+CD44^+$ CM, and $CD62L^-CD44^-$ naïve cells in $CD4^+$ **(A)** and $CD8^+$ **(B)** T cell subsets were analyzed in the spleens from normal BALB/c mice and leukemia-bearing mice treated with BMT alone, BMT+ATT, and BMT+DLI. Analyses were performed at the same time as for the experiment in Fig. 3. $^*P<0.01$ compared with BALB/c, BMT, and BMT+ATT. $^{**}P<0.01$ compared with BALB/c and BMT. $^{\#}P<0.05$ compared with BMT+ATT and BMT+DLI. $^{\#\#}P<0.01$ compared with BMT+DLI. $^{\S}P<0.01$ compared with BMT, BMT+ATT, and BMT+DLI. $^{\S\S}P<0.01$ compared with BMT+ATT and BMT+DLI. $^{\ddagger}P<0.01$ compared with BALB/c, BMT, and BMT+ATT. $^{\ddagger\ddagger}P<0.01$ compared with BALB/c and BMT. $^{\ddagger\ddagger\ddagger}P<0.01$ compared with BALB/c. $^{\P}P<0.05$ compared with BMT, BMT+ATT, and BMT+DLI. $^{\P\P}P<0.05$ compared with BMT+ATT and BMT+DLI. $^{\P\P\P}P<0.01$ compared with BMT+DLI. $^{f}P<0.01$ compared with BMT, BMT+ATT, and BMT+DLI. $^{ff}P<0.05$ compared with BMT+ATT and BMT+DLI. Data are shown as means±SD. Normal BALB/c ($n=5$), BMT ($n=5$), BMT+ATT ($n=5$), and BMT+DLI ($n=5$). CM, central memory; EM, effector memory.

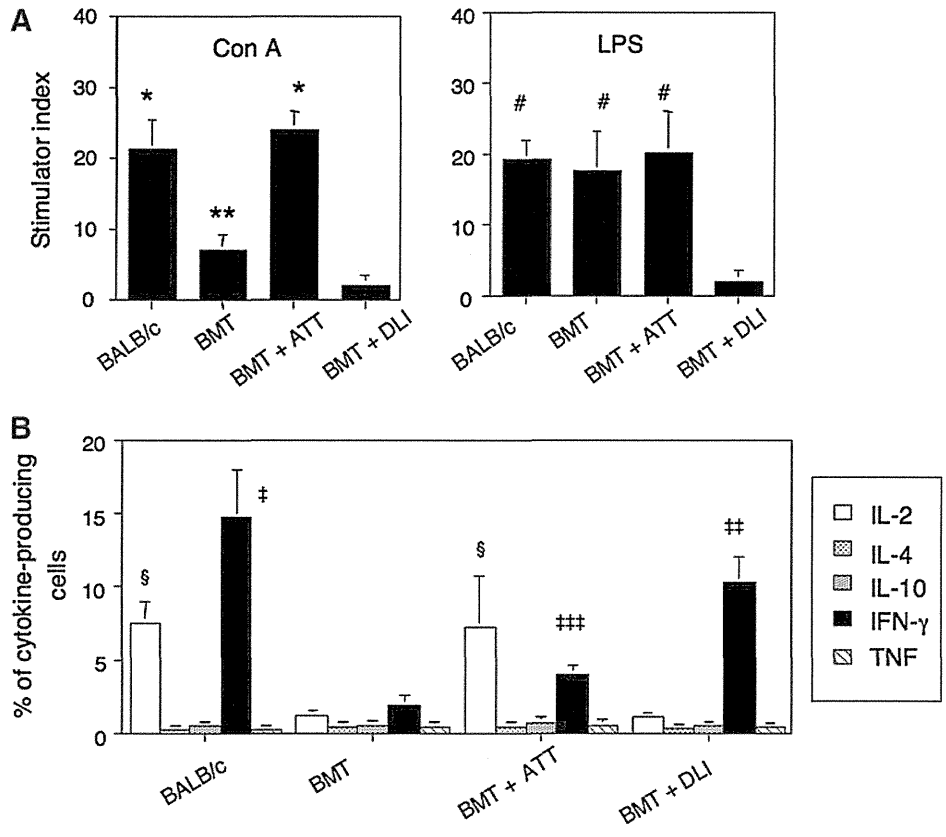


FIG. 5. Mitogen responses and percentages of cytokine-producing cells in the spleens from leukemia-bearing mice treated with BMT and ATT Mitogen responses: Con A and LPS **(A)** and percentages of cytokine-producing cells **(B)** in the spleen were evaluated in the spleens from normal BALB/c mice and leukemia-bearing mice treated with BMT alone, BMT+ATT, and BMT+DLI. Analyses were performed at the same time as for the experiment in Fig. 3. $^*P<0.05$ compared with BMT and BMT+DLI. $^{**}P<0.05$ compared with BMT+DLI. $^{\#}P<0.05$ compared with BMT+DLI. $^{\S}P<0.05$ compared with BMT and BMT+DLI. $^{\ddagger}P<0.05$ compared with BMT, BMT+ATT, and BMT+DLI. $^{\ddagger\ddagger}P<0.05$ compared with BMT and BMT+ATT. $^{\ddagger\ddagger\ddagger}P<0.05$ compared with BMT. Data are shown as means±SD. Normal BALB/c ($n=4$), BMT ($n=4$), BMT+ATT ($n=4$), and BMT+DLI ($n=4$). LPS, lipopolysaccharide.

an organ involved in the maintenance of homeostasis itself and regulates the production of not only T cells but also several cytokines and hormones in a feedback mechanism [30]. Thus, TT is different from DLI, which supplies mature lymphocytes in one direction. Hence, TT is a type of functional organ transplantation, and may represent an approach that significantly regulates the immune function of T cells in vivo for the benefit of the host.

Overall, we found that allogeneic IBM–BMT + ATT induces strong GVL effects with mild GVHD. Although it may be both ethically and technically difficult to obtain adequate thymus tissue in clinical cases (including the question of donor age), grafts could be obtained from patients with congenital heart diseases or from aborted fetuses, as previously utilized [31,32]. We have recently found that even if the thymus donor is different from the BMC donor, then the effect is comparable to that seen with transplantation from the same donor using triple chimeric mice [33]. In addition, a method of regenerating the thymus has also been developed [34–37]. Therefore, IBM–BMT + ATT could become a viable strategy for the treatment of malignant hematological tumors in humans.

## Acknowledgments

This work was supported by a grant from Haiteku Research Center of the Ministry of Education; a grant from the Millennium Program of the Ministry of Education, Culture, Sports, Science, and Technology; a grant from the Science Frontier Program of the Ministry of Education, Culture, Sports, Science, and Technology; a grant from The 21st Century Center of Excellence (COE) Program of the Ministry of Education, Culture, Sports, Science, and Technology; a Research Grant B from Kansai Medical University, Health and Labor Sciences (Research on Human Genome, Tissue Engineering Food Biotechnology); a grant from the Department of Transplantation for Regeneration Therapy (sponsored by Otsuka Pharmaceutical Co., Ltd.); a grant from the Molecular Medical Science Institute (Otsuka Pharmaceutical Co., Ltd.); and a grant from Japan Immunoresearch Laboratories Co., Ltd. (JIMRO). The project was supported by Guangdong Natural Science Foundation. The authors wish to thank Ms. Y. Tokuyama and Ms. A. Kitajima for their technical assistance, and Mr. Hilary Eastwick-Field and Ms. K. Ando for their help in the preparation of this article.

## Author Disclosure Statement

The authors disclose no commercial association that might create a conflict in connection with the submitted article. No competing financial interests exist.

## References

1. Chao NJ. (1997). Graft-versus-host disease. The view point from the donor T cell. Biol Blood Marrow Transplant 3:1–10.
2. Watson JG. (1983). Problems of infection after bone marrow transplantation. J Clin Pathol 36:683–692.
3. Russell NH, JL Byrne, RD Faulkner, M Gilyead, EP Das-Gupta and AP Haynes. (2005). Donor lymphocyte infusions can result in sustained remissions in patients with residual or relapsed lymphoid malignancy following allogeneic haemopoietic stem cell transplantation. Bone Marrow Transplant 36:437–441.
4. Deol A and LG Lum. (2010). Role of donor lymphocyte infusions in relapsed hematological malignancies after stem cell transplantation revisited. Cancer Treat Rev 36:528–538
5. Kushida T, M Inaba, H Hisha, N Ichioka, T Esumi, R Ogawa, H Iida and S Ikehara. (2001). Intra-bone marrow injection of allogeneic bone marrow cells: a powerful new strategy for treatment of intractable autoimmune diseases in MRL/lpr mice. Blood 97:3292–3299.
6. Ikehara S. (2008). A novel method of bone marrow transplantation (BMT) for intractable autoimmune diseases. J Autoimmun 30:108–113.
7. Nakamura K, M Inaba, K Sugiura, T Yoshimura, AH Kwon, Y Kamiyama and S Ikehara. (2004). Enhancement of allogeneic hematopoietic stem cell engraftment and prevention of graft-versus-host diseases (GvHD) by intra-bone marrow-bone marrow transplantation plus donor lymphocyte infusion. Stem Cells 22:125–134.
8. Hosaka N, M Nose, M Kyogoku, N Nagata, S Miyashima, RA Good and S Ikehara. (1996). Thymus transplantation, a critical factor for correction of autoimmune disease in aging MRL/+mice. Proc Natl Acad Sci U S A 93:8558–8562.
9. Hosaka N, T Ryu, T Miyake, W Cui, T Nishida, T Takaki, M Inaba and S Ikehara. (2007). Treatment of autoimmune diseases in MRL/lpr mice by allogeneic bone marrow transplantation plus adult thymus transplantation. Clin Exp Immunol 147:555–663.
10. Ryu T, N Hosaka, T Miyake, W Cui, T Nishida, T Takaki, M Li, K Kawamoto and S Ikehara. (2008). Transplantation of newborn thymus plus hematopoietic stem cells can rescue supralethal irradiated mice. Bone Marrow Transplant 41:659–666.
11. Li M, NG Abraham, L Vanella, Y Zhang, M Inaba, N Hosaka, S Hoshino, M Shi, YM Ambrosini, ME Gershwin and S Ikehara. (2010). Successful modulation of mice with intrabone marrow—bone marrow transplantation plus concurrent thymic transplantation. J Autoimmun 35:414–423.
12. Nishida T, N Hosaka, T Takaki, T Miyake, W Cui, M Inaba, H Kinoshita, T Matsuda and S Ikehara. (2009). Allogeneic intra-BM-BMT plus adult thymus transplantation from same donor has benefits for long-term survival even after sublethal irradiation or low-dose BM cell injection. Bone Marrow Transplant 43:829–837.
13. Miyake T, N Hosaka, W Cui, T Nishida, T Takaki, M Inaba, Y Kamiyama and S Ikehara. (2009). Adult thymus transplantation with allogeneic intra-bone marrow-bone marrow transplantation from same donor induces high thymopoiesis, mild graft-versus-host reaction and strong graft-versus-tumour effects. Immunology 126:552–564.
14. Hosaka N, W Cui, Y Zhang, T Takaki, M Inaba and S Ikehara. (2010). Prolonged survival in mice with advanced tumors treated with syngeneic or allogeneic intra-bone marrow-bone marrow transplantation plus fetal thymus transplantation. Cancer Immunol Immunother 59:1121–1130.
15. Sakaguchi S, M Ono, R Setoguchi, R Setoguchi, H Yagi, S Hori, Z Fehervari, J Shimizu, T Takahashi and T Nomura. (2006). Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease. Immunol Rev 212:8–27.
16. Hoffmann P, J Ermann, M Edinger, CG Fathman and S Strober. (2002). Donor-type CD4(+)CD25(+) regulatory T cells suppress lethal acute graft-versus-host disease after allogeneic bone marrow transplantation. J Exp Med 196:389–399.

17. Sallusto F, D Lenig, R Förster, M Lipp and A Lanzavecchia. (1999). Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 401:708–712.
18. Blander JM, DB Sant'Angelo, D Metz, SW Kim, RA Flavell, K Bottomly and CA Janeway Jr. (2003). A pool of central memory-like CD4 T cells contains effector memory precursors. J Immunol 17:2940–2948.
19. Wingard JR, S Piantadosi, GB Vogelsang, ER Farmer, DA Jabs, LS Levin, WE Beschorner, RA Cahill, DF Miller and D Harrison. (1989). Predictors of death from chronic graft-versus-host disease after bone marrow transplantation. Blood 74:1428–1435.
20. Graze PR and RP Gale. (1979). Chronic graft versus host disease: a syndrome of disordered immunity. Am J Med 66:611–620.
21. Edinger M, P Hoffmann, J Ermann, K Drago, CG Fathman, S Strober and RS Negrin. (2003). CD4+CD25+ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. Nat Med 9:1144–1150.
22. Trenado A, F Charlotte, S Fisson, M Yagello, D Klatzmann, BL Salomon and JL Cohen. (2003). Recipient-type specific CD4+CD25+ regulatory T cells favor immune reconstitution and control graft-versus-host disease while maintaining graft-versus-leukemia. J Clin Invest 112:1688–1696.
23. Dutt S, J Baker, HE Kohrt, N Kambham, M Sanyal, RS Negrin and S Strober. (2011). CD8+ CD44hi but not CD4+ CD44hi memory T cells mediate potent graft anti-lymphoma activity without GVHD. Blood 117:3230–3239.
24. Zheng H, C Matte-Martone, D Jain, J McNiff and WD Shlomchik. (2009). Central memory CD8+ T cells induce graft-versus-host disease and mediate graft-versus-leukemia. J Immunol 182:5938–5948.
25. Zhang Y, G Joe, E Hexner, J Zhu and SG Emerson. (2005). Alloreactive memory T cells are responsible for the persistence of graft-versus-host disease. J Immunol 174:3051–3058.
26. Weiss L, I Lubin, I Factorowich, Z Lapidot, S Reich, Y Reisner and S Slavin. (1994). Effective graft-versus-leukemia effects independent of graft-versus-host disease after T cell-depleted allogeneic bone marrow transplantation in a murine model of B cell leukemia/lymphoma. Role of cell therapy and recombinant IL-2. J Immunol 153:2562–2567.
27. Klimpel GR, CR Annable, MG Cleveland, TR Jerrells and JC Patterson. (1990). Immunosuppression and lymphoid hypoplasia associated with chronic graft versus host disease is dependent upon IFN-gamma production. J Immunol 144:84–93.
28. Tosolini M, A Kirilovsky, B Mlecnik, T Fredriksen, S Mauger, G Bindea, A Berger, P Bruneval, WH Fridman, F Pagès and J Galon. (2011). Clinical impact of different classes of infiltrating T cytotoxic and helper cells (Th1, th2, treg, th17) in patients with colorectal cancer. Cancer Res 71:1263–1271.
29. Baum PD, JJ Young and JM McCune. (2011). Measurement of absolute T cell receptor rearrangement diversity. J Immunol Methods 368:45–53.
30. Mehr R, AS Perelson, M Fridkis-Hareli and A Globerson. (1997). Regulatory feedback pathways in the thymus. Immunol Today 18:581–585.
31. Markert ML, A Boeck, LP Hale, AL Kloster and TM McLaughlin. (1999). Transplantation of thymus tissue in complete DiGeorge syndrome. N Engl J Med 341:1180–1189.
32. Markert ML, CB Hicks, JA Bartlett and JL Harmon. (2000). Hale Effect of highly active antiretroviral therapy and thymic transplantation on immunoreconstitution in HIV infection. AIDS Res Hum Retroviruses 16:403–413.
33. Cui W, N Hosaka, T Miyake, X Wang, K Guo, Y Cui, Q Li, C Song, W Feng, et al. (2008). Analysis of tolerance induction using triple chimeric mice: MHC-disparate thymus, hemopoietic cells and microenvironment. Transplantation 85:1151–1158.
34. Zhang L, L Sun and Y Zhao. (2007). Thymic epithelial progenitor cells and thymus regeneration: an update. Cell Res 17:50–55.
35. Lai L and J Jin. (2009). Generation of thymic epithelial cell progenitors by mouse embryonic stem cells. Stem Cells 27:3012–3020.
36. Takaki T, N Hosaka, T Miyake, W Cui, T Nishida, M Inaba and S Ikehara. (2008). Presence of donor-derived thymic epithelial cells in [B6—>MRL/lpr] mice after allogeneic intra-bone marrow-bone marrow transplantation (IBM-BMT). J Autoimmun 31:408–415.
37. Rossi SW, WE Jenkinson, G Anderson and EJ Jenkinson. (2006) Clonal analysis reveals a common progenitor for thymic cortical and medullary epithelium. Nature 441:988–991.

Address correspondence to:
*Dr. Susumu Ikehara*
*Department of Stem Cell Disorders*
*Kansai Medical University*
*10-15 Fumizono-cho*
*Moriguchi*
*Osaka 570-8506*
*Japan*

*E-mail:* ikehara@takii.kmu.ac.jp

Received for publication July 7, 2011
Accepted after revision August 19, 2011
Prepublished on Liebert Instant Online August 23, 2011

IVYSPRING INTERNATIONAL PUBLISHER

*International Journal of Biological Sciences*

2012; 8(10):1335-1344. doi: 10.7150/ijbs.4654

Research Paper

# Stem Cell Transplantation Increases Antioxidant Effects in Diabetic Mice

**Ming Li [1], Luca Vanella [2], Yuming Zhang [3], Ming Shi [1], Takashi Takaki[4], Joseph I Shapiro[2], Susumu Ikehara[1]✉**

1. Department of Stem Cell Disorders, Kansai Medical University, Moriguchi City, Osaka, Japan.
2. School of Medicine, University of Marshall, 1600 Medical Center Drive, Huntington, WV 25701, USA.
3. Department of Pediatrics, Nanfang Hospital, Guangzhou, China.
4. JEOL Ltd., Tokyo, Japan.

✉ Corresponding author: Susumu Ikehara, M.D., Ph.D., Department of Stem Cell Disorders, Kansai Medical University, Moriguchi City, Osaka 570-8506, Japan. Telephone: 81-6-6993-9625; Fax: 81-6-6994-9627; e-mail: ikehara@takii.kmu.ac.jp.



Received: 2012.05.26; Accepted: 2012.09.13; Published: 2012.10.27

## Abstract

Intra bone marrow-bone marrow transplantation (IBM- BMT) + thymus transplantation (TT) has been shown to reduce the incidence of graft versus host disease (GVHD) and restore donor-derived T cell function. In addition, an increase in insulin sensitivity occurred in db/db mice after IBM-BMT+TT treatment. Heme oxygenase (HO)-1 is a stress inducible enzyme which exert antioxidant, antiapoptotic, and immune-modulating properties. We examined whether IBM-BMT+TT could modulate the expression of HO-1 in the kidneys of db/db mice. Six-week-old db/db mice with blood glucose levels higher than 250 mg/dl were treated with IBM-BMT+TT. Six weeks later, the db/db mice showed decreased body weight, blood glucose levels and insulin, and increased plasma adiponectin levels. The upregulation of HO-1 was associated with significantly ($p<0.05$) increased levels of peNOS and pAKT, but decreased levels of iNOS in the kidneys of db/db mice. Plasma creatinine levels also decreased ($p<0.05$), and the expression of type IV collagen was improved. Thus IBM-BMT+TT unregulated the expression of HO-1, peNOS and pAKT, while decreasing iNOS levels in the kidney of db/db mice. This was associated with an improvement in renal function.

Key words: IBM-BMT+TT, antioxidant, HO-1, diabetic nephropathy.

## Introduction

Heme oxygenase (HO)-1 is a stress inducible enzyme that catalyzes the degradation of heme proteins into free iron, CO and biliverdin, which is then rapidly converted into bilirubin. These catabolic end products exert antioxidant, antiapoptotic, and immune-modulating properties, rendering the overall function of HO-1 to be cytoprotective [1, 2]. Graft versus host disease (GVHD) is a major side effect of allogenic bone marrow transplantation (BMT) [3]. Intra bone marrow- BMT (IBM-BMT) appears to offer the best approach for allogenic BMT, as manifested by a reduced incidence of GVHD and the restoration of donor-derived T cell function [4-6]. We recently reported that, in db/db mice treated with IBM-BMT+ thymus (TT) transplantation, CD4/CD8 ratios were normalized with a consequent increase in plasma adiponectin levels and insulin sensitivity. In addition, the expression of pancreatic phosphorylated AK transforming factor (pAKT), phosphorylated liver kinase B1 (pLKB1), phosphorylated adenosine monophosphate-activated protein kinase (pAMPK) and HO-1 increased in thus-treated db/db mice [7].

The db/db mouse presents with renal abnormalities including proteinuria, glomerular hypertrophy, and glomerulosclerosis [8, 9], thereby making it an ideal animal model in which to study renal and

vascular dysfunction in diabetic nephropathy (DN). DN is the major cause of end-stage renal failure, mainly due to the increased incidence of type 2 diabetes mellitus [10]. Oxidative stress (OS) is considered a significant pathogenic factor in the development of diabetic vascular complications, including nephropathy [11, 12]. The beneficial effect of increased HO-1 expression and HO activity further suggests that the abnormality in endothelial progenitor cells is due to a mesenchymal stem cell (MSC) disorder exacerbated by OS and decreased levels of adiponectin [13].

In the present study, we examined whether allogenic IBM-BMT+TT modulated the expression of HO-1 in the kidneys of db/db mice and show that this intervention does indeed upregulate the expression of HO-1, phosphorylated endothelial nitric oxide synthase (peNOS) and pAKT, decrease inducible NOS (iNOS), and improve renal function, therefore providing a mechanistic basis for the therapeutic use of IBM-BMT+TT.

## Materials and methods

### Animals

Six-, 12- and 30-wk-old BKS.Cg-m+Lepr$^{db}$/+Lepr$^{db}$/J (H-2K$^{d}$) (db/db) mice and age-matched lean mice were purchased from Charles River Laboratories (Yokohama, Japan) and maintained in animal facilities under specific pathogen-free conditions. All procedures were performed under protocols approved by the Institutional Animal Care and Use Committee at Kansai Medical University. Body weight and blood glucose levels were measured each week. Blood glucose levels higher than 250 mg/dl on two consecutive measurements were considered to indicate the onset of diabetes. Each experiment was repeated three times.

### IBM-BMT+TT

Six-wk-old db/db mice with blood glucose levels greater than 250 mg/dl and proteinuria greater than ++ on two consecutive measurements received fractionated irradiation twice a day (5.0 Gy×2, 4-hour interval). One day after irradiation, whole BMCs from B6 mice were injected into the recipient mice (1×10$^{7}$/mouse) by IBM-BMT using our previously-described method [7]. Simultaneously, the thymus from newborn B6 mice was grafted under the renal capsule of the left kidney of the recipient mice. All treated mice were sacrificed 6 wks after IBM-BMT+TT treatment.

### Immunochemistry and measurement of nitric oxide (NO) levels

The pancreata, livers, kidneys and bones of lean and db mice were removed at each time point. After the tissues were fixed in 10% formalin for 24 hours at room temperature, they were embedded in paraffin. The sections (3-μm thick) were stained with hematoxylin and eosin. The pancreata were stained with polyclonal guinea pig anti-swine insulin antibody (N1542, Dako Cytomation, CA). The kidneys were stained with collagen type IV (Sigma-Aldrich, MO), pAKT, iNOS and eNOS (Abcam PLC, Cambridge, UK) antibodies. The stained sections were examined under a microscope.

NO levels in the kidneys were measured according to the manufacturer's protocol using a Nitric Oxide Assay Kit (BioAssay Systems, Hayward, CA).

### Electron microscopy

The fixed renal cortices were embedded in epoxy resin using conventional methods [14]. Kidney tissue was fixed in 2.5% glutaraldehyde in 0.1M PBS, pH 7.4. Samples were washed and post fixed with 2% aqueous $OsO_4$. The samples were then dehydrated with serially-increasing concentrations of ethanol (60 to 100%), and were infiltrated with epon mixed with methyl nadic anhydride (TAAB Laboratories, UK), Dodecenyl succinic anhydride (TAAB Laboratories), and Quetol-812 and DMP-30 (Nishin Company, Japan). Ultrathin sections were prepared, stained using uranyl acetate and lead citrate, and then examined with a JEM-1400A electron microscope (JEOL, Tokyo, Japan). The measurements were taken from electron micrographs with magnifications of x2,500 and x12,000.

### Adiponectin, insulin, creatinine and pancreatic insulin content measurements

Adiponectin was determined in mouse plasma using an ELISA assay (R&D Systems, Inc. MN and Invitrogen Corporation CA). Insulin was measured using an ELISA kit (Morinaga, Yokohama, Japan).

Plasma creatinine was measured according to the manufacturer's protocols using a plasma creatinine detection kit (Arbor Assays, Michigan).

For insulin content measurement, the pancreas was isolated and weighed, and then placed in 3ml cold acid ethanol (0.18M HCl in 70% ethanol) and homogenated. This was kept overnight at 4°C and centrifuged at 2400rpm for 30min at 4°C. The supernatant was transferred and stored at -20°C for measurement using ELISA.

### Western blot analysis of kidney HO-1, peNOS, AKT, pAKT, and iNOS

At sacrifice, kidneys were dissected, then pooled for each mouse in order to measure signaling molecules. Specimens were stored at -140°C until assayed. Frozen kidney tissues were pulverized under liquid nitrogen and placed in a homogenization buffer (mmol/l: 10 phosphate buffer, 250 sucrose, 1 EDTA, 0.1 PMSF and 0.1% v/v tergitol, pH 7.5). Homogenates were centrifuged at 27,000 g for 10 min at 4°C, and the supernatant was then isolated and protein levels were obtained by immunoblotting with the relevant antibodies. Antibodies against HO-1, AKT, phosphorylated AKT (pAKT), eNOS, peNOS and iNOS were obtained from Cell Signaling Technology, Inc. (Beverly, MA). Antibodies were prepared by dilution as described previously [15, 16].

### Flow cytometric analyses

Peripheral blood mononuclear cells were obtained from the tail vein of mice 30 days after transplantation. These cells were stained with antibodies against PE-H-2K$^{d}$, PE-CD4, PE-CD8a and FITC-H-2K$^{b}$ (BD Bioscience Pharmingen, San Diego, CA) for 30 min on ice. After washing twice with 2% FCS / PBS and lysing red blood cells, the 10000 events acquired were analyzed by FACScan (BD Bioscience). Isotype-matched immunoglobulins were used as controls.

### Statistical analysis

Statistical significance between experimental groups was determined by the Fisher method of analysis of multiple comparisons. For comparison between treatment groups, the null hypothesis was tested by either a single-factor ANOVA for multiple groups or unpaired t test for two groups. Statistical significance was regarded as significant at $p<0.05$.

## Results

### Body weight, thymus weight, and morphology of pancreas and bone, and insulin content of pancreas islet

We first confirmed the parameters (body weight, thymus weight and morphology of pancreas and bone marrow) of the db/db mice and lean mice (as control) maintained in our animal facilities (Fig.1), since food and environment may affect some parameters of db/db mice. As seen in Fig. 1A, the body weight of 12-wk-old lean mice was significantly higher than that of 6-wk-old lean mice (30.4 ±0.5 vs 22.6±0.1 g, $p< 0.001$). Similar results occurred in the db/db mice (53.2 ±1.7 vs 33.5±0.03 g, $p< 0.001$). However, although the body weight of 30-wk-old db/db mice was significantly higher than that of 12-wk-old db/db mice (61.2±0.6 vs 53.2± 1.7 g, $p< 0.01$), there was no significant difference in body weight between 30-wk-old and 12-wk-old lean mice (32.4±0.5 vs 30.4± 0.5 g). As shown in Fig. 1B, thymus weight was significantly lower in the 6-wk-, 12-wk and 30-wk-old db/db mice than in the age-matched lean mice (51.7 ±4.7 vs 75.7±3.3, 21.3 ±0.9 vs 49±4.0, 20.5 ±1.2 vs 43±4.1mg, $p < 0.05$ at each time point).

On HE staining, 12-wk- and 30-wk-old db/db mice (Fig. 1E and F) exhibited larger islets than 6 wk-old db/db mice and lean mice (Fig. 1C and D). More adipocytes were present in the bone marrow of 12-wk- and 30-wk-old db/db mice (Fig. 1H-J) than in lean mice (Fig. 1G).

The db/db mouse shows insulin resistance and insulin-positive cell failure. The insulin content (brown color) was much lower in the larger islets of 6-wk-, 12-wk- and 30-wk-old db/db mice (Fig. 1L-N) than in those of lean mice (Fig. 1K), suggesting that more insulin was secreted into the peripheral blood in the db/db mice. Pancreatic insulin contents of lean mice (2336±84.5 pg/ml per mg pancreas), and 6-wk- (1149±37.5 pg/ml per pancreas), 12-wk- (521±21.7 pg/ml per mg pancreas), and 30-wk- (180±21.3 pg/ml per mg pancreas) old db/db mice were measured by ELISA.

### Plasma creatinine levels and histology of the kidneys of db/db mice

As shown in Fig. 1O, plasma creatinine levels were significantly higher in 12-wk-old when compared to 6-wk-old db/db mice (3.41±0.20 vs 1.47±0.46 mg/dl, $p<0.05$), and in 30-wk-old compared to 12-wk-old db/db mice (4.65±0.03 vs 3.41±0.20 mg/dl, $p<0.05$). Electron microscopic images of the kidneys are shown in Fig. 1P-S. 12-wk and 30-wk db/db mice exhibited severe podocyte foot fusion. Electron dense deposits and increased numbers of mesangium cells were observed in the 30-wk-old db/db mice (Fig. 1R and S).

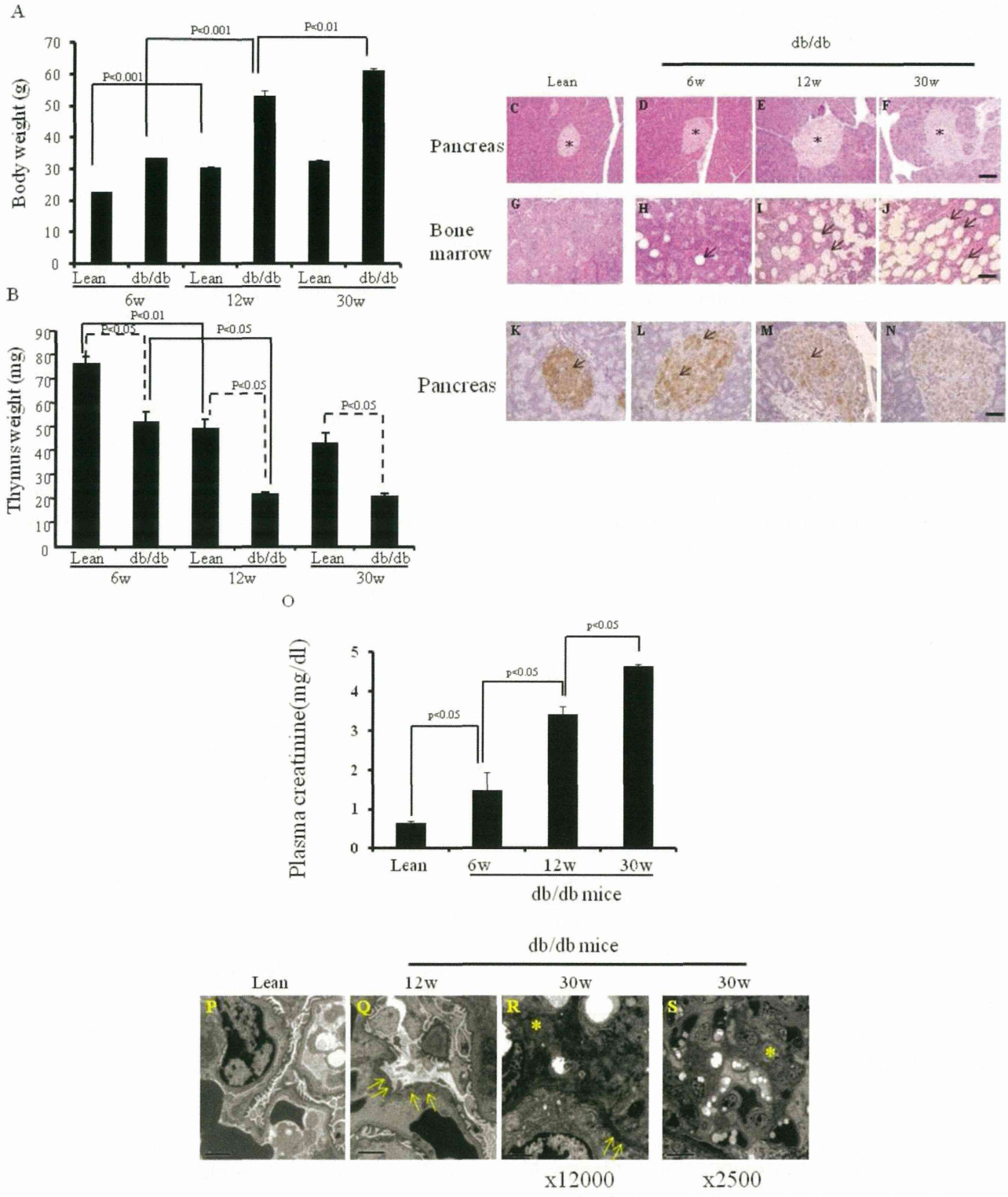


**Figure 1. Body weight, thymus weight and the morphology of pancreas and bone marrow, and expression of insulin on the pancreata, and plasma creatinine levels and images of electron microcopy.** (A) Body weights are shown (*p< 0.01). (B) Thymus weights are shown (*p<0.05). (C-J) HE staining of pancreas and bone marrow of the lean and db/db mice at each age. The islets are shown (* in Fig.C-F). The adipocytes are shown in db/db mice (arrows in Fig. H-J). (K-N) Immunochemistry staining for insulin (arrows) was performed. Scale bar =50μm in Fig. C-J, Scale bar =25μm in Fig. K-N. (O) Plasma creatinine levels of lean, (p< 0.05). (P-S) EM of kidneys. Podocyte foot fusion is shown in Fig. 3Q and R (arrows). Electron dense deposits and increased mesangium cells (*) are shown in Fig. 1R and S. Scale bar =2μm in Fig. P-R, Scale bar =10μm in Fig. S. The results are mean±SE, n=6 in each group.

### Improved body weight, blood glucose and plasma adiponectin, and insulin levels 6 weeks after treatment with IBM-BMT +TT

As seen in Fig.2A, body weight was significantly ($p<0.01$) lower in the IBM-BMT+TT treated db/db mice than in the age-matched non-treated db/db mice. In addition, fasting blood glucose levels (Fig. 2B) were significantly ($p<0.01$) lower in the treated db/db mice than in the non-treated db/db mice. Indeed IBM-BMT+TT reduced blood glucose to the levels found in age-matched lean mice. As shown in Fig. 2C, the non-treated db/db mice exhibited a significantly ($p<0.05$) lower level of plasma adiponectin than age-matched lean mice. However, the plasma adiponectin levels were significantly ($p<0.05$) increased in the treated db/db mice when compared to the non-treated db/db mice (Fig. 2C). Plasma insulin levels were higher ($p<0.05$) in the non-treated db/db mice than in the age matched lean mice. IBM-BMT+TT treatment resulted in a decrease ($p<0.05$) in plasma insulin levels to the levels found in age-matched lean animals (Fig. 2D).

### Lymphocyte subpopulations in peripheral blood and cell number of thymus 6 weeks after treatment with IBM-BMT +TT

The relative percentage of the total cell number in the thymus was significantly ($p<0.05$) lower in the non-treated db/db mice than in the lean mice (65.13% of that of lean mice). The total cell number of the thymus was significantly higher ($p<0.05$) increased in the treated mice (97.9% of lean mice) (Fig. 3A). Figure 3B shows the percentages of donor-derived cells ($H\text{-}2K^b$) in the peripheral blood of the treated db/db mice. The percentages of donor-derived $CD4^+$ and $CD8^+$ were 10.16% and 7.81% respectively, Figure 3C and D.

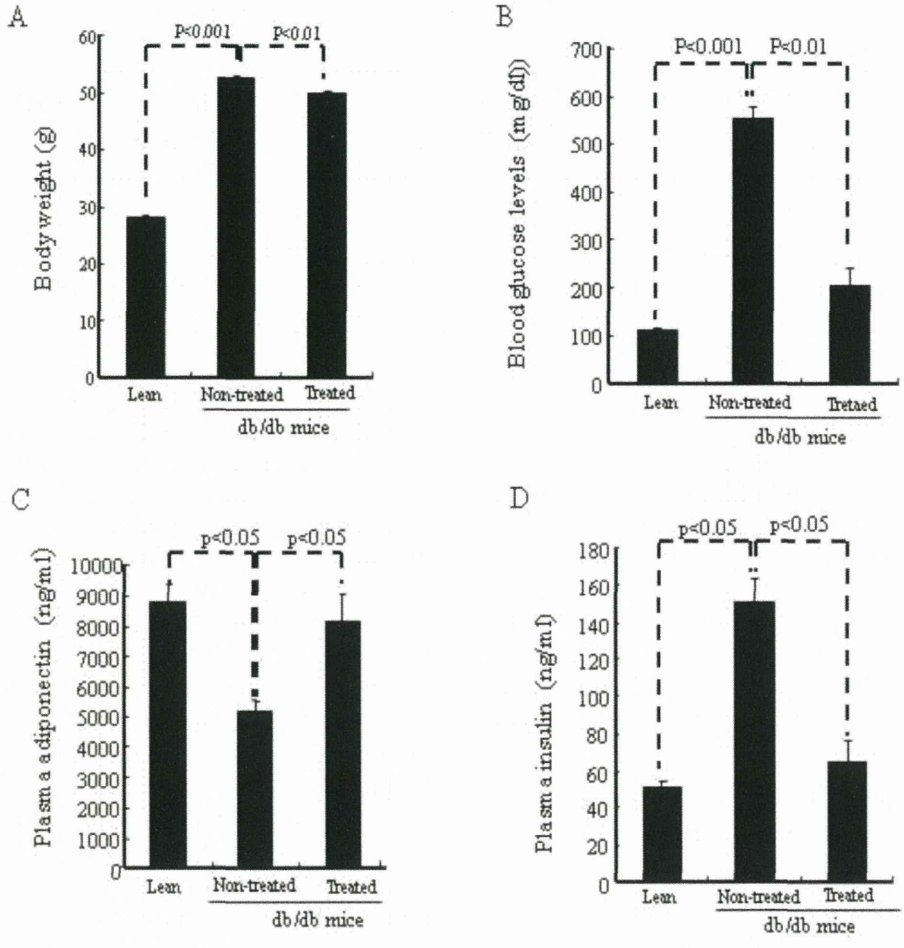


**Figure 2. Body weight, blood glucose levels, plasma adiponectin, insulin.** (A) Body weights are shown. (B) Blood glucose levels are shown. (C) Plasma adiponectin. (D) Plasma insulin.

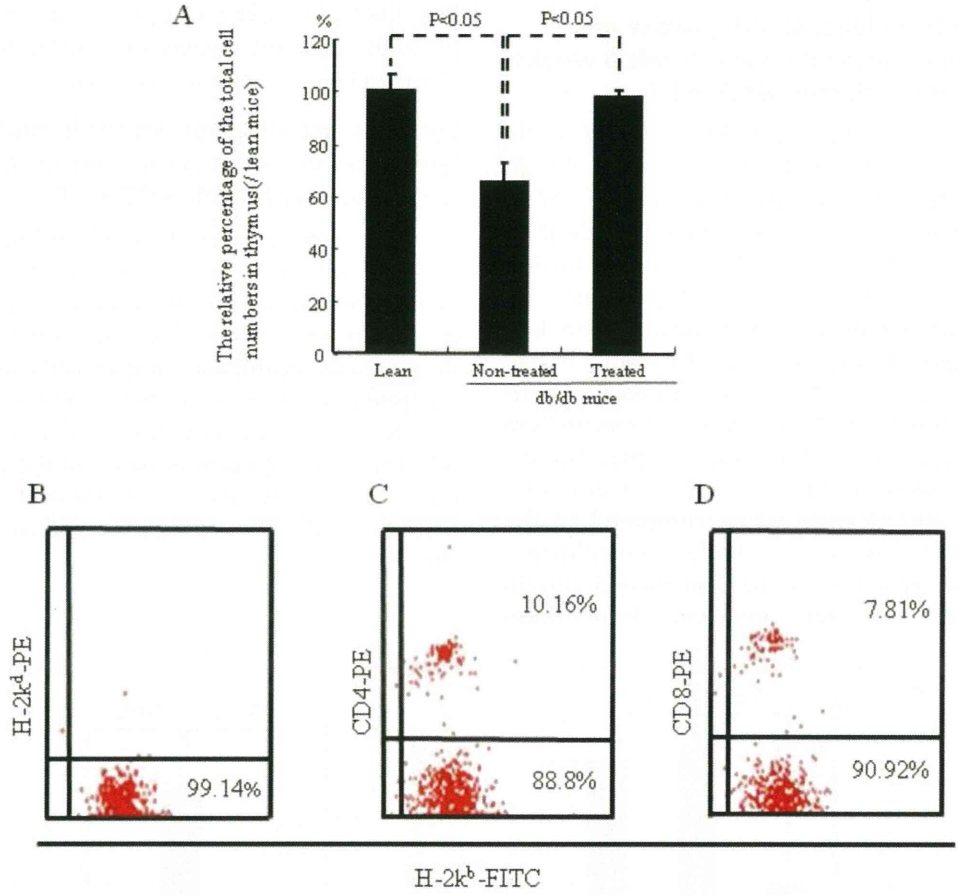


**Figure 3. Cell number of thymus and lymphocyte subpopulations in peripheral blood.** (A) The relative percentage of total cell numbers in the thymus, which was significantly increased in the db/db mice treated with IBM-BMT+TT. (B-D) Donor-derived cells in peripheral blood, $CD4^+$ cells and $CD8^+$ cells are shown. The results are mean±SE, n=6 in each group.

## Lymphocyte function

Approximately 98% of hematolymphoid cells were of donor-origin in the peripheral blood of the recipients one month after IBM-BMT+TT treatment. Spleen cells of the recipient animals demonstrated mitogen responses to lipopolysaccharide, in contrast to those of non-treated db/db mice: 26296±2780.93 vs 19292±1310.42, respectively, $p<0.05$. In lean mice the mitogen response was 31406±2780.93. These findings suggest that IBM-BMT+TT treatment restores lymphocyte function in the db/db mice.

## Expression of HO-1, iNOS, peNOS and pAKT in the kidney of db/db mice treated with IBM-BMT+TT

The expression of renal HO-1 was significantly lower ($p<0.05$) in the non-treated db/db mice when compared to lean mice, but significantly increased ($p<0.05$) in db/db mice treated with IBM-BMT+TT, when compared with non-treated db/db mice (Fig. 4A and B). The expression of iNOS was the receptacle of HO-1 expression and was significantly ($p<0.05$) higher in the non-treated db/db mice than in the lean mice. Densitometry analyses revealed a significant decrease in iNOS ($p<0.05$) in the kidney of treated db/db mice when compared with non-treated db/db mice (Fig. 4A and C).

The expression of pAKT and peNOS in the kidney was significantly lower ($p<0.05$) in the non-treated db/db mice when compared to the lean mice, but there was a significant increase in the expression of both pAKT and peNOS in the IBM-BMT+TT treated db/db mice, when compared with non-treated db/db mice (Fig. 4D-G, $p<0.05$). The expression of type IV collagen (brown color) was greater in the kidney of the non-treated db/db mice (Fig. 4I) than in the lean mice (Fig. 4H). However, it was significantly lower in the kidney (arrows in Fig. 4J) in the treated db/db mice than in the non-treated db/db mice.

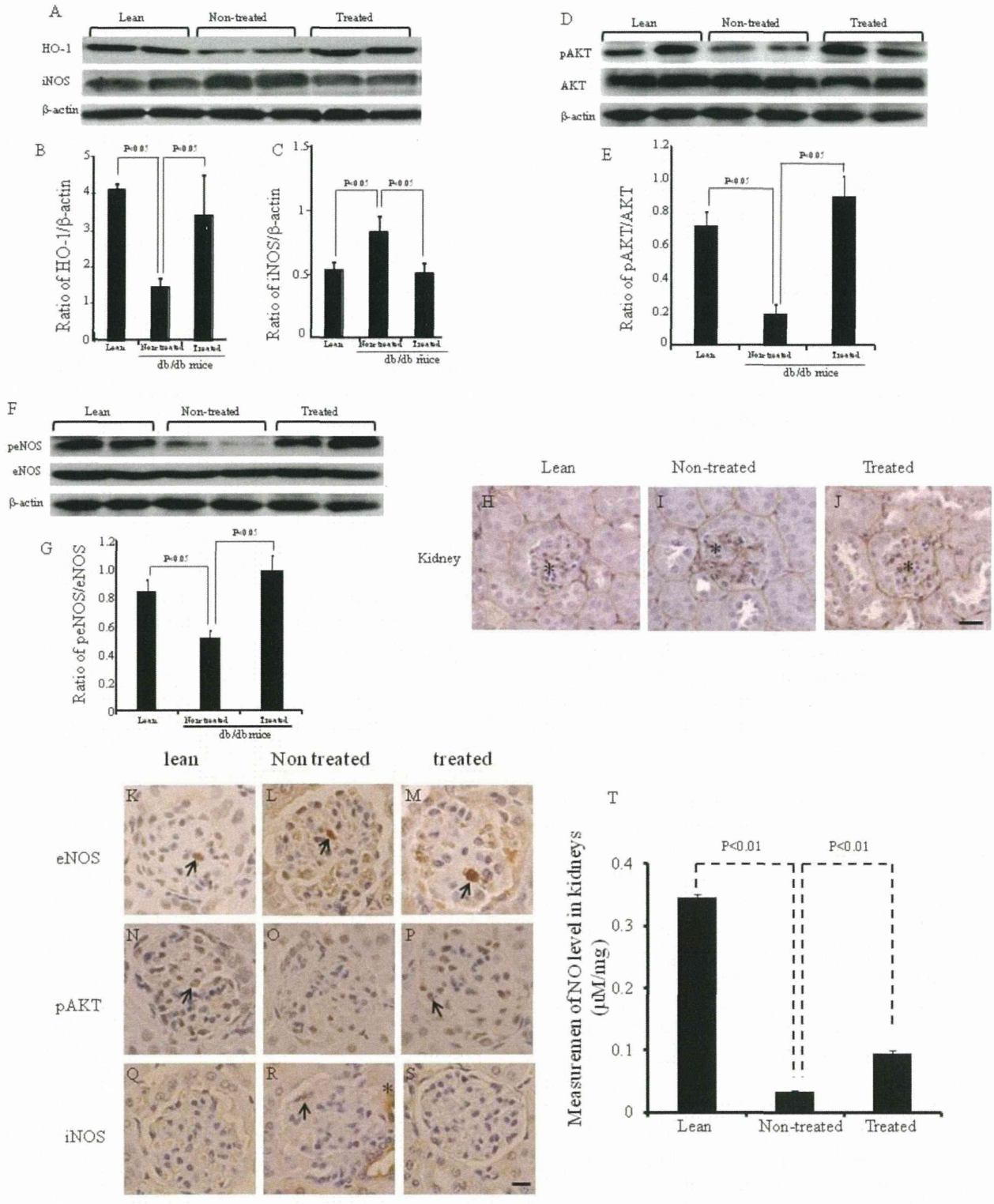


**Figure 4. Expression of HO-1, iNOS AKT, pAKT, eNOS and peNOS in the kidney, and expression of type IV collagen in the kidneys.** (A-C) Western blot and densitometry analysis of HO-1, iNOS and actin proteins in kidneys of lean, non-treated db/db mice and treated db/db mice. (D and E) Western blot and densitometry analyses of AKT and pAKT and actin proteins in kidney of lean, non-treated db/db mice and treated db/db mice are shown. (F and G) Western blot and densitometry analyses of eNOS and peNOS and actin proteins in kidney of lean, non-treated db/db mice and treated db/db mice are shown. Representative immunoblots are shown. There were significant differences between non-treated and treated groups ($p<0.05$). (H-J) Immunochemistry staining for type IV collagen (*). (K-S) Immunochemistry staining for eNOS, pAKT and iNOS. Scale bar =25μm. (T) NO levels in the kidney.

The expression of peNOS, pAKT and iNOS was confirmed by immunochemistry staining (Fig. 4K-T). eNOS positive cells were identified in the kidneys of lean, non-treated and treated db/db mice ( arrow in Fig. 4K-M). pAKT positive cells were identified in the kidney of lean and treated db/db mice (arrow in Fig. 4N and P). However, none were found in the non-treated db/db mice. iNOS positive cells were identified in the kidney of non-treated db/db mice (arrow and *in Fig. 4R).

As shown in Fig. 4T, NO levels in the kidney were significantly lower in non-treated db/db mice when compared to lean mice (0.35±0.004 vs 0.03±0.002 μM/mg, $p<0.01$). However, the NO levels were significantly higher in the kidney (0.09±0.005 vs 0.03±0.002 μM/mg, $p<0.01$) in the treated db/db mice than in the non-treated db/db mice.

## Discussion

Leptin receptor-deficient db/db mice exhibit severe hereditary obesity [17] and display hormonal imbalances and hematolymphoid defects [18, 19]. db/db mice exhibit a marked reduction in the size and cellularity of the thymus [20, 21]. Thus we firstly confirmed the body weight and thymus weight of db/db and lean mice at each age that were maintained in our animal facilities. Meantime, the morphology of the pancreas, kidney and bone marrow was also confirmed (Figure 1- 3). There were significant differences in body weight and thymus weight between 6-wk-and 12-wk-old mice. The pancreas, kidney and bone marrow showed differences in morphology at each time point. Previous studies have shown that IBM-BMT+TT improved insulin sensitivity in db/db mice 7 wks after treatment [7]. However, there are no reports indicating whether IBM-BMT+TT increased HO-1 expression, or benefited the kidney in db/db mice, although a protective effect of BMT on renal pathology has been reported [22].

HO-1 is the inducible form of HO (HO-2 is the constitute form) and is increased in response to OS. Increased levels of HO-1 slow the rate of weight gain and decrease levels of TNF-α and IL-6, but increase serum levels of adiponectin in obese diabetic mice [15]. db/db mice treated with IBM-BMT+TT exhibited a larger decrease in body weight and plasma insulin levels than age-matched non-treated db/db mice. Insulin administration to treated db/db mice produced a rapid decrease in blood glucose levels, suggesting improved insulin sensitivity after IBM-BMT+TT treatment. In the present study, HO-1 expression increased in the kidney after IBM-BMT+TT and was accompanied by an enhanced expression of peNOS and pAKT. A reduction in the production of nitric oxide [23] and a decrease in the expression of eNOS are reportedly associated with advanced DN [24]. An increase in AKT signaling is considered an important metabolic response to OS through the attenuation of ROS-mediated endothelial dysfunction [25]. The up regulation of renal HO-1 expression with a resultant increase in signaling molecules, including adiponectin via the pAKT-pAMPK-peNOS pathway, prevents the development of metabolic syndrome and improves both vascular and renal function [26].

HO has been shown to be important for attenuating the overall production of reactive oxygen species (ROS) through its ability to degrade heme and to produce carbon monoxide (CO), biliverdin/bilirubin, and the release of free iron, which possess potent antioxidant properties and antiapoptotic effects [26]. Recently, the induction of HO-1 has been found to reduce diabetic induced-glomerular injury and apoptosis, and these effects are associated with decreased NF-κB-induced inflammation and oxidative stress [27]. MSCs have the capacity to repair renal injury, accelerate tubular proliferation and improve renal function, and upregulate HO-1 expression and increase HO activity, all are essential for MSC growth and differentiation to the osteoblast lineage, which is consistent with the role of HO-1 in hematopoietic stem cell differentiation [28]. Induction of HO-1 by cobalt-protoporphyrin IX in recipient mice before conditioning and bone marrow transplantation (BMT) results in a reduction of GVHD and improved survival [29]. One report has indicated that HO-1 in dendritic cells may function as an inhibitor of the alloimmune response mediated by CD4+ T cells. HO-1 could thus play a key role in the design of therapies to prolong allograft function, based on the potent alloimmunity modulating capacity of this enzyme [30].

Plasma adiponectin levels are also significantly higher in treated db/db mice than in non-treated db/db mice. Adiponectin is a key regulator of albuminuria, suppressing not only i) OS and albuminuria but also ii) high levels of adiponectin phosphorylase and iii) active AMPK, presumably via adipoR1, which prevents OS and the fusion of the podocyte foot processes [31]. There is a report supporting the hypothesis that adiponectin is critical for both endothelial cell survival and function via the activation of eNOS and crosstalk between pAKT and pAMPK [32].

Bone marrow cells have the capacity to differentiate into mesangial cells [33, 34] and to transdifferentiate into podocytes. This is accompanied by the re-expression of the defective collagen chains and improved renal histology and function [35]. MSCs have the capacity to repair renal injury, accelerate tubular proliferation and improve renal function [36,

37]. Nephrons are of mesenchymal origin and stroma cells are of crucial importance for signaling, leading to the differentiation of both nephrons and collecting ducts [38]. Bone marrow-derived mesangial cell progenitors may play a crucial role in the development and progression of extracellular matrix accumulation and mesangial cell proliferation in the db/db mouse. Future studies to clarify extra-cellular matrix accumulation will focus on the donor-derived BM cells in the kidneys of the recipient.

Type IV collagen is a major structural component of all basement membranes, including the glomerular basement membrane of the kidney in vertebrates and invertebrates [39, 40]. Our results show that the expression of type IV collagen in the glomerulus was attenuated after IBM-BMT+TT. BM-derived cells fuse with existing glomerular cells and thereby provide therapeutic benefit or, alternatively, transfer their nuclei to damaged podocytes and thereby enable repair. Bone marrow-derived stem cells repair basement membrane collagen defects and reverse genetic kidney disease [35].

Age-related hematologic changes are associated with a decline in BM cellularity and a decline in adaptive immunity [41, 42]. The thymus involutes steadily with increasing age, resulting in a decreased release of new naïve T cells to the periphery, thereby affecting adaptive immunity [43]. The thymus also undergoes age-related progressive involution with decreased thymic lymphopoiesis, reduced thymic size and disrupted thymic architecture. Our previous studies demonstrated that the thymus is significantly lighter in db/db mice than in age-matched lean mice. When thymi from newborn C57BL6 mice were transplanted into db/db mice, the percentage of double-positive, double-negative and CD4+ cells in the thymus was normalized, as was the CD4/CD8 ratio in the peripheral blood [7], and the total cell number of the thymus, suggesting that IBM-BMT+TT is capable of restoring the immune repertoire and overcoming the autoimmune response, which is considered to be partly responsible for the development of diabetes.

IBM-BMT appears to be the most appropriate strategy for allogenic BMT [4]. Moreover, allogenic IBM-BMT+TT normalizes T cell subsets, cytokine imbalance and insulin sensitivity in db/db mice [7]. Allogenic IBM-BMT+TT upregulates the expression of HO-1 in kidney. This is followed by the upregulation of peNOS and pAKT and a reduction in iNOS levels, resulting in an improvement in renal function. This therapeutic approach offers decided advantages in the treatment of both autoimmune and hematological diseases.

## Acknowledgments

We would like to thank Mr. Hilary Eastwick-Field and Ms. Keiko Ando for their help in the preparation of the manuscript. This study was supported by Otsuka Pharmaceutical Company, Ltd and by NIH grants DK068234, HL55601, HL34300 (NGA). We thank Ms Aiko Kitajima for her excellent technical assistance regarding the morphology studies.

## Conflict of interest

The authors declare no conflict of interest.

## Abbreviations

DN: diabetic nephropathy; GVHD: Graft versus host disease; HO: Heme oxygenase; IBM-BMT: Intra bone marrow-bone marrow transplantation; iNOS: inducible nitric oxide synthase; NO: Nitric oxide; OS: Oxidative stress; pAKT: phosphorylated AK transforming; peNOS: phosphorylated endothelial nitric oxide synthase; pLKB1: phosphorylated liver kinase B1; pAMPK: phosphorylated adenosine monophosphate-activated protein kinase; TT: thymus transplantation.

## Competing Interests

The authors have declared that no competing interest exists.

## References

1. Otterbein LE, Soares MP, Yamashita K, Bach FH. Heme oxygenase-1: unleashing the protective properties of heme. Trends Immunol. 2003; 24: 449-55.
2. Orozco LD, Kapturczak MH, Barajas B, Wang X, Weinstein MM, Wong J, et al. Heme oxygenase-1 expression in macrophages plays a beneficial role in atherosclerosis. Circ Res. 2007; 100: 1703-11.
3. Goulmy E, Schipper R, Pool J, Blokland E, Falkenburg JH, Vossen J, et al. Mismatches of minor histocompatibility antigens between HLA-identical donors and recipients and the development of graft-versus-host disease after bone marrow transplantation. N Engl J Med. 1996; 334: 281-5.
4. Ikehara S. A novel strategy for allogeneic stem cell transplantation: perfusion method plus intra-bone marrow injection of stem cells. Exp Hematol. 2003; 31: 1142-6.
5. Nakamura K, Inaba M, Sugiura K, Yoshimura T, Kwon AH, Kamiyama Y, et al. Enhancement of allogeneic hematopoietic stem cell engraftment and prevention of GVHD by intra-bone marrow bone marrow transplantation plus donor lymphocyte infusion. Stem Cells. 2004; 22: 125-34.
6. Hosaka N, Nose M, Kyogoku M, Nagata N, Miyashima S, Good RA, et al. Thymus transplantation, a critical factor for correction of autoimmune disease in aging MRL/+mice. Proc Natl Acad Sci U S A. 1996; 93: 8558-62.
7. Li M, Abraham NG, Vanella L, Zhang Y, Inaba M, Hosaka N, et al. Successful modulation of type 2 diabetes in db/db mice with intra-bone marrow--bone marrow transplantation plus concurrent thymic transplantation. J Autoimmun. 2010; 35: 414-23.
8. Breyer MD, Bottinger E, Brosius FC, 3rd, Coffman TM, Harris RC, Heilig CW, et al. Mouse models of diabetic nephropathy. J Am Soc Nephrol. 2005; 16: 27-45.
9. Ziyadeh FN, Hoffman BB, Han DC, Iglesias-De La Cruz MC, Hong SW, Isono M, et al. Long-term prevention of renal insufficiency, excess matrix gene expression, and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-beta

antibody in db/db diabetic mice. Proc Natl Acad Sci U S A. 2000; 97: 8015-20.
10. Ritz E, Tarng DC. Renal disease in type 2 diabetes. Nephrol Dial Transplant. 2001; 16 Suppl 5: 11-8.
11. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes. 1991; 40: 405-12.
12. Kakimoto M, Inoguchi T, Sonta T, Yu HY, Imamura M, Etoh T, et al. Accumulation of 8-hydroxy-2'-deoxyguanosine and mitochondrial DNA deletion in kidney of diabetic rats. Diabetes. 2002; 51: 1588-95.
13. Abraham NG, Li M, Vanella L, Peterson SJ, Ikehara S, Asprinio D. Bone marrow stem cell transplant into intra-bone cavity prevents type 2 diabetes: role of heme oxygenase-adiponectin. J Autoimmun. 2008; 30: 128-35.
14. Toki J, Adachi Y, Jin T, Fan T, Takase K, Lian Z, et al. Enhancement of IL-7 following irradiation of fetal thymus. Immunobiology. 2003; 207: 247-58.
15. Li M, Kim DH, Tsenovoy PL, Peterson SJ, Rezzani R, Rodella LF, et al. Treatment of obese diabetic mice with a heme oxygenase inducer reduces visceral and subcutaneous adiposity, increases adiponectin levels, and improves insulin sensitivity and glucose tolerance. Diabetes. 2008; 57: 1526-35.
16. Peterson SJ, Kim DH, Li M, Positano V, Vanella L, Rodella LF, et al. The L-4F mimetic peptide prevents insulin resistance through increased levels of HO-1, pAMPK, and pAKT in obese mice. J Lipid Res. 2009; 50: 1293-304.
17. Chua SC, Jr., Chung WK, Wu-Peng XS, Zhang Y, Liu SM, Tartaglia L, et al. Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. Science. 1996; 271: 994-6.
18. Fantuzzi G, Faggioni R. Leptin in the regulation of immunity, inflammation, and hematopoiesis. J Leukoc Biol. 2000; 68: 437-46.
19. Matarese G, Moschos S, Mantzoros CS. Leptin in immunology. J Immunol. 2005; 174: 3137-42.
20. Fernandes G, Handwerger BS, Yunis EJ, Brown DM. Immune response in the mutant diabetic C57BL/Ks-dt+ mouse. Discrepancies between in vitro and in vivo immunological assays. J Clin Invest. 1978; 61: 243-50.
21. Kimura M, Tanaka S, Isoda F, Sekigawa K, Yamakawa T, Sekihara H. T lymphopenia in obese diabetic (db/db) mice is non-selective and thymus independent. Life Sci. 1998; 62: 1243-50.
22. Flaquer M, Franquesa M, Barquinero J, Lloberas N, Gutierrez C, Torras J, et al. Bone marrow transplantation induces normoglycemia in a type 2 diabetes mellitus murine model. Transplant Proc. 2009; 41: 2282-5.
23. Amiri F, Shaw S, Wang X, Tang J, Waller JL, Eaton DC, et al. Angiotensin II activation of the JAK/STAT pathway in mesangial cells is altered by high glucose. Kidney Int. 2002; 61: 1605-16.
24. Marrero MB, Banes-Berceli AK, Stern DM, Eaton DC. Role of the JAK/STAT signaling pathway in diabetic nephropathy. Am J Physiol Renal Physiol. 2006; 290: F762-8.
25. Schulz E, Dopheide J, Schuhmacher S, Thomas SR, Chen K, Daiber A, et al. Suppression of the JNK pathway by induction of a metabolic stress response prevents vascular injury and dysfunction. Circulation. 2008; 118: 1347-57.
26. Abraham NG, Cao J, Sacerdoti D, Li X, Drummond G. Heme oxygenase: the key to renal function regulation. Am J Physiol Renal Physiol. 2009; 297: F1137-52.
27. Elmarakby AA, Faulkner J, Baban B, Saleh MA, Sullivan JC. Induction of hemeoxygenase-1 reduces glomerular injury and apoptosis in diabetic spontaneously hypertensive rats. Am J Physiol Renal Physiol. 2012; 302: F791-800.
28. Vanella L, Sanford C, Jr., Kim DH, Abraham NG, Ebraheim N. Oxidative stress and heme oxygenase-1 regulated human mesenchymal stem cells differentiation. Int J Hypertens. 2012; 2012: 890671.
29. Gerbitz A, Ewing P, Wilke A, Schubert T, Eissner G, Dietl B, et al. Induction of heme oxygenase-1 before conditioning results in improved survival and reduced graft-versus-host disease after experimental allogeneic bone marrow transplantation. Biol Blood Marrow Transplant. 2004; 10: 461-72.
30. Cheng C, Noorderloos M, van Deel ED, Tempel D, den Dekker W, Wagtmans K, et al. Dendritic cell function in transplantation arteriosclerosis is regulated by heme oxygenase 1. Circ Res. 2010; 106: 1656-66.
31. Sharma K, Ramachandrarao S, Qiu G, Usui HK, Zhu Y, Dunn SR, et al. Adiponectin regulates albuminuria and podocyte function in mice. J Clin Invest. 2008; 118: 1645-56.
32. Ouchi N, Kobayashi H, Kihara S, Kumada M, Sato K, Inoue T, et al. Adiponectin stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and Akt signaling in endothelial cells. J Biol Chem. 2004; 279: 1304-9.
33. Ito T, Suzuki A, Imai E, Okabe M, Hori M. Bone marrow is a reservoir of repopulating mesangial cells during glomerular remodeling. J Am Soc Nephrol. 2001; 12: 2625-35.
34. Wong CY, Cheong SK, Mok PL, Leong CF. Differentiation of human mesenchymal stem cells into mesangial cells in post-glomerular injury murine model. Pathology. 2008; 40: 52-7.
35. Sugimoto H, Mundel TM, Sund M, Xie L, Cosgrove D, Kalluri R. Bone-marrow-derived stem cells repair basement membrane collagen defects and reverse genetic kidney disease. Proc Natl Acad Sci U S A. 2006; 103: 7321-6.
36. Lange C, Togel F, Ittrich H, Clayton F, Nolte-Ernsting C, Zander AR, et al. Administered mesenchymal stem cells enhance recovery from ischemia/reperfusion-induced acute renal failure in rats. Kidney Int. 2005; 68: 1613-7.
37. Morigi M, Imberti B, Zoja C, Corna D, Tomasoni S, Abbate M, et al. Mesenchymal stem cells are renotropic, helping to repair the kidney and improve function in acute renal failure. J Am Soc Nephrol. 2004; 15: 1794-804.
38. Anglani F, Forino M, Del Prete D, Tosetto E, Torregrossa R, D'Angelo A. In search of adult renal stem cells. J Cell Mol Med. 2004; 8: 474-87.
39. Kalluri R, Shield CF, Todd P, Hudson BG, Neilson EG. Isoform switching of type IV collagen is developmentally arrested in X-linked Alport syndrome leading to increased susceptibility of renal basement membranes to endoproteolysis. J Clin Invest. 1997; 99: 2470-8.
40. Hudson BG, Tryggvason K, Sundaramoorthy M, Neilson EG. Alport's syndrome, Goodpasture's syndrome, and type IV collagen. N Engl J Med. 2003; 348: 2543-56.
41. Hakim FT, Gress RE. Immunosenescence: deficits in adaptive immunity in the elderly. Tissue Antigens. 2007; 70: 179-89.
42. Linton PJ, Dorshkind K. Age-related changes in lymphocyte development and function. Nat Immunol. 2004; 5: 133-9.
43. Aspinall R. Age-associated thymic atrophy in the mouse is due to a deficiency affecting rearrangement of the TCR during intrathymic T cell development. J Immunol. 1997; 158: 3037-45.

Hindawi Publishing Corporation
Stem Cells International
Volume 2013, Article ID 132642, 8 pages
http://dx.doi.org/10.1155/2013/132642

*Review Article*

# Bone-Marrow-Derived Mesenchymal Stem Cells for Organ Repair

**Ming Li and Susumu Ikehara**

*Department of Stem Cell Disorders, Kansai Medical University, Moriguchi, Osaka 570-8506, Japan*

Correspondence should be addressed to Susumu Ikehara; ikehara@takii.kmu.ac.jp

Received 5 October 2012; Accepted 28 January 2013

Academic Editor: Rangnath Mishra



Mesenchymal stem cells (MSCs) are prototypical adult stem cells with the capacity for self-renewal and differentiation with a broad tissue distribution. MSCs not only differentiate into types of cells of mesodermal lineage but also into endodermal and ectodermal lineages such as bone, fat, cartilage and cardiomyocytes, endothelial cells, lung epithelial cells, hepatocytes, neurons, and pancreatic islets. MSCs have been identified as an adherent, fibroblast-like population and can be isolated from different adult tissues, including bone marrow (BM), umbilical cord, skeletal muscle, and adipose tissue. MSCs secrete factors, including IL-6, M-CSF, IL-10, HGF, and PGE2, that promote tissue repair, stimulate proliferation and differentiation of endogenous tissue progenitors, and decrease inflammatory and immune reactions. In this paper, we focus on the role of BM-derived MSCs in organ repair.

## 1. Introduction

The shortage of donor organs and the need of lifelong immunosuppression for the thousands of patients suffering from end-stage diseases worldwide are problems that need to be resolved. The repair, replacement, and regeneration of organs can restore impaired functions and are regarded as a potential solution to allotransplantation [1]. The bone marrow (BM) is an invaluable source of adult pluripotent stem cells, including hematopoietic stem cells (HSCs), endothelial progenitor cells (EPCs), and mesenchymal stem cells (MSCs). MSCs are prototypical adult stem cells with the capacity for self-renewal and differentiation with a broad tissue distribution. MSCs have been identified as an adherent, fibroblast-like population, originally isolated from BM [2]. These multipotent cells can be differentiated *in vitro* and *in vivo* into various cell types of mesenchymal origin, such as osteoblasts, adipocytes, and chondrocytes [3, 4]. Recently, more reports have demonstrated that MSCs secrete a variety of factors that promote tissue repair, stimulate proliferation and differentiation of endogenous tissue progenitors, and decrease inflammatory and immune reactions [5–7]. Because MSCs do not evoke an immune response, they are useful for allogenic organ and tissue repair.

## 2. Source, Multilineage Potential and Definition of MSCs

MSCs were first isolated from BM and have since been isolated from different adult tissues, including skeletal muscle [8], adipose tissue [9], umbilical cord [10], synovium [11], the circulatory system [12], dental pulp [13], amniotic fluid [14], fetal blood [15], lung [16], liver, and BM [17]. Friedenstein and coworkers first reported the existence of adherent, fibroblast-like cells isolated from BM [2], and that these cells could differentiate into mesodermal lineage such as osteoblasts, adipocytes, and chondrocytes *in vitro* [18] and cardiomyocytes [19]. Also, MSCs have been reported to differentiate into types of cells of endodermal and ectodermal lineages, including lung [20], retinal pigment [21], skin [22], sebaceous duct cells [23], renal tubular cells [24], and neural cells [25, 26], hepatocytes [27], and pancreatic islets [28]. There has hitherto been no specific surface marker for the identification of MSCs. For the isolation of human MSCs, the International Society for Cell Therapy proposed criteria [18] that comprise (1) adherence to plastic in standard culture conditions; (2) expression of the surface molecules CD73, CD90, and CD105 in the absence of CD34, CD45, HLA-DR, CD14 or CD11b, CD79a, or CD19 surface molecules as

assessed by fluorescence-activated cell sorter analysis; (3) a capacity for differentiation to osteoblasts, adipocytes, and chondroblasts *in vitro*. Similarly, murine MSCs have been shown to differ from human MSCs in terms of marker expression and behavior and have been identified as an adherent, fibroblast-like population, negative for CD45, CD11b, and CD 31, and positive for Scal1 and CD106 [29].

## 3. MSCs and the Immune System

MSCs have the ability to modify and influence almost all the cells of the innate and adaptive immune systems, to interfere with and affect cellular proliferation, differentiation, maturation, and function to induce an anti-inflammatory phenotype, and to modulate the immune response mediated by MSC soluble factors, including IL-6, M-CSF, IL-10, TGF$\beta$, HGF, and PGE2 [7, 30, 31]. The innate immune cells include neutrophils, dendritic cells (DCs), natural killer (NK) cells, eosinophils, mast cells, and macrophages. MSCs modulate DC function, indirectly regulate T and B cell activities, delay and prevent the development of acute graft versus host disease (GVHD) [32], and suppress DC function during allogeneic islet transplantation [33]. MSCs have been shown to suppress these inflammatory cells [34] and to alter NK cell phenotype and suppress proliferation, cytokine secretion, and cytotoxicity against HLA class I expressing targets [35]. MSCs mediated NK cell suppression via soluble factors such as indoleamine 2,3-dioxygenase, PGE2, and TGF$\beta$ [36]. The adaptive immune system, which is composed of T and B lymphocytes generates specific immune responses to pathogens with the production of memory cells. It has been reported that MSCs upregulate anti-inflammatory Th2 cytokines, including IL-3, -5, -10, and -13, and downregulate proinflammatory Th1 cytokines, including IL-1$\alpha$ and $\beta$, IFN$\gamma$, and TNF$\alpha$ [37]. MSCs induced an alteration of DC cytokine secretion, inducing a decreased secretion of pro-inflammatory cytokines such as TNF$\alpha$, IFN$\gamma$, and IL-12, and increased IL-10, which is a suppressive cytokine and inducer of reg T cells [38]. MSCs exert an inhibitory effect on B cells, but MSCs have stimulatory effect in low doses [39]. Concerning the immunomodulatory properties of MSCs in a mouse model, one report [40] has suggested that allogeneic MSCs are not intrinsically immunoprivileged, and under appropriate conditions, allogeneic MSCs induce a memory T-cell response resulting in rejection of an allogeneic stem cell graft. Another report [41] has suggested that MSCs could potentially improve experimental autoimmune encephalomyelitis in mice.

## 4. Homing of MSCs

Intravenously injected MSCs can migrate to the BM [42, 43] in the steady state and home to the inflammation site by migrating across the endothelium and then entering the injured organ [20, 44–47]. The fact that MSCs confer protection cannot be entirely attributed to their ability to home and engraft to the site of damage, suggesting that they are also capable of mediating protection in an endocrine manner [1]. MSCs have many chemokine receptors that assist in their migration to inflammatory sites via the SDF1/CXCR4 pathway [48]. Moreover, studies have demonstrated that platelet-derived growth factor-AB, IGF-1, and CD44 are the most potent chemoattractants for MSCs [44, 49].

## 5. BM-Derived MSCs (BMMSCs) and Organ Repair

Many reports have indicated that MSCs have the capacity to differentiate into endodermal, mesodermal, and ectodermal lineage cells. Recently, a report has indicated that the ability of MSCs to alter the tissue microenvironment via the secretion of soluble factors may contribute more significantly than their capacity for differentiation in tissue repair [50]. Adipose tissue and BM are the most readily available sources of MSCs because they are easy to harvest, and because of their relative abundance of progenitors and the lack of ethical concerns. Although adipose tissue-derived MSCs and BMMSCs show the same immunoregulatory and supporting hematopoiesis [51], BMMSCs have a higher degree of commitment to differentiate into chondrogenic and osteogenic lineages than adipose tissue-derived MSCs [52]. BMMSCs have been shown to ameliorate tissue damage and to improve function after lung injury [53–55], kidney disease [56, 57], diabetes [58, 59], myocardial infarction [60, 61], liver injury [62, 63], and neurological disorders [64].

*5.1. BMMSCs and Lung.* The lung is an organ that is highly susceptible to edema and endothelial permeability after traumatic injury. BMMSCs inhibit endothelial cell barrier permeability and preserve pulmonary endothelial cell integrity by preserving adherent junctions, tight junctions and decreasing inflammation. BMMSCs address both components of endothelial permeability and inflammation induced by hemorrhagic shock [54]. Interstitial lung diseases are characterized by epithelial injury, fibroblast proliferation, expansion of the lung matrix, and dyspnea. Of these diseases, idiopathic pulmonary fibrosis (IPF) is the most frequent and lethal. Proinflammatory cytokines IL-1 and TNF-$\alpha$ induce endothelial cells to express adhesion molecules and chemokines that attract other white cells from the blood to the site of injury [65]. IL-1 and TNF-$\alpha$ also stimulate proliferation of endothelial cells and fibroblasts that increase the blood supply at the site of injury and repair damage by the formation of scar tissue [66]. BMMSCs protect lung tissue from bleomycin-induced injury by blocking TNF-$\alpha$ and IL-1, two fundamental proinflammatory cytokines in the lung [53]. BMMSCs enhance the restoration of systemic oxygenation and lung compliance and decrease lung inflammation and histological lung injury. They also secrete cytokines, enhance lung repair, and attenuate the inflammatory response following ventilator-induced lung injury [55].

*5.2. BMMSCs and Kidney.* Acute and chronic kidney injuries after transplantation have a complex pathophysiology involving ischemic, inflammatory, and immunologic mechanisms, and adult stem cells have been used in the treatment of

these kidney diseases. Adult BM stem cells and the kidney precursors have been demonstrated to have an ability to differentiate into the kidney's specialized structures [67]. Nephrons are of mesenchymal origin, and stromal cells are of crucial importance for signaling, leading to the differentiation of both nephrons and collecting ducts [67]. Ischemic acute renal failure (ARF), characterized by a sharp decline in the glomerular filtration rate, is a very common complication in hospitalized patients and particularly in patients with multiorgan failure. When BMMSCs are injected after ARF, they can histologically become located in the kidney and significantly enhance the recovery of renal function by transdifferentiation into renal tubular or vascular endothelial cells [24, 68]. A single intrarenal administration of BMMSCs 7 days after ischemia-reperfusion significantly improved renal function and modified renal remodeling. The improvement of renal function was associated with a reduction in extracellular matrix accumulation. In addition, MSC administration also reduced tubular dilation, which is a classical feature of progressive renal failure in a renal ischemia rat model [57].

*5.3. BMMSCs and Pancreas.* Diabetes is caused by absolute insulin deficiency due to autoimmune destruction of insulin-secreting pancreatic $\beta$-cells (type 1 diabetes) or by relative insulin deficiency due to decreased insulin sensitivity, usually observed in overweight individuals (type 2 diabetes). In both types of the disease, an inadequate mass of functional $\beta$-cells is the major determinant for the onset of hyperglycemia and the development of overt disease. BM and BMMSCs induce the regeneration of recipient-derived pancreatic insulin-secreting cells, and MSCs inhibit T-cell-mediated immune responses against newly formed $\beta$-cells, which are able to survive in this altered immunological milieu [69].

Acute pancreatitis (AP) is characterized by a rapid onset and disease progression, with high fatality. Pancreatic acinar cells are the functional unit for the external secretion of the pancreas, which accounts for 80% of pancreatic tissue. During the process of severe AP, inflammatory mediators, metabolic products of arachidonic acid, and oxygen-derived free radicals enhance vascular permeability and cause tissue thrombosis and hemorrhage, thereby inducing necrosis of the pancreas [70]. BMMSCs can effectively relieve injury to pancreatic acinar cells and small intestinal epithelium, promote the proliferation of enteric epithelium and repair of the mucosa, and attenuate systemic inflammation in rats with severe acute peritonitis [71].

Human BM stem cells are able to differentiate into insulin-expressing cells *in vitro* by a mechanism involving several transcription factors of the $\beta$-cell developmental pathway when cultured in an appropriate microenvironment [72]. Human BMMSCs can be induced to express insulin in sufficient quantities to to reduce blood glucose in a diabetic mouse model [73] and to protect human islets from proinflammatory cytokines [74]. The use of human BMMSCs could be developed as a cell therapy for pancreatitis because of the ability, as shown in a rat model of acute pancreatitis, to reduce inflammation and damage to pancreatic tissue by reducing levels of cytokines and inducing Foxp3(+) regulatory T cells [75].

*5.4. BMMSCs and Heart.* Cardiovascular diseases are the first cause of death worldwide, and myocardial infarction (MI) is responsible for 12.8% of all deaths [76]. BMMSCs have been shown to differentiate into myogenic phenotype [77] and show a potent antifibrotic action, as their conditioned medium decreases cardiac fibroblast proliferation and the expression of collagen types I and III [78, 79] and increases the secretion of antifibrotic molecules such as matrix metalloproteinases 2, 9, and 14 [80]. BMMSCs exhibit the ability to differentiate into cardiomyocytes, smooth muscle cells, and endothelium in a swine model of chronic ischemic cardiomyopathy [81]. They have been shown to prolong survival compared with controls when hearts of Wistar rats were transplanted to Fisher 344 rats with intravenous MSC infusion [82]. Intravenous fusion of MSCs is the easiest and most practical method for delivery, though the MSCs must travel through the pulmonary circulation, where entrapment of cells is a concern [83]. Intracoronary infusion of stem cells is delivered with a standard over-the-wire balloon angioplasty catheter placed into the target coronary artery [84]. Injected BMMSCs improve cardiac function and reduce scar size in acute MI [85, 86]. Early-phase clinical trial data demonstrate that MSC therapy for post-MI is safe and has favorable effects on cardiac structure and function [87, 88].

*5.5. BMMSCs and Liver.* FGF-4 is one of the most important members of the fibroblast growth factor family; it can initiate the proliferation of mesodermal and endodermal cells and improve the development of fetal liver [89]. HGF is essential for the development of several epithelial organs and has been one of the most well-characterized cytokines for the stimulation of DNA synthesis in primary hepatocyte cultures and for liver development [90]. Oncostatin M is a member of the interleukin-6 family produced by hematopoietic cells and induces the differentiation of fetal hepatic cells, conferring various metabolic activities of adult liver [91]. These three factors participate in different developmental stages of the liver. FGF4, HGF, and oncostatin M have been shown to be key cytokines for hepatic differentiation from mouse BMMSCs [92]. Transplantation of BMMSCs alleviates GalN-induced acute liver injury in rats and stimulates the recovery systems, as evidenced by an earlier surge of cellular proliferation and differentiation into functional hepatocytes. IL-6 exerts hepatoprotective and mitogenic effects by stimulating the induction of acute-phase proteins as well as by suppressing apoptosis. Transplantation of BMMSCs could ameliorate acute liver injury. It promotes cell proliferation and organ repair, and the activation of the IL-6/gp130-mediated STAT3 signaling pathway via soluble IL-6 receptor is crucial in hepatic differentiation of BMMSCs [93].

Liver fibrosis is the excessive accumulation of extracellular matrix proteins, including collagen, that occurs in most types of chronic liver disease. Advanced liver fibrosis results in cirrhosis, liver failure, and portal hypertension, and often requires liver transplantation [94]. Although liver

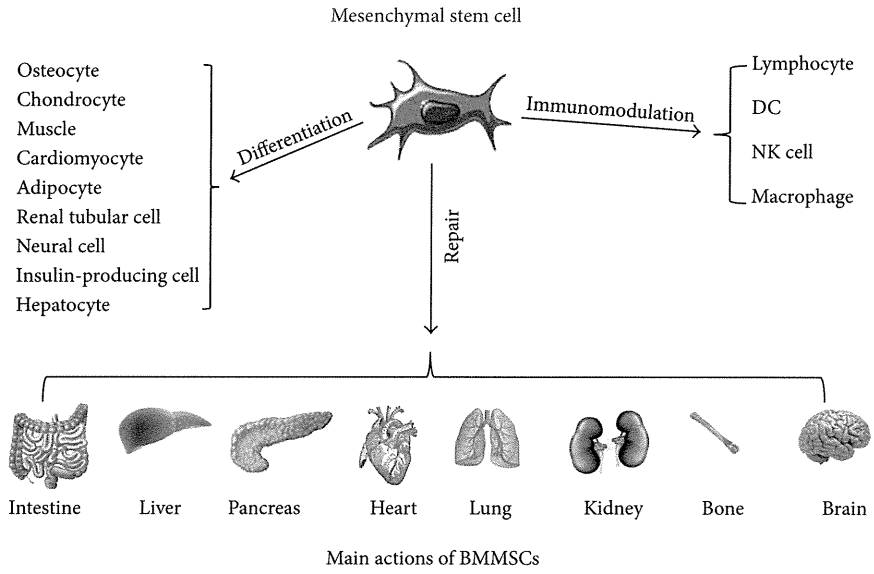


FIGURE 1: Main actions of BMMSCs.

transplantation is by far the most effective treatment for liver cirrhosis, extensive clinical application of the technique is limited by the lack of donor organ availability [95]. Cell-based hepatocyte transplantation, a potential interventional procedure, provides an effective strategy and holds great promise for the treatment of impaired livers. BMMSCs can protect against experimental liver fibrosis through promotion of IL-10 expression in CCl4- or dimethylnitrosamine-induced rats [63, 96].

*5.6. BMMSCs and Brain.* The development of effective treatments for human brain and spinal cord injury remains a serious challenge. In this regard, the transplantation of stem cells may help repair injured nerve tissue through the replacement of damaged cells, neuroprotection, or the creation of an environment conductive to regeneration by endogenous cells [97]. BMMSCs have been shown to promote cell proliferation and neurotrophic function of Schwann cells *in vitro* and *in vivo* [98]. Transplantation of BMMSCs can significantly reduce the behavioral abnormalities of these animals during the six weeks after engraftment [64]. Intravenously transplanted MSCs are capable of improving functional recovery and restoring neurological deficits in experimental intracerebral hemorrhage. The mechanisms are associated with enhanced survival and differentiation of neural cells and increased expression of antiapoptotic proteins and atrophic factors [99]. Human BMMSCs can improve neurological functional recovery in mice with experimental autoimmune encephalitis, possibly via a reduction of inflammatory infiltrates and areas of demyelination, stimulation of oligodendrogenesis, and by elevating brain-derived neurotrophic factor (BDNF) expression [41, 100]. Human BMMSCs transfected with the BDNF gene also showed improved functional recovery and reduced infarct size through a reduction in apoptosis [101]. Patients with Parkinson's disease transplanted with BMMSCs in the early stages of the disease (less than 5 years) showed greater improvement than in the later stages (11–15 years) [102].

*5.7. BMMSCs and Intestine.* Inflammatory bowel disease comprises a spectrum of chronic and relapsing diseases, including Crohn's disease (CD) and ulcerative colitis [103]. CD is characterized by a background of mucosal T-cell dysfunction, inflammatory cell infiltration, and abnormal cytokine production leading to uncontrolled and persistent intestinal transmural inflammation. Intraperitoneally injected cryopreserved BMMSCs home to and engraft into the inflamed colon and ameliorate trinitrobenzene sulfonic acid-induced colitis in rats [104]. Similarly, the injection of adipose-derived MSCs facilitated colonic mucosal repair and reduced the infiltration of inflammatory cells in the experimental colitis model [105].

Small intestinal permeability and villi injuries were significantly reduced in an MSC-administered group compared with the control group. MSC administration accelerated the recovery of the intestinal barrier dysfunction in a rat model of ischemia/reperfusion injury [106].

*5.8. BMMSCs and Bone.* Bone is regarded as an organ, and small bone damage can repair spontaneously without intervention. However, bone transplantation and surgery are required when there is extensive bone damage. As adult stem cells, BMMSCs possess a number of characteristics that make them appropriate for use in promoting bone regeneration [107]. BMMSCs may differentiate into tissue cells in order to restore lost morphology as well as function and to secrete a wide spectrum of bioactive factors that help to create a repair environment through their antiapoptotic effects, immunoregulatory function, and the stimulation of endothelial progenitor cell proliferation [108]. One report shows that

BMMSCs stimulate growth with osteogenesis imperfecta when children received allogeneic BMMSCs [109].

## 6. Conclusion

Figure 1 summarizes the main actions of BMMSCs. The original use of BMMSCs was to accelerate hematopoiesis, since they have the potential to differentiate into various cells, and to secrete cytokines and growth factors. BMMSCs have immunomodulatory properties through paracrine and endocrine mechanisms to repair damaged tissue. Homing and immunomodulation are important aspects of MSC functioning and their clinical effects. It has been proposed that the anti-inflammatory and antiapoptotic effects of MSCs may promote tissue regeneration. The use of allogenic nonimmunogenic BMMSCs would be a more acceptable strategy clinically. The potential role of BMMSCs to promote engraftment of organs and prevent rejection may be multifactorial and might be dependent on secretion of soluble growth factors, increasing angiogenesis, suppressing alloreactive T cells, and interacting with several arms of the immune system. However, the long-term safety of transplanted BMMSCs for organ repair needs to be proven prior to their clinical application.

## Conflict of Interests

None of the authors has conflict of interests to declare.

## Acknowledgments

The authors would like to thank Mr. Hilary Eastwick-Field and Ms. Keiko Ando for their help in the preparation of the paper.

## References

[1] B. Bi, R. Schmitt, M. Israilova, H. Nishio, and L. G. Cantley, "Stromal cells protect against acute tubular injury via an endocrine effect," *Journal of the American Society of Nephrology*, vol. 18, no. 9, pp. 2486–2496, 2007.

[2] A. J. Friedenstein, R. K. Chailakhjan, and K. S. Lalykina, "The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells," *Cell and Tissue Kinetics*, vol. 3, no. 4, pp. 393–403, 1970.

[3] A. J. Friedenstein, R. K. Chailakhyan, and N. V. Latsinik, "Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo," *Transplantation*, vol. 17, no. 4, pp. 331–340, 1974.

[4] A. I. Caplan, "Mesenchymal stem cells," *Journal of Orthopaedic Research*, vol. 9, no. 5, pp. 641–650, 1991.

[5] M. D. Nicola, C. Carlo-Stella, M. Magni et al., "Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli," *Blood*, vol. 99, no. 10, pp. 3838–3843, 2002.

[6] L. Raffaghello, G. Bianchi, M. Bertolotto et al., "Human mesenchymal stem cells inhibit neutrophil apoptosis: a model for neutrophil preservation in the bone marrow niche," *Stem Cells*, vol. 26, no. 1, pp. 151–162, 2008.

[7] S. Aggarwal and M. F. Pittenger, "Human mesenchymal stem cells modulate allogeneic immune cell responses," *Blood*, vol. 105, no. 4, pp. 1815–1822, 2005.

[8] J. T. Williams, S. S. Southerland, J. Souza, A. F. Calcutt, and R. G. Cartledge, "Cells isolated from adult human skeletal muscle capable of differentiating into multiple mesodermal phenotypes," *American Surgeon*, vol. 65, no. 1, pp. 22–26, 1999.

[9] P. A. Zuk, M. Zhu, H. Mizuno et al., "Multilineage cells from human adipose tissue: implications for cell-based therapies," *Tissue Engineering*, vol. 7, no. 2, pp. 211–228, 2001.

[10] A. Erices, P. Conget, and J. J. Minguell, "Mesenchymal progenitor cells in human umbilical cord blood," *British Journal of Haematology*, vol. 109, no. 1, pp. 235–242, 2000.

[11] C. De Bari, F. Dell'Accio, P. Tylzanowski, and F. P. Luyten, "Multipotent mesenchymal stem cells from adult human synovial membrane," *Arthritis & Rheumatism*, vol. 44, no. 8, pp. 1928–1942, 2001.

[12] S. A. Kuznetsov, M. H. Mankani, S. Gronthos, K. Satomura, P. Bianco, and P. G. Robey, "Circulating skeletal stem cells," *Journal of Cell Biology*, vol. 153, no. 5, pp. 1133–1139, 2001.

[13] S. Gronthos, M. Mankani, J. Brahim, P. G. Robey, and S. Shi, "Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 25, pp. 13625–13630, 2000.

[14] P. S. In 't Anker, S. A. Scherjon, C. Kleijburg-van der Keur et al., "Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation," *Blood*, vol. 102, no. 4, pp. 1548–1549, 2003.

[15] W. A. Noort, A. B. Kruisselbrink, P. S. In't Anker et al., "Mesenchymal stem cells promote engraftment of human umbilical cord blood-derived $CD_{34}^{+}$ cells in NOD/SCID mice," *Experimental Hematology*, vol. 30, no. 8, pp. 870–878, 2002.

[16] C. G. Fan, F. W. Tang, Q. J. Zhang et al., "Characterization and neural differentiation of fetal lung mesenchymal stem cells," *Cell Transplantation*, vol. 14, no. 5, pp. 311–321, 2005.

[17] C. Campagnoli, I. A. G. Roberts, S. Kumar, P. R. Bennett, I. Bellantuono, and N. M. Fisk, "Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow," *Blood*, vol. 98, no. 8, pp. 2396–2402, 2001.

[18] M. Dominici, K. Le Blanc, I. Mueller et al., "Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement," *Cytotherapy*, vol. 8, no. 4, pp. 315–317, 2006.

[19] S. Makino, K. Fukuda, S. Miyoshi et al., "Cardiomyocytes can be generated from marrow stromal cells in vitro," *Journal of Clinical Investigation*, vol. 103, no. 5, pp. 697–705, 1999.

[20] L. A. Ortiz, F. Gambelli, C. McBride et al., "Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 14, pp. 8407–8411, 2003.

[21] S. Arnhold, Y. Absenger, H. Klein, K. Addicks, and U. Schraermeyer, "Transplantation of bone marrow-derived mesenchymal stem cells rescue photoreceptor cells in the dystrophic retina of the rhodopsin knockout mouse," *Graefe's Archive for Clinical and Experimental Ophthalmology*, vol. 245, no. 3, pp. 414–422, 2007.

[22] H. Nakagawa, S. Akita, M. Fukui, T. Fujii, and K. Akino, "Human mesenchymal stem cells successfully improve skin-substitute wound healing," *British Journal of Dermatology*, vol. 153, no. 1, pp. 29–36, 2005.

[23] X. Fu, L. Fang, X. Li, B. Cheng, and Z. Sheng, "Enhanced wound-healing quality with bone marrow mesenchymal stem cells autografting after skin injury," *Wound Repair and Regeneration*, vol. 14, no. 3, pp. 325–335, 2006.

[24] M. Morigi, B. Imberti, C. Zoja et al., "Mesenchymal stem cells are renotropic, helping to repair the kidney and improve function in acute renal failure," *Journal of the American Society of Nephrology*, vol. 15, no. 7, pp. 1794–1804, 2004.

[25] G. C. Kopen, D. J. Prockop, and D. G. Phinney, "Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 19, pp. 10711–10716, 1999.

[26] G. Muñoz-Elias, A. J. Marcus, T. M. Coyne, D. Woodbury, and I. B. Black, "Adult bone marrow stromal cells in the embryonic brain: engraftment, migration, differentiation, and long-term survival," *Journal of Neuroscience*, vol. 24, no. 19, pp. 4585–4595, 2004.

[27] R. E. Schwartz, M. Reyes, L. Koodie et al., "Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells," *Journal of Clinical Investigation*, vol. 109, no. 10, pp. 1291–1302, 2002.

[28] D. Q. Tang, L. Z. Cao, B. R. Burkhardt et al., "In vivo and in vitro characterization of insulin-producing cells obtained from murine bone marrow," *Diabetes*, vol. 53, no. 7, pp. 1721–1732, 2004.

[29] A. Peister, J. A. Mellad, B. L. Larson, B. M. Hall, L. F. Gibson, and D. J. Prockop, "Adult stem cells from bone marrow (MSCs) isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential," *Blood*, vol. 103, no. 5, pp. 1662–1668, 2004.

[30] S. Beyth, Z. Borovsky, D. Mevorach et al., "Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness," *Blood*, vol. 105, no. 5, pp. 2214–2219, 2005.

[31] R. Ramasamy, H. Fazekasova, E. W. F. Lam, I. Soeiro, G. Lombardi, and F. Dazzi, "Mesenchymal stem cells inhibit dendritic cell differentiation and function by preventing entry into the cell cycle," *Transplantation*, vol. 83, no. 1, pp. 71–76, 2007.

[32] B. Zhang, R. Liu, D. Shi et al., "Mesenchymal stem cells induce mature dendritic cells into a novel Jagged-2 dependent regulatory dendritic cell population," *Blood*, vol. 113, no. 1, pp. 46–57, 2009.

[33] A. Aldinucci, L. Rizzetto, L. Pieri et al., "Inhibition of immune synapse by altered dendritic cell actin distribution: a new pathway of mesenchymal stem cell immune regulation," *Journal of Immunology*, vol. 185, no. 9, pp. 5102–5110, 2010.

[34] A. Uccelli, L. Moretta, and V. Pistoia, "Mesenchymal stem cells in health and disease," *Nature Reviews Immunology*, vol. 8, no. 9, pp. 726–736, 2008.

[35] P. A. Sotiropoulou, S. A. Perez, A. D. Gritzapis, C. N. Baxevanis, and M. Papamichail, "Interactions between human mesenchymal stem cells and natural killer cells," *Stem Cells*, vol. 24, no. 1, pp. 74–85, 2006.

[36] G. M. Spaggiari, A. Capobianco, H. Abdelrazik, F. Becchetti, M. C. Mingari, and L. Moretta, "Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2," *Blood*, vol. 111, no. 3, pp. 1327–1333, 2008.

[37] J. A. Potian, H. Aviv, N. M. Ponzio, J. S. Harrison, and P. Rameshwar, "Veto-like activity of mesenchymal stem cells: functional discrimination between cellular responses to alloantigens and recall antigens," *Journal of Immunology*, vol. 171, no. 7, pp. 3426–3434, 2003.

[38] W. Zhang, W. Ge, C. Li et al., "Effects of mesenchymal stem cells on differentiation, maturation, and function of human monocyte-derived dendritic cells," *Stem Cells and Development*, vol. 13, no. 3, pp. 263–271, 2004.

[39] A. Corcione, F. Benvenuto, E. Ferretti et al., "Human mesenchymal stem cells modulate B-cell functions," *Blood*, vol. 107, no. 1, pp. 367–372, 2006.

[40] A. J. Nauta, G. Westerhuis, A. B. Kruisselbrink, E. G. A. Lurvink, R. Willemze, and W. E. Fibbe, "Donor-derived mesenchymal stem cells are immunogenic in an allogeneic host and stimulate donor graft rejection in a nonmyeloablative setting," *Blood*, vol. 108, no. 6, pp. 2114–2120, 2006.

[41] J. Zhang, Y. Li, J. Chen et al., "Human bone marrow stromal cell treatment improves neurological functional recovery in EAE mice," *Experimental Neurology*, vol. 195, no. 1, pp. 16–26, 2005.

[42] S. M. Devine, A. M. Bartholomew, N. Mahmud et al., "Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion," *Experimental Hematology*, vol. 29, no. 2, pp. 244–255, 2001.

[43] R. F. Wynn, C. A. Hart, C. Corradi-Perini et al., "A small proportion of mesenchymal stem cells strongly expresses functionally active CXCR4 receptor capable of promoting migration to bone marrow," *Blood*, vol. 104, no. 9, pp. 2643–2645, 2004.

[44] M. B. Herrera, B. Bussolati, S. Bruno et al., "Exogenous mesenchymal stem cells localize to the kidney by means of CD44 following acute tubular injury," *Kidney International*, vol. 72, no. 4, pp. 430–441, 2007.

[45] A. Chapel, J. M. Bertho, M. Bensidhoum et al., "Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome," *Journal of Gene Medicine*, vol. 5, no. 12, pp. 1028–1038, 2003.

[46] A. Mahmood, D. Lu, M. Lu et al., "Treatment of traumatic brain injury in adult rats with intravenous administration of human bone marrow stromal cells," *Neurosurgery*, vol. 53, no. 3, pp. 697–703, 2003.

[47] D. Orlic, J. Kajstura, S. Chimenti et al., "Mobilized bone marrow cells repair the infarcted heart, improving function and survival," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 18, pp. 10344–10349, 2001.

[48] J. M. Fox, G. Chamberlain, B. A. Ashton, and J. Middleton, "Recent advances into the understanding of mesenchymal stem cell trafficking," *British Journal of Haematology*, vol. 137, no. 6, pp. 491–502, 2007.

[49] A. L. Ponte, E. Marais, N. Gallay et al., "The in vitro migration capacity of human bone marrow mesenchymal stem cells: comparison of chemokine and growth factor chemotactic activities," *Stem Cells*, vol. 25, no. 7, pp. 1737–1745, 2007.

[50] D. G. Phinney and D. J. Prockop, "Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair—current views," *Stem Cells*, vol. 25, no. 11, pp. 2896–2902, 2007.

[51] A. Poloni, G. Maurizi, P. Leoni et al., "Human dedifferentiated adipocytes show similar properties to bone marrow-derived mesenchymal stem cells," *Stem Cells*, vol. 30, no. 5, pp. 965–974, 2012.

[52] J. M. Gimble, A. J. Katz, and B. A. Bunnell, "Adipose-derived stem cells for regenerative medicine," *Circulation Research*, vol. 100, no. 9, pp. 1249–1260, 2007.