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Intrabone marrow injection enhances placental mesenchymal stem cell mediated support of hematopoiesis in mice

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Background/aim: In order to determine the synergistic effects of human placental mesenchymal stem cells (PMSCs) on hematopoiesis in vivo, we compared the intrabone marrow injection (IBMI) with the conventional intravenous injection (IVI).

Materials and methods: C57BL/6 recipient mice conditioned with lethal doses of irradiation were transplanted with bone marrow mononuclear cells (MNCs) and bone marrow-derived mesenchymal stem cells (BMSCs) from BALB/c mice by IBMI or IVI. NOD/SCID recipient mice conditioned with sublethal doses of irradiation were transplanted with human umbilical cord blood MNCs (UCB-MNCs) and PMSCs by IBMI or IVI.

Results: The number of hematopoietic cells was significantly higher in mice transplanted with BMSCs by IBMI than in those transplanted by IVI in a murine transplantation model (BALB/c→C57BL/6). Moreover, the percentage of human hematopoietic cells in the tibiae of the NOD/SCID mice that were transplanted with PMSCs plus UCB-MNCs was higher than that in mice transplanted with UCB-MNCs alone. In addition, in mice that were transplanted with PMSCs, PMSCs injected by IBMI were more efficient than those injected by IVI.

Conclusion: Our results not only elucidated the role of PMSCs in promoting hematopoiesis, but also revealed the therapeutic potential of the combination of PMSCs and IBMI in transplantation.

Key words: Placental mesenchymal stem cells, mononuclear cells, intrabone marrow injection, hematopoietic reconstruction

1. Introduction

Bone marrow transplantation (BMT) has been widely used for the treatment of a variety of diseases, including hematological diseases, autoimmune diseases, and cancers (1–3). A healthy bone marrow hematopoietic microenvironment (HME) is of great significance for the implantation of hematopoietic stem cells (HSCs) and hematopoietic recovery. In clinical BMT, the bone marrow HME is often damaged by chemotherapy prior to transplantation, resulting in a slow recovery of the recipient's HME. Mesenchymal stem cells (MSCs) are an important element of the bone marrow HME and can differentiate into various stromal cells of mesenchymal origin. In this microenvironment, MSCs supply an appropriate scaffold for hematopoiesis and a complex network of cytokines, adhesion molecules, and extracellular matrix proteins that regulate survival, proliferation, growth, and differentiation of HSCs (4–6). Studies have demonstrated that mesenchymal stem cells (MSCs) support hematopoiesis and enhance the engraftment of hematopoietic cells after cotransplantation (7,8). The cotransplantation of human MSCs and HSCs results in increased chimerism and accelerates hematopoietic recovery in both patient and animal models (9–12).

The classic BMT approach is to inject bone marrow (BM) or HSCs into the recipient's blood, where HSCs find "niches" in the BM to "seed," a process commonly referred to as "homing." In the case of conventional intravenous BMT (IV-BMT), the majority of bone marrow cells (BMCs), both HSCs and MSCs, are trapped in the lung and liver, where host cells kill the BMCs (13–15). To overcome this problem, transplantation using an intrabone marrow injection (IBMI) of hematopoietic stem/progenitor cells (HS/PCs) has been developed. Studies have demonstrated that intrabone marrow transplantation (IBMT) is an advantageous strategy for allogeneic BMT over the conventional IV-BMT (16). Nonetheless, in terms of MSCs, it is not clear whether transplantation through IBMI

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would enhance hematopoiesis better than transplantation through IVI. In the present study, we established two transplantation models to compare the efficacy of hematopoiesis reconstruction after transplanting MSCs by IBMI or by IVI.

Unlike all of the above-mentioned studies, which used bone marrow-derived MSCs (BMSCs), we also used placenta-derived MSCs (PMSCs) in our transplantation models. It has been shown that the placenta yields a large amount of MSCs from the fetal membrane with high plasticity that is capable of differentiating into both natural mesodermal and nonmesodermal lineages (17,18). These characteristics are similar to those of BMSCs (19). However, the PMSCs do not have the drawbacks associated with using BMSCs in clinical application. For example, large quantities of infused cells are required for treatment but the amount of donor BM is limited. Furthermore, the collection of donor BM is an invasive procedure. There is also the possibility that BMSCs might be contaminated with malignant cells if harvested from patients with a hematological malignancy (e.g., leukemia). Therefore, the PMSCs may be the ideal succedaneum of BMSCs. It is not known, however, whether PMSCs are able to support hematopoiesis in vivo.

In the present study we determined the effects of human PMSCs on hematopoiesis in vivo. We also compared IBMI to IVI to assess their synergistic effect with PMSCs on hematopoiesis.

2. Materials and methods

2.1. Cell isolation and culture

BABL/c mouse BMSC cell line BABL-5043 was purchased from American Cell Biologics Company, USA. Cell lines were grown in α-MEM medium supplemented with 10% fetal bovine serum. The PMSCs were generated from full-term placentae of healthy volunteers and collected according to procedures approved by the local ethics committee. The cells were isolated as previously reported (19). Briefly, the placental tissue was dissected, digested in enzymatic mixtures, and homogenized. A single-cell suspension was cultured in a complete medium comprising Dulbecco's modified Eagle's medium-low glucose (Gibco, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco) at 37 °C in 5% CO_2. The medium was replaced one to two times each week. After three passages, the cells were ready for use in the experiment. PMSCs were identified by morphology under a light microscope and assayed routinely for the presence of MSC-related cell surface antigens by flow cytometry analysis. Additionally, the PMSCs were cultured in adipogenic and osteogenic medium to estimate their potential for differentiation.

BMCs were flushed from the medullary cavities of the humeri, femora, and tibiae of BALB/c mice, using a 26-G needle attached to a 1-mL syringe, into phosphate-buffered saline with 2% fetal calf serum and filtered through a 70-μm nylon mesh. Bone marrow mononuclear cells (BM-MNCs) were isolated from the BMCs on a Ficoll–Hypaque density gradient (1.077 g/mL, Pharmacia, USA) after centrifugation for 25 min at 400 × *g*.

Human umbilical cord blood (hUCB) samples were obtained from full-term deliveries after informed consent of the mother and were used in accordance with the local ethics committee. The hUCB was collected in bags containing heparin (Shandong Weigao Group Co., Ltd., China) and processed within 24 h. Samples were diluted 1:2 in PBS. Human umbilical cord blood mononuclear cells (UCB-MNCs) were collected after centrifugation on Ficoll–Hypaque density gradient (1.077 g/mL, Pharmacia) and washed in PBS.

2.2. Mice

Seven-week-old female C57BL/6 (H-2b), BALB/c (H-2d), and NOD/SCID mice were purchased from the Animal Experiment Center of Wuhan University (China) and maintained under pathogen-free conditions in our animal facility throughout the study. C57BL/6 and NOD/SCID mice were irradiated lethally (8 Gy) and sublethally (2.5 Gy), respectively, 16–18 h before transplantation. Gamma-irradiation was delivered with a Gammacell 40 Exactor with ^{60}Co sources at a dose of 1.0 Gy/min.

2.3. Flow cytometry assay

Cells were harvested from the cell culture, BM, spleen, or blood and washed with PBS. Cells were then stained with fluorochrome-conjugated antibodies against some cell surface markers. The staining of an IgG isotype was used as a control. After washing, the cells were analyzed by flow cytometry for the expression of each surface marker.

2.4. Intrabone marrow and intravenous injection

For IBMI, mice were anesthetized with an intraperitoneal injection of 0.05 g/g body weight of a 1% solution of pentobarbital sodium and the knee joint was flexed. A hole was drilled into the tibia with a short, 27-gauge needle attached to a 3-mL syringe filled with PBS. The first needle was then removed and replaced with a 28-gauge needle attached to a 0.3-mL insulin syringe containing the indicated number of cells in 30 μL of substrate for delivery. For IVI, cells were injected into the tail vein of the recipient mice.

2.5. Experimental protocols of transplantation

Two transplantation models were established in the study. First, with the lethal dose of irradiation, the C57BL/6 recipient mice were transplanted with BM-MNCs and BMSCs from the BALB/c mice by IBMI or IVI (Figure 1). Sixty recipient mice were randomly divided into three groups. In the IBM1 group, 1×10^6 BMSCs were injected into the left tibial marrow cavity and 2×10^7 BM-MNCs

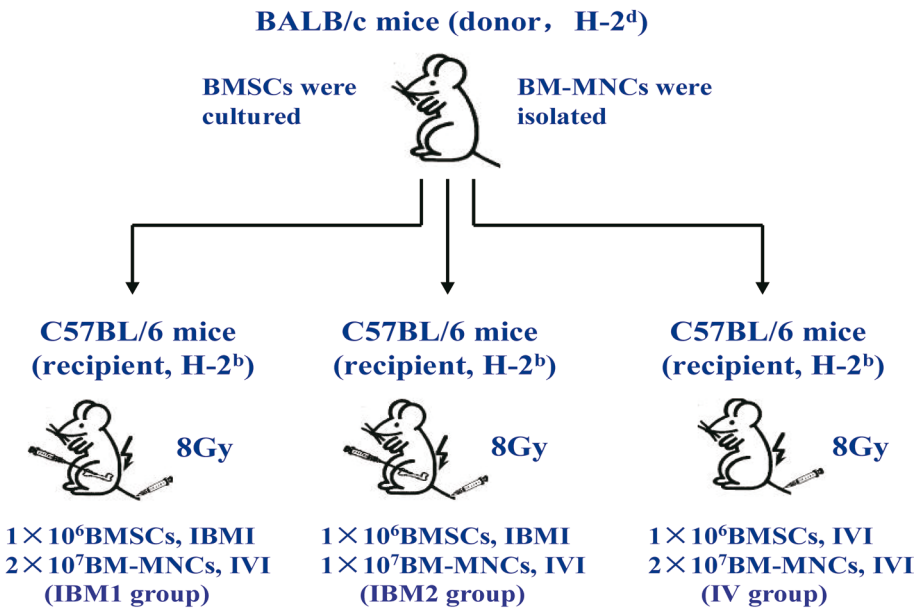

Figure 1. Experiment protocol of the BALB/c→C57BL/6 model. The number and infusion way of BMSCs and BM-MNCs are shown.

were injected into the C57BL/6 mice by IVI. In the IBM2 group, 1 × 10^6 BMSCs were injected into the left tibial marrow cavity and 1 × 10^7 BM-MNCs were injected into the C57BL/6 mice by IVI. In the IV group, 1 × 10^6 BMSCs and 2 × 10^7 BM-MNCs were injected into the C57BL/6 mice by IVI.

Second, with the sublethal dose of irradiation, recipient NOD/SCID mice were transplanted with human UCB-MNCs and PMSCs by IBMI or IVI (Figure 2). Thirty-six recipient mice were randomly divided into the following three groups: group a (PMSCs by IBMI; UCB-MNCs by IVI), group b (PMSCs + UCB-MNCs by IVI), and group c (UCB-MNCs by IVI). In group a, 1 × 10^6 PMSCs were injected into the left tibia by IBMI, and 3 × 10^7 UCB-MNCs were injected into the same recipient mice by IVI. In group b, 1 × 10^6 PMSCs and 3 × 10^7 UCB-MNCs were injected into recipient mice by IVI. In group c, only 3 × 10^7 UCB-MNCs were injected into recipient mice by IVI.

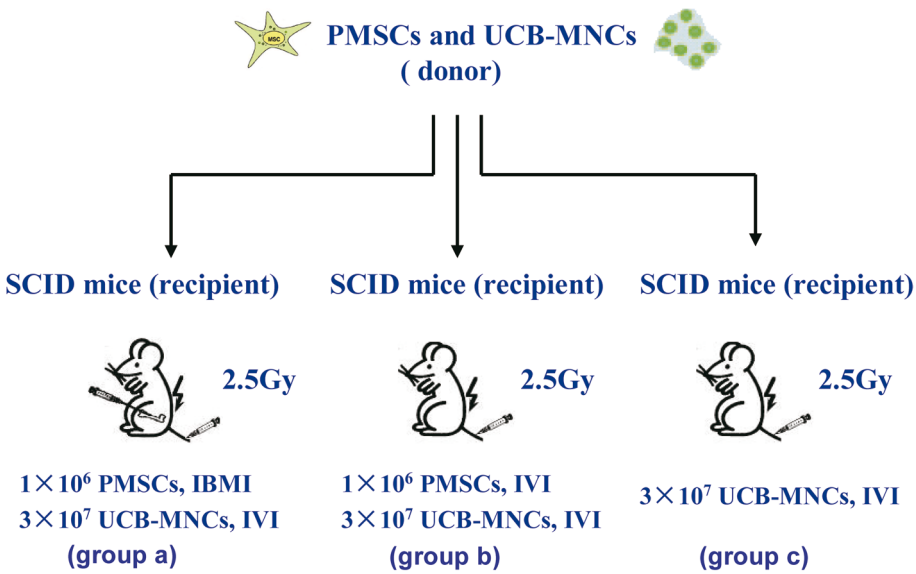

Figure 2. Experiment protocol of the human PMSCs→NOD/SCID model. The number and infusion way of PMSCs and UCB-MNCs are shown.

2.6. Detection of hematopoietic reconstruction in recipient mice

For BALB/c→C57BL/6 transplantation, C57BL/6 recipient mice were sacrificed on days 1, 3, 6, or 9 after transplantation, and the BM was flushed from the injected and contralateral tibiae. Lysis of the red cells was performed using a red blood cell lysis buffer (Sigma, USA). The nucleated cells were stained with a PE-conjugated anti-H-2K^d mAb (BD Pharmingen, USA) to identify the BALB/c-derived cells. To detect the hematopoietic reconstruction in the recipient mice, the nucleated cells were stained with an FITC-conjugated anti-CD45 mAb and biotinylated anti-Gr-1, anti-CD11b, and anti-B220 mAb, followed by streptavidin-cychrome (BD Pharmingen). The stained cells were analyzed using FACScan (BD Biosciences, USA) equipped with CellQuest software.

For human PMSCs→NOD/SCID transplantation, recipient mice were sacrificed on day 14 after transplantation. BMCs were flushed from the injected and contralateral tibiae. The nucleated cells were counted and stained with FITC-conjugated antihuman CD45 and PE-conjugated antihuman CD34 mAbs. At week 6, recipient mice were sacrificed, cells were recovered from the tibiae, spleens, and peripheral blood. To obtain splenocytes, spleens were ruptured, homogenized, filtered through a 40-μm nylon mesh, centrifuged, and subjected to red blood cell lysis. The nucleated cells were stained with FITC-conjugated human anti-CD45, anti-CD34, anti-CD14, anti-CD19, and anti-CD56 mAbs (eBioscience, USA). The percentage of human-derived hematopoietic cells was analyzed by flow cytometry.

2.7. Statistical analysis

The results represent samples from more than three mice per experiment. Statistical analysis was performed with the use of SPSS 19.0. Statistical comparisons between the two groups were performed by using Student's t test. Probability (P) values of < 0.05 were considered significant.

3. Results

3.1. Identification of PMSCs

PMSCs were characterized by plastic adhesion and fibroblastic morphology by light microscopy. These cells are able to differentiate into osteocytes, chondrocytes, and adipocytes under specific differentiation conditions. During the culture of primary cells, we saw that a few attached MSCs stretched from a small tissue mass and had obtained the shape of a typical fibroblast in 4–6 days (Figure 3A, I). After 10 to 14 days, the cells formed a monolayer of homogeneous, bipolar, spindle-like cells in a whirlpool-like array (Figure 3A, II). These cells grew, were firmly attached to the bottom of the flask, and required tryptic digestion for cell passage.

To phenotypically characterize these freshly isolated placental MSCs, we stained the cells with a panel of surface markers and analyzed them by flow cytometry. As shown in Figure 3B, these cells did not express CD45, suggesting that they were not hematopoietic cells. The cells also lacked CD34 expression, indicating that they differed from hematopoietic stem cells. However, these cells expressed mesenchymal cell markers such as CD29, CD44, CD105, CD106, and CD166. To further confirm whether these placental cells were MSCs, we assessed their potential to differentiate into several tissue lineages. To do this, we cultured these placental cells in an OsteoDiff or AdipoDiff induction medium (Miltenyi Biotec GmBH) for 3 weeks and measured osteogenesis and adipogenesis by alkaline phosphatase (ALP) and oil red O staining, respectively. As shown in Figure 3C, the cells stained positively for ALP after a 3-week culture, suggesting extracellular calcium deposition, a key marker of osteogenesis. When cultured in the AdipoDiff induction medium for 3 weeks, cells stained positively for fat droplets, a feature of adipogenesis. Taken together, these results indicated that these placental cells displayed a similar morphology, phenotype, and function as MSCs did. Therefore, the cells were identified as PMSCs.

3.2. Effects of transplantation BMSCs by IBMI on hematopoiesis in mice

We next employed a mouse transplantation model to test whether transplantation BMSCs by IBMI enhance the growth of hematopoietic cells in vivo. BM-MNCs from the BALB/c mice were transplanted into C57BL/6 mice by IVI. BMSCs from the BALB/c mice were transplanted into C57BL/6 mice by IBMI or by IVI. The recipient C57BL/6 mice were sacrificed at various times (days 1, 3, 6, and 9) after IBMI or IVI. BMCs were prepared from the injected and contralateral tibiae, double-stained with anti-H-2K^d mAb (donor-type) and some hematopoietic cell markers, and analyzed by FACScan. As shown in Figure 4A, the total number of BMCs increased in a time-dependent manner after transplantation in all groups, with a higher number of BMCs in the IBM1 or IBM2 group than in the IV group ($P < 0.05$) on day 6. This difference was reflected in the donor-derived cells (H-2K^{d+}) as we observed that the number of H-2K^{d+} cells in the IBM1 or IBM2 group was significantly higher than that in the IV group ($P < 0.05$) on day 6 or 9 (Figure 4B). Similarly, after transplantation, the number of H-2K^{d+} cells increased in a time-dependent manner in all groups with the lowest increased rate in the IV group.

To trace the myeloid and lymphoid lineage cells, we measured the number of H-2K^{d+}/Gr-1$^+$ (Figure 4C), H-2K^{d+}/CD11b$^+$ (Figure 4D), and H-2K^{d+}/B220$^+$ cells in donor-derived BMCs from recipient mice. Similar to H-2K^{d+} cells, Gr-1$^+$ or CD11b$^+$ cells proliferated over time after transplantation. Again, we found that the number of Gr-1$^+$ or CD11b$^+$ cells was significantly higher in the IBM1

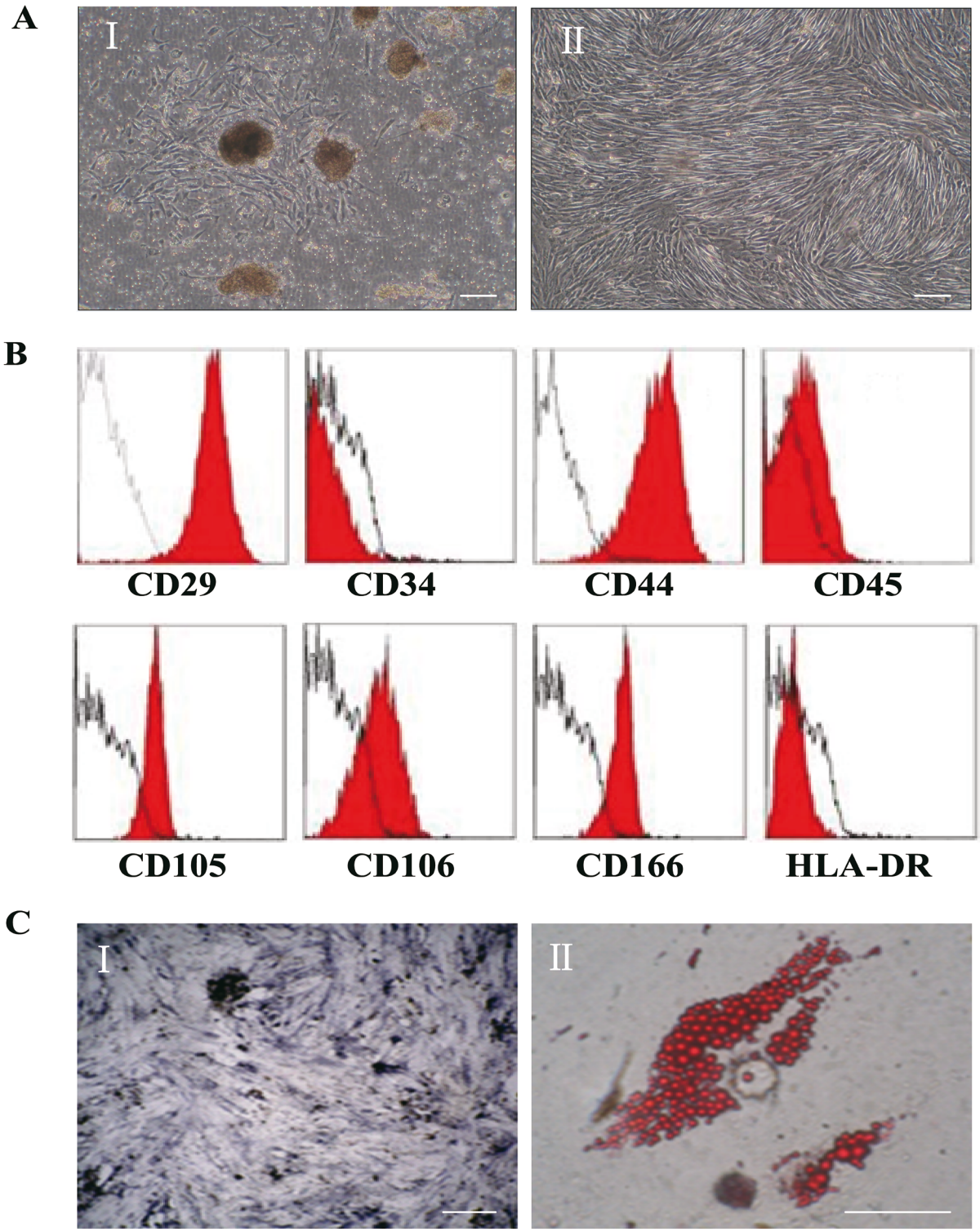

Figure 3. Isolation and characterization of placenta-derived cells. (A) Morphology of placenta-derived cells under light microscopy. Freshly isolated placental cells were cultured for 4–6 days (I) or 10–14 days (II). Fibroblast-like colonies were seen. Bar = 50 μm. (B) Immunophenotype of placenta-derived cells. Cells were stained with fluorochrome-conjugated antibodies against CD29, CD34, CD44, CD45, CD105, CD106, CD166, HLA-DR, or immunoglobulin isotype control antibodies and analyzed by flow cytometry. (C) Differentiation potential of placenta-derived cells. Cells were cultured in an osteogenic (I) or adipogenic (II) medium for 3 weeks and stained with ALP for calcium deposition or oil red O. Magnification: I, ×100; II, ×400. Bar = 10 μm.

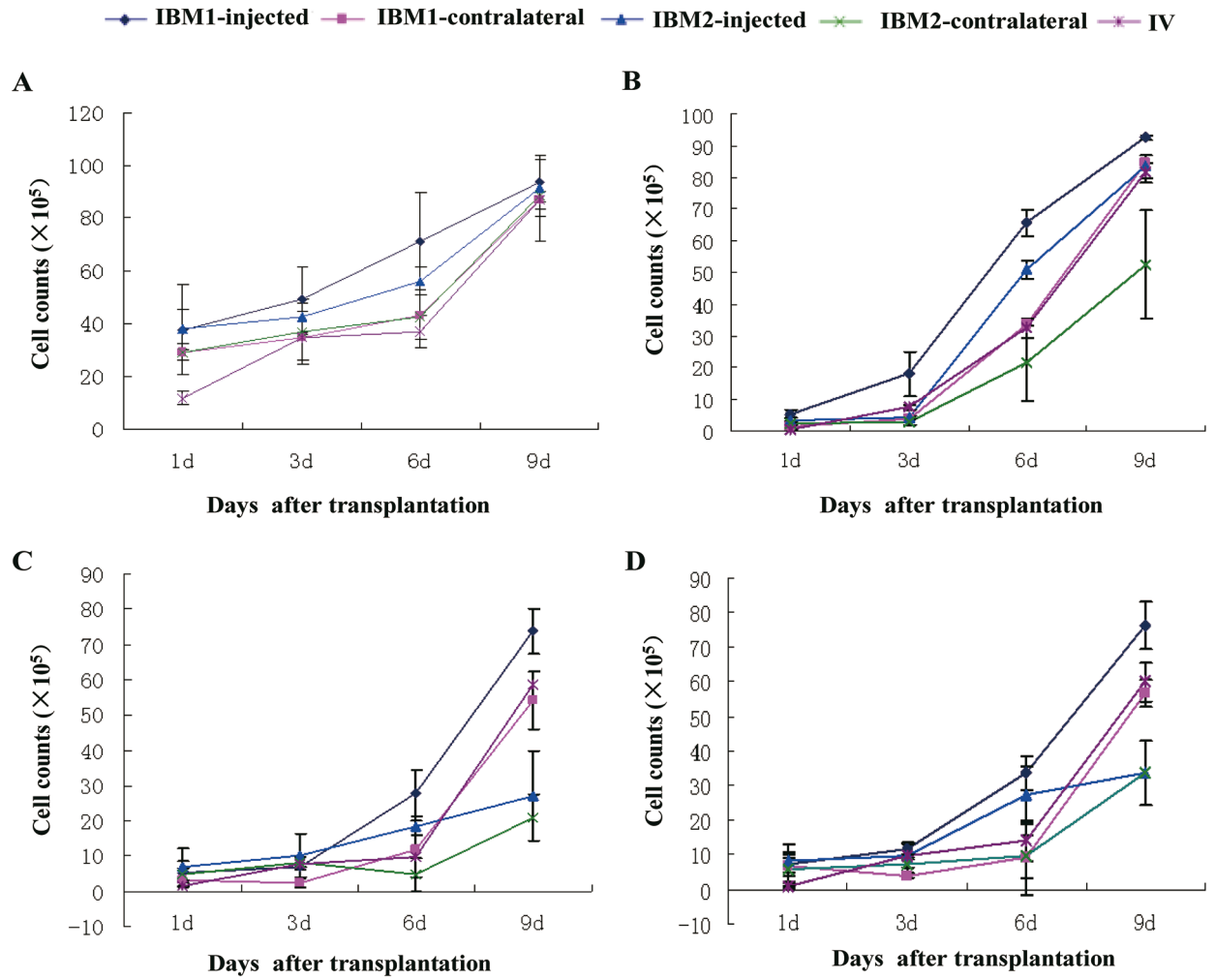

Figure 4. Effects of transplantation of BMSCs through IBMI and IVI on hematopoiesis in mice. BMSCs and BM-MNCs from the BALB/c mice were transplanted into C57BL/6 mice through IBMI or IV. The recipient C57BL/6 mice were then sacrificed at the indicated time points. A graph showing the total BMC (A), donor-derived (B), donor-derived Gr-1$^+$ (C), and donor-derived CD11b$^+$ cell numbers (D).

or IBM2 group than in the IV group ($P < 0.01$) on day 6 or 9. Among the BMCs, the number of H-2K^{d+}/B220$^+$ cells was always very low and no difference was observed between the IBM and IV groups. Summarized results from multiple samples clearly showed that MSC transplanted by IBMI enhanced hematopoietic cell growth more strongly than those transplanted by IVI. The numbers of total BMCs, donor-derived BMCs, Gr-1$^+$ cells, and CD11b$^+$ cells were higher in the IBM group than they were in the IV group on day 6 after transplantation ($P < 0.05$) (Figure 5A). We observed in a representative transplanted mouse that the percentage of donor-derived nucleated cells (H-2K^{d+}/CD45$^+$) and myeloid cells (H-2K^{d+}/Gr-1$^+$ and H-2K^{d+}/CD11b$^+$ cells) was higher in the IBM groups than in the IV group. This increase was more obvious in the IBM1- or IBM2-injected tibia of mice (Figure 5B). Taken together, these results clearly indicated that MSCs transplanted by IBMI were superior to those transplanted by IVI in facilitating hematopoietic cell growth.

3.3. Effects of human PMSCs transplantation on hematopoietic reconstruction in NOD/SCID mice

Next, we wanted to examine hematopoietic reconstruction in sublethal-dose-irradiated NOD/SCID mice transplanted with human PMSCs and UCB-MNCs by IBMI or IVI. BMCs in tibiae (injected or contralateral) were harvested 14 days after transplantation and analyzed by flow cytometry (Figure 6A). The transplantation of PMSCs promoted hematopoietic reconstitution in NOD/SCID mice, as the percentage of human CD45$^+$ cell in the injected or contralateral tibiae of the mice cotransplanted with PMSCs and UCB-MNCs (groups a and b, 63.5% and 20.6%, respectively) was higher than that of mice transplanted with UCB-MNCs alone (group c, 8.6%). Moreover, the percentage of human CD34$^+$ cells in the

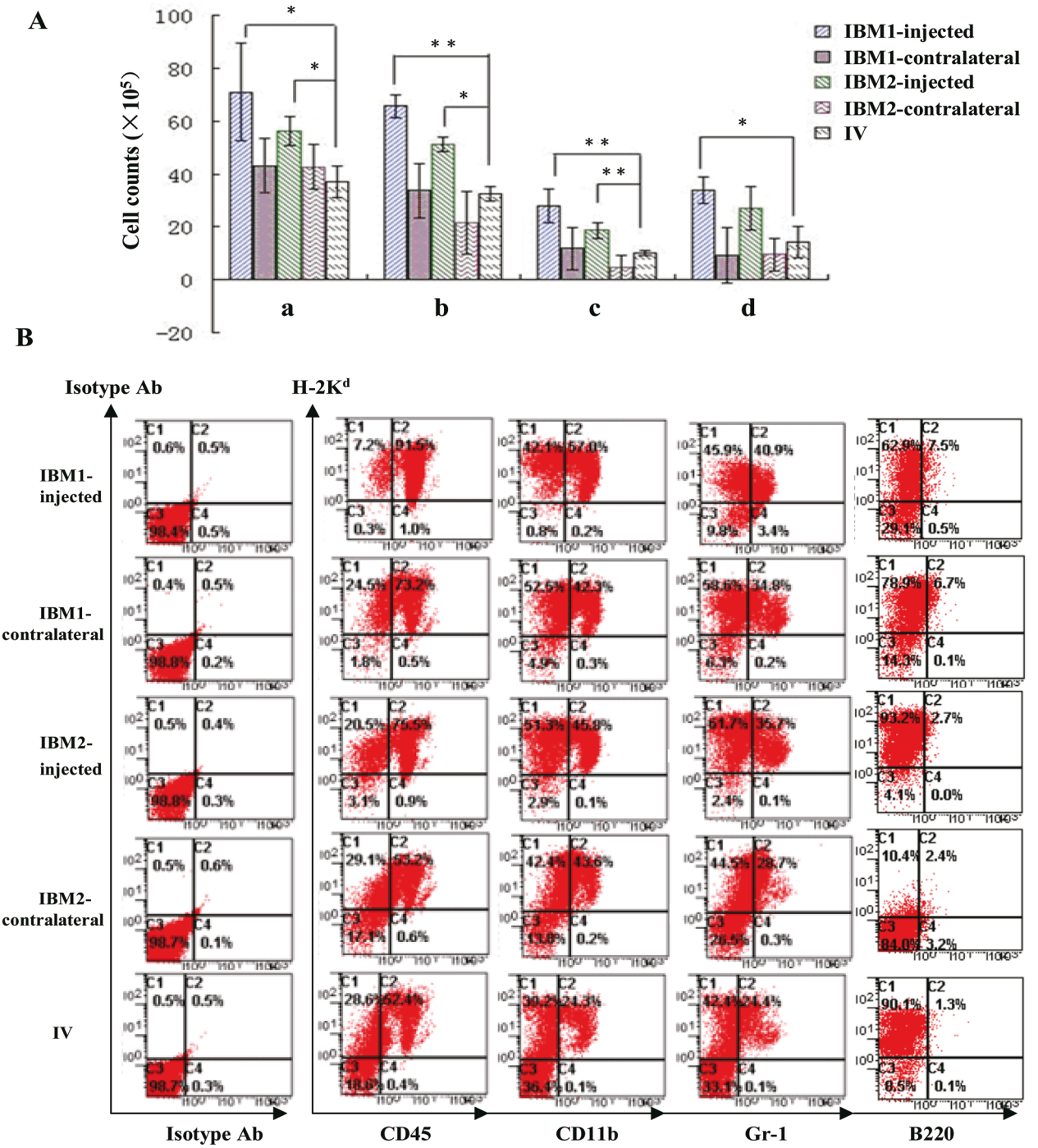

Figure 5. A summary of donor cell engraftment levels in the BM of recipient mice 6 days after transplantation. (A) A graph showing the total BMC (a), total donor-derived (b), donor-derived Gr-1$^+$ (c), and donor-derived CD11b$^+$ (d) cell numbers. (**P < 0.01; *P < 0.05). (B) Representative dot plots showing the expression of CD45, CD11b, Gr-1, or B220 in recipient C57BL/6 mice transplanted with BMSCs and BM-MNCs from the BALB/c mice by IBMI or IVI.

injected or contralateral tibiae of the mice in group a and group b (9.6% and 2.5%, respectively) was higher than that in the mice of group c (0.9%). Actually, human cells were hardly detected, and the number of CD45$^+$ and CD34$^+$ cells was extremely low in group c (Figure 6B). Supporting this finding, when transplanted with PBMCs by IBMI and UCB-MNCs through a vein (group a), NOD/SCID mice had a significant increase in the percentage of CD45$^+$ and

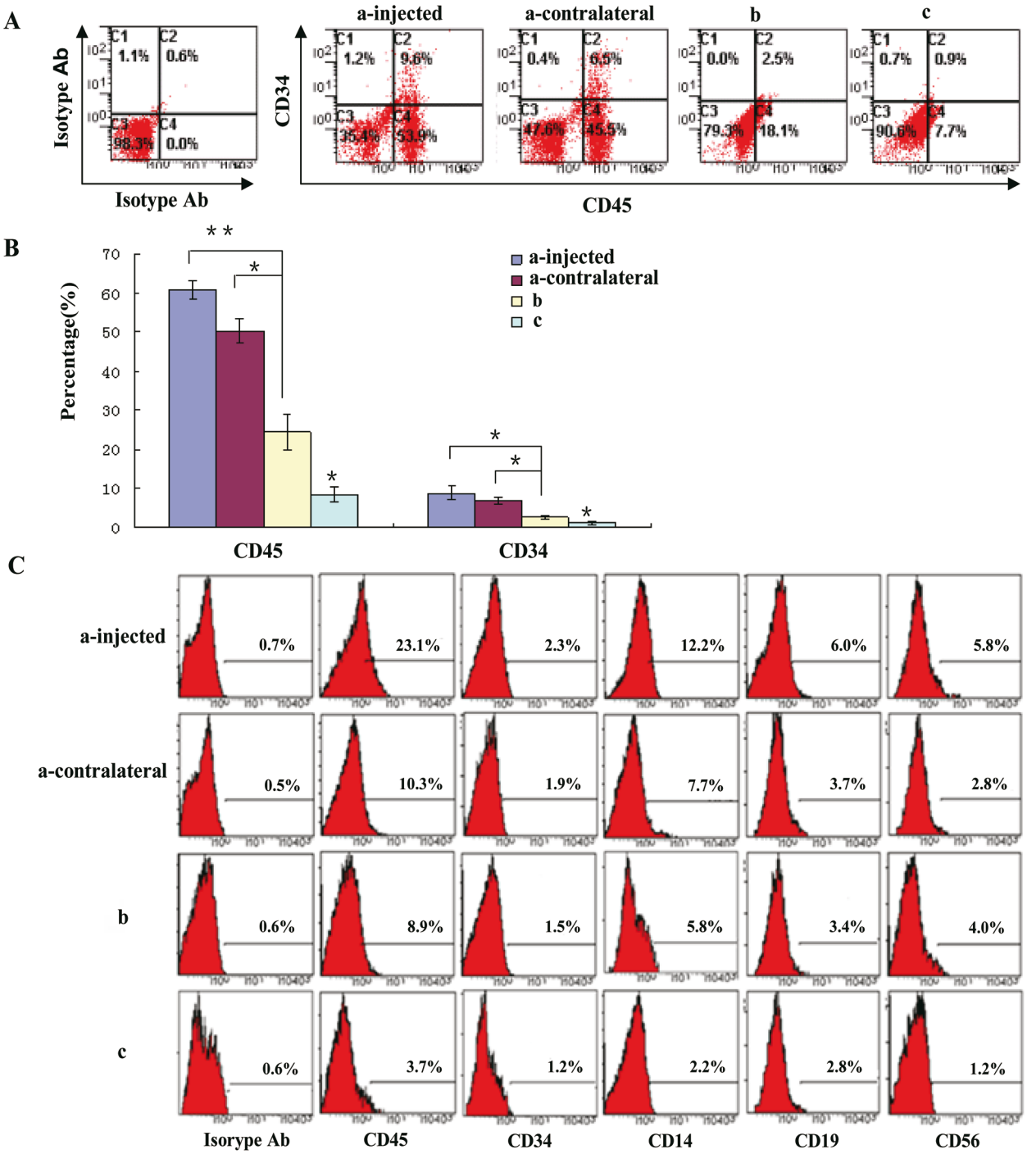

Figure 6. Effects of human PMSCs transplantation on hematopoietic reconstruction in NOD/SCID mice. (A) Representative dot plots showing growth of human $CD45^+$ or $CD34^+$ cells in the tibia of NOD/SCID mice on day 14 after transplantation by flow cytometry. (B) A graph showing the percentage of human $CD45^+$ and $CD34^+$ cells in the tibia of NOD/SCID mice on day 14 after transplantation. Group c was compared with group a or group b; $^*P < 0.05$. Group a was compared with group b, $^*P < 0.05$; $^{**}P < 0.01$. (C) Representative histograms showing growth of human hematopoietic cell in the tibia of NOD/SCID mice at week 6 after transplantation.

$CD34^+$ cells as compared with mice in group c (Figure 6B). In addition, in group a the percentage of human $CD45^+$ and $CD34^+$ cells was higher than that in group b, demonstrating that PMSCs transplanted by IBMI were more efficient than cells transplanted by IVI.

To investigate the later hematopoietic reconstitution of NOD/SCID mice, we measured the percentage of human $CD45^+$, $CD34^+$, $CD14^+$, $CD19^+$, and $CD56^+$ cells in the BM, spleen, and blood 6 weeks after transplantation by flow cytometry. As shown in the Table, we again found that transplantation with PMSCs plus UCB-MNCs significantly enhanced the growth of these human cells as compared with transplantation with UCB-MNCs alone ($P < 0.05$). Moreover, the percentages of all these human cells in bone marrow were higher when PMSCs were transplanted by IBMI than when cells were transplanted by IVI. As shown in Figure 6C, we displayed the FACS pictures of representative transplanted mice in the three groups. However, it was difficult to detect the presence of these hematopoietic cells in the spleen or blood. These results suggest that the reconstruction of hematopoiesis was more promising in the BM than in the peripheral lymphoid organs.

4. Discussion

In the present study, we evaluated the effects of IBMI on human PMSCs-mediated hematopoietic reconstruction in NOD/SCID mice and found that mouse BMSCs transplanted by IBMI were superior to those transplanted by IVI. In addition, we found that the PMSCs isolated in the present study demonstrated features similar to BMSCs for improving hematopoietic cells implanted in vivo (20). In the (BALB/c→C57BL/6) mouse model, the reconstruction of BMCs, donor-derived cells, and donor-derived myeloid lineage cells in the tibial cavity was quicker in the IBM group than it was in the IV group. The percentage of donor-derived nucleated cells reached over 90% on posttransplantation day 6, consistent with a previous report (21). Even though they were transplanted with half of the cells, IBM-transplanted mice experienced a faster hematopoietic cell reconstruction than IV-transplanted mice, confirming that MSCs transplanted by IBMI were more efficient than those transplanted by IVI. Similar results were seen in myeloid ($Gr\text{-}1^+$; $CD11b^+$) hematopoietic reconstruction. An explanation for this observation is that if BMSCs are directly injected into the BM cavity, MSCs could remain at the BM site and could proliferate and differentiate. MSCs are the precursor cells of mesenchymal cell types in the hematopoietic niche, and adipocytes, osteoblasts, and fibroblasts represent an important, defining cell type of this niche (22). Through IBMI, MSCs repaired the injuries of the hematopoietic niche caused by irradiation and provided a supportive role for the hematopoietic microenvironment that enabled the engraftment of HSCs. Moreover, when an osteoblastic niche was generated through cotransplantation with MSCs, the engraftment of HSCs was enhanced (23). However,

Table. Analyses of surface antigens on donor-derived cells in NOD/SCID mice 6 weeks posttransplantation.

Mice	Cell surface antigen (%)				
	CD45	CD34	CD14	CD19	CD56
Group a					
BM(injected)	24.6(±5.6)**△	2.1(±1.4)*△	10.4 (±4.0)**△	5.9(±3.1)*△	4.7(±2.1)*
BM (contralateral)	15.9 (±4.3)*	1.8(±1.1)	7.4 (±3.7)*△	3.5(±2.3)*	2.6(±1.8)*
Spleen	1.7 (±1.2)	2.7(±1.8)	3.6 (±1.9)	ND	1.7(±1.1)
Blood	1.9(±0.9)	ND	ND	ND	ND
Group b					
BM	11.0(±3.8)*	1.6(±0.9)	5.0 (±3.9)*	3.3 (±1.8)	4.1 (±2.3)*
Spleen	1.9(±0.7)	1.2 (±1.1)	2.7(±1.3)	1.9 (±0.7)	2.0 (±1.1)
Blood	0.6(±0.4)	0.6(±0.5)	ND	ND	ND
Group c					
BM	3.9(±2.8)	1.3(±0.7)	2.3 (±1.9)	2.8 (±1.8)	1.2 (±0.6)
Spleen	2.4 (±1.1)	ND	1.8 (±0.6)	ND	ND
Blood	1.4 (±0.8)	ND	ND	ND	ND

Results are expressed as means ± SD for 3–6 mice. Asterisks represent the P values of BM of group a and group b versus BM of group c;*$P < 0.05$;**$P < 0.01$; Triangles represent the P values of group a injected and contralateral versus BM of group b; △$P < 0.05$.

MSCs cannot home to or engraft in the BM if transplanted through the veins (24). In addition, MSCs in the bone marrow facilitated HSC homing to the BM and might play an important role in promoting hematopoiesis. It has been shown that CXCR4 is expressed on the surface of MSCs and BM stromal cells (25,26) and the SDF-1/CXCR4 axis plays an essential role in directing engraftment of HSCs into the BM after transplantation (27,28). Thus, through IBMI, MSCs did not only dwell in bone marrow to support the hematopoietic microenvironment, but also promoted the HSCs to home to BM and accelerate the hematopoietic recovery by generating some cytokines.

Although BM-MSCs may have promoting effects on HSC engraftment and repopulation (29), BM collection involves invasive procedures. In addition, the number of BM-MSCs decreases with age. The easy access, the ability to repeatedly use it, and the uncomplicated enzyme-based isolation procedure make placental tissue the most attractive source of MSCs. However, the capacity of PMSCs to support hematopoiesis in vivo was not clear. In the present study, we found that human PMSCs improve hematopoietic recovery in vivo. Therefore, PMSCs may be an ideal alternative source of BMSCs for basic research and clinical applications. Further studies are required to better understand the supportive effects of PMSCs on hematopoiesis in vitro and in vivo as well as its great therapeutic potential.

In addition, the IBMI is safe and effective for the clinical application of PMSCs in the future. IBMI has been used clinically as a technique to deliver medications (30), blood products, and fluids to critically ill children and wounded soldiers (31–33). In these studies, this technique was safely performed in thousands of patients. Interest in IBMI has now been renewed in the field of hematopoietic cell transplantation. Both, in clinical settings and in murine models, the IBMI delivery of hematopoietic grafts and lymphocytes may reduce the risk of acute graft-versus-host disease (GVHD) (34). Intra-BM cord blood transplants in patients with malignant hematopoietic diseases are associated with high rate of engraftment and early and robust platelet recovery (35). The available clinical data are encouraging and at this point IBMI can be recommended for clinical application.

In conclusion, we generated MSCs from placental tissue and found that PMSCs supported hematopoiesis in vivo. Our data clearly showed that, for enhancing the PMSC-mediated support of hematopoiesis, transplantation through IBMI was superior to that through IVI.

Acknowledgments

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