Allogeneic Compact Bone-Derived Mesenchymal Stem Cell Transplantation Attenuates the Severity of Idiopathic Pneumonia Syndrome in a Murine Bone Marrow Transplantation Model

Shu-kai Qiao\textsuperscript{a,b} Han-yun Ren\textsuperscript{a} Yong-jin Shi\textsuperscript{a} Wei Liu\textsuperscript{a}

\textsuperscript{a}Department of Hematology, Peking University First Hospital, Beijing, \textsuperscript{b}Department of Hematology, the Second Hospital of Hebei Medical University, Shijiazhuang, China

Key Words
Mesenchymal stem cell • Hematopoietic stem cell transplantation • Idiopathic pneumonia syndrome • Mouse model • Chemokine receptors

Abstract

\textbf{Background/Aims:} Idiopathic pneumonia syndrome (IPS) is a serious and life-threatening lung complication following allogeneic hematopoietic stem cell transplantation (allo-HSCT) and currently no effective therapies exist. This study was designed to determine whether transplantation of allogeneic murine compact bone derived mesenchymal stem cells (CB-MSCs) could prevent the development of IPS. \textbf{Methods:} We tested the effects of CB-MSCs transplantation on IPS using an established murine model of C57BL/6 (H-2b)→BALB/c (H-2d). Survival rates, body weight change, clinical GVHD scores, lung histological changes were assessed after IPS induction. Mechanistically, concentrations of cytokines (TNF-\(\alpha\), IFN-\(\gamma\) and IL-4) and chemokines (CCL5, CXCL9 and CXCL10) in bronchoalveolar lavage fluid (BALF) from the recipient mice were measured at different time point post-transplantation. CD4\(^+\)CD25\(^+\)Foxp3\(^+\) regulatory T cell (Treg) percentage, CCR5, CXCR3 and CCR7 expression on CD3\(^+\) T cells, and lung CXCR3, CCR5, CCR7, T-bet and GATA-3 mRNA levels were also evaluated at different time point post-transplantation. \textbf{Results:} Co-transplantation of CB-MSCs significantly attenuated the severity of lung injuries and increased survival rate of mice compared to non-cotransplanted mice. A higher Treg percentage, reduction of TNF-\(\alpha\), IFN-\(\gamma\), CCL5, CXCL9 and CXCL10 levels, down-regulation of CXCR3 and CCR5, as well as up-regulation of CCR7, were observed in MSCs co-transplantation mice. Also, the prophylactic effect of CB-MSCs was associated with a shift of Th1/Th2 balance toward anti-inflammatory Th2 polarization. \textbf{Conclusions:} Allogeneic CB-MSCs effectively controlled the occurrence of IPS due to its profound immunomodulatory capacity. This may offer a novel prophylactic approach for IPS after allo-HSCT.

Han-yun Ren, MD & PhD

Department of Hematology, Peking University First Hospital, Beijing, 100034, (China)
Tel. +86-10-83575082, Fax +86-10-66551811, E-Mail renhy0813@163.com

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Introduction

Although the clinical success rate of allogeneic hematopoietic stem cell transplantation (allo-HSCT) has increased steadily in the past decades, pulmonary complications remain serious threats to survival in some patients after transplantation [1, 2]. Idiopathic pneumonia syndrome (IPS) is a noninfectious fatal lung complication following allo-HSCT, which usually develops within the first 100 days after transplantation [3]. Despite many studies have reported a lower incidence (3%-15%), IPS responds poorly to conventional therapy and the mortality rates are alarmingly high [4, 5]. Currently, the underlying mechanism responsible for IPS is not completely understood. The data obtained from clinic experience and experimental models indicated that some risk factors, including the conditioning intensity of HSCT, the use of total body irradiation (TBI), the mismatch of major histocompatibility complex (MHC) between donor and recipient, older recipient age and acute graft-versus-host disease (GVHD) occurring etc, have been associated with the lung injuries of IPS [4-8]. Some researchers suggested that the pathogenesis of IPS is similar to GVHD, alloreactive donor T lymphocytes recruitment to the lung might play a critical role in the development of IPS after allo-HSCT, thus IPS may be considered as a pulmonary manifestation of GVHD [9, 10]. Many experimental and clinical evidences have indicated that dys-regulation of cytokines and chemokines networks may be a primary cause for the development of IPS. Previous studies showed that several chemokines, such as CCL5, CXCL9-11, and their receptors CCR5 and CXCR3 have been known to increase in animal IPS models [11, 12]. Hildebrandt et al. [12] demonstrated that blocking interactions between CXCR3 and its ligand reduce T cells recruitment to the lung and prevent the generation of IPS. Therefore, to search the effectively therapeutic approach for IPS will help to further improve the success rate of allo-HSCT.

Mesenchymal stem cells (MSCs) are pluripotent nonhematopoietic progenitor cells, which have been isolated from many different tissues, including bone marrow, adipose tissue, umbilical cord blood and amniotic fluid etc. [13]. Several recent studies have independently reported MSCs purification from human or mouse trabecular bone [14-16]. Their findings and our previous work confirm that the MSCs obtained from collagenase-treated bone fragments are of similar or greater amount and purity as their marrow-derived counterparts. MSCs not only possess the ability of self-renewal, but also have the potential to differentiate into a variety of mesenchymal lineages tissues. Due to the low expression of major histocompatibility complex (MHC) class II antigen and B7 family co-stimulatory molecules in their cell surface that are required to initiate an immune response, MSCs hold an immunoprivileged capacity, and exert immunosuppressive effects in a number of situations [17]. Although the hypo-immunogenicity of MSCs facilitates the use of “off-the-shelf” allogeneic MSC-based therapies in clinical trials, the immunoprivileged status of allogeneic MSCs remains ambiguous and questions their utility for treating some human disease [18, 19]. A large number of studies have confirmed the anti-inflammatory and immunosuppressive characteristics of MSCs both in vitro and in vivo [20-22]. MSCs express a variety of cytokines and chemokine receptors and can home to sites of inflammation by migrating towards inflammatory chemokines and cytokines [23]. These biological characteristics promote MSCs as a promising therapeutic modality in various inflammatory diseases and GVHD. Some clinical trials have presented the clinical benefits of MSCs therapy in GVHD [24-26]. But so far, there has been no studies published about whether MSCs has a prophylactic effect on IPS.

In this study, with an established murine model of IPS, we observed the effect of allogeneic CB-MSCs transplantation on lung injuries in the BALB/c recipient mice after IPS induction. Furthermore, we also sought to investigate the potentially therapeutic mechanisms of CB-MSCs and make progress in clinical treatment and prevention of IPS in the future.
Materials and Methods

Animals

Healthy female C57BL/6 (H-2b) and male BALB/c (H-2d) mice were purchased from the Animal Experiment Center of Peking Medical University. The C57BL/6 mice used for MSCs cultivation were 6-8 weeks old and 18-23g each. The BALB/c recipient mice were 8-10 weeks old and 23-25g each. All experimental protocols were approved by the Animal Care and Use Committee of Peking Medical College.

Isolation and identification of CB-MSCs

CB-MSCs were isolated and cultivated from C57BL/6 mice using collagenase-treated bone fragment methods as our previously described [27]. The cells were shown to express high levels of CD44, CD29, CD34, and Sca-1, but not CD45 and CD117. We confirmed that CB-MSCs successfully differentiated into osteogenic, adipogenic, and chondrogenic cells under different in vitro induction conditions. The cells of passage 5 (P5) were harvested for use in the following experiments.

Experimental groups of animal model

Animals were divided into four groups: simple irradiation group (A, n = 20), IPS group (B, n = 20); IPS with MSC cotransplantation group (C, n = 20); normal control group [healthy age-matched male BALB/c (H-2d) mice] (D, n = 20).

The induction of IPS is according to previously published literatures [8, 28]. Briefly, BALB/c recipient mice received 8 Gy of total body x-ray irradiation (0.5Gy/min) as simple irradiation group. At 6 hours after TBI, 0.2ml cell mixtures of 1.0 × 10^7 bone marrow cells and 2.0 × 10^7 spleen cells from C57BL/6 mice were transplanted into irradiated mice via tail veins infusion as IPS group. Except for same number of bone marrow cells and spleen cells transplanted as mentioned in IPS group, additional 1 × 10^7 CB-MSCs were simultaneously infused via veins (IPS with MSCs group). Healthy age-matched BALB/c mice presented as normal control. Mice in all groups were subsequently housed in sterilized microisolator cages and received normal chow and autoclaved hyperchlorinated water after transplantation. All experimental groups were performed in duplicate. One was used to observe survival rates and body weight changes for 14 days after TBI and the other was used to explore the immunological mechanisms in the following experiments.

Assessment of GVHD

The severity of GVHD was assessed at 7 and 14 days post-transplantation using a scoring system involving five clinical parameters: weight loss (1, 10-25%; 2, >25%), posture (1, hunching only at rest; 2, severe hunching impairs movement), activity (1, stationary>45% of the time; 2, stationary unless stimulated), fur texture (1, mild to moderate ruffling; 2, ruffling entire body) and skin integrity (1, scaling paws/tails; 2, obvious areas of denuded skin), as previously described by Cooke et al. [28]. A clinical GVHD score was calculated by summation of the five criteria scores (scores ranging 0-10).

Lung histopathological analysis

Lungs were obtained from surviving animals at days 3 and 7 post-transplantation. Lung tissues were fixed in 10% formalin overnight at room temperature, and then paraffin sections were prepared and stained with hematoxylin and eosin for morphometric analyses. Pathological severity of lung injury was assessed according to the following four categories: alveolar capillary congestion, hemorrhage, leukocyte infiltration into the airspace or vessel wall, and alveolar wall thickness/hyaline membrane formation. Each category was graded according to a five-point scale: 0, minimal damage; 1, mild damage; 2, moderate damage; 3, severe damage; and 4, maximal damage [29]. A total score of lung injury was calculated by summation of the four criteria scores (scores ranging from 0-16). Also, the lungs were excised and immediately placed into a micro-tube to weigh. Then the lungs were dried at 60°C for 72 h and weighed again. The wet-dry lung weight ratio was calculated (wet lung mass divided by the dry lung mass), which indicates the fraction of wet lung weight due to water.

To determine the infiltration and distribution of T cells in the lungs of IPS mice, immunohistochemical analysis for CD4+ and CD8+ were performed on the lung tissues of recipient mice at 7 days post-transplantation according to the manufacturer’s instructions. Briefly, paraffin-embedded tissue sections (5 μm thick) were

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mounted on silanized glass slides, dewaxed, rehydrated, and treated with 0.5% hydrogen peroxide solution in methanol to quench endogenous tissue peroxidase. After antigen retrieval, slides were blocked for 1h with 1% BSA in PBS. Then, slides were treated with the primary antibodies of CD4 and CD8 to stain their respective antigens, as well as the appropriate horseradish peroxidase-conjugated secondary antibody for 90 and 60 min, respectively. For quantification of immunostaining, the number of stained cells per high-power field (HPF) was counted at 400× magnification.

Measurements of cytokines and chemokines in BALF

The bronchoalveolar lavage fluid (BALF) samples were obtained from each group mice at different time post-transplantation. BALF was collected after euthanizing the mice and placing a 20-gauge catheter into the trachea through which 0.7 ml of cold PBS was flushed back and forth three times. The protein levels of TNF-α, IFN-γ, IL-4, CCL5, CXCL9-10 were measured in BALF samples with Enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer’s protocol (R&D Systems, Minneapolis, MN). All samples and standards were measured in triplicate.

Analysis of Treg cells and CXCR3, CCR5 and CCR7 expressions on T cells

Peripheral blood (PB) and BALF were obtained from each group mice at 7 days post-transplantation. Treg cells and expression of chemokine receptors were evaluated by flow cytometry. Briefly, the blood and BALF samples were treated according to the manufacturer’s protocol and stained with fluorescein-conjugated monoclonal antibodies against mouse CD4-CD25-FOXP3, CD3ε, CXCR3, CCR5 and CCR7 (Biolegend, San Diego, CA), respectively, and followed by analyzing with flow cytometry.

Measurement of CXCR3, CCR5, CCR7, T-bet and GATA-3 mRNA in the lung tissues

Total lung RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA) at different time post-transplantation, and then cDNA was synthesized from 2μg of total RNA by using a first-strand cDNA synthesis kit (Invitrogen, Carlsbad, CA). Relative quantitation of chemokine receptors, T-bet and GATA-3 to β-tubulin were performed on ABI 7500 using SYBR Green chemistry. PCR primers were designed according to the corresponding structure of the mouse genes. Primers used for the determination of CXCR3, CCR5, CCR7, T-bet and GATA-3 were listed in Table 1. The real-time RT-PCR assays were performed in triplicate.

Statistical analysis

The comparisons of treatment effects among different groups were performed using a one-way analysis of variance. Kaplan Meier Survival curve were constructed and survival rates were compared using a log rank (Mantel-Cox) test. All data were analyzed using GraphPad Prism Version 5.0 (San Diego, CA, USA). p<0.05 was considered statistically significant.

Table 1. Primers for transcription factor gene and chemokine receptors gene by real-time RT-PCR. Abbreviations: T-bet, T-box expressed in T-cells; GATA-3, GATA-binding protein-3; CCR5, C-C chemokine receptor 5; CCR7, C-C chemokine receptor 7; CXCR3, CXC chemokine receptor 3

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<th>Gene</th>
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<td>Transcription factor gene</td>
<td>Sense: 5′-TCAACCAGCAGACAGACAGAG-3′</td>
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<td></td>
<td>Antisense: 5′-AACACCTGCTATGCTGTTG-3′</td>
</tr>
<tr>
<td>T-bet</td>
<td>Sense: 5′-GGTTCCTCTGTCGCCCGTGTA-3′</td>
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<td>Antisense: 5′-AGCACAGCGACAGCAGACAT-3′</td>
</tr>
<tr>
<td>GATA-3</td>
<td>Sense: 5′-CGAAAACAACATGCTAAAAGC-3′</td>
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<tr>
<td></td>
<td>Antisense: 5′-GTTGCTTTGCTTGGATCGTCA-3′</td>
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<tr>
<td>Chemokine receptors gene</td>
<td>Sense: 5′-TTTCCAGATGCCTGCTGTCG-3′</td>
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<td>CCR5</td>
<td>Antisense: 5′-GAGCACAGCAGACAGACAGCAGACATC-3′</td>
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</tr>
<tr>
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Results

**CB-MSCs improve the survival rate and decrease body weight loss of IPS mice**

Except for IPS mice suffered obviously diarrhea, the mice undergoing TBI had the similar manifestation during the first week post-irradiation. They showed rapid weight loss and common signs including lassitude, ruffled fur, and a hunched posture. Subsequently, body weights began to gradually rise among the mice with MSC cotransplantation. The mice of simple irradiation group had all died at 13 days post-irradiation. The median survival rate in simple irradiation, IPS and IPS with MSCs groups were 7.8 ± 0.7, 16 ± 2.4 and 52 ± 4.8 days, respectively. There was a significantly lower survival rate in IPS group (55%, 11/20 mice surviving) than the 100% survival rate of normal control. CB-MSCs co-transplantation significantly increased the survival rate of IPS mice compared to non-MSC co-transplantation group (80% vs. 55%, p < 0.05; Fig. 1A). Body weight changes of mice from each group at different time point post-TBI were shown in Fig. 1B. CB-MSCs significantly reduced the weight loss of the recipient mice after IPS induction (p < 0.05).

**CB-MSCs lowered the clinical GVHD scores of IPS mice**

The recipient mice in IPS group developed progressive acute GVHD, with a higher clinical GVHD scores than those of IPS+MSCs infused group at 7 days (p < 0.01) and 14 days (p < 0.01) post-HSCT (Fig. 1C). These results indicated that CB-MSCs co-transplantation significantly attenuated the severity of GVHD.

**CB-MSCs alleviate lung injury and lung water content**

Lung tissues from the normal control and simple irradiation group showed a normal structure or minor histopathological changes. However, the recipient mice suffered a varied degree of alveolar collapse, lung edema, alveolar hemorrhage, inflammatory cells infiltration and alveolar wall thickening after IPS induction. The representative images of lung injury in mice from each group at 3 and 7 days post-transplantation were shown in Fig. 2(A-H). The lung injury scores were clearly higher in IPS group than control group at 3 (p < 0.01) and 7 days (p < 0.01) post-transplantation. The lung injury scores were also significantly increased in IPS group compared with simple irradiation group at 3 (p < 0.01) and 7 days (p < 0.01) post-transplantation. Importantly, CB-MSCs significantly decreased the lung injury scores of IPS mice at 3 (p < 0.05) and 7 days (p < 0.01) post-transplantation (Fig. 2I).
Wet/Dry (W/D) lung weight ratio was used to determine lung water content, which is an evaluation indicator of pulmonary vascular barrier. Compared with control mice, W/D weight ratios in IPS group were significantly increased at 3 ($p < 0.01$) and 7 ($p < 0.01$) days post-transplantation. The W/D weight ratio was also significantly higher in IPS group than simple irradiation group at 3 ($p < 0.01$) and 7 days ($p < 0.05$) post-transplantation. CB-MSCs co-transplantation clearly prevented this increase of lung water content ($p < 0.05$) and ($p < 0.05$) days post-transplantation. However, there was no significant difference in W/D weight ratio among four groups at 1 day post-transplantation (Fig. 2J). These results indicated that CB-MSCs attenuated the severity of lung injury and reduced the increase of lung water content in IPS mice.

Through the immunohistochemical analysis of CD4$^+$ and CD8$^+$ for lung tissues, we attempted to confirm whether the pathogenesis of IPS was involved in the abnormal infiltration of CD4$^+$ and/or CD8$^+$ T cells. The representative images of CD4 and CD8 staining for lung tissues in mice from each group at 7 days post-transplantation were shown in Fig. 3(A-H). Both CD4 and CD8 staining in IPS and IPS+MSCs group were a varied degree positive, but they were very low expression in simple irradiation group and normal control group. The number of CD4 and CD8-positive cells per HPF in IPS+MSCs group is less than IPS group (Fig. 3I). Our findings showed that CB-MSCs clearly reduced the infiltration of CD4$^+$ and CD8$^+$ T cell in the lung of IPS mice.
CB-MSCs decreased the levels of inflammatory cytokines and chemokines in BALF

The concentrations of TNF-α and IFN-γ, but not IL-4 in BALF were dramatically increased at 3 and 7 days, and peaked at 3 days after IPS induction. IFN-γ levels in simple irradiation, IPS and IPS with MSC groups were significantly higher than the normal control group. The concentrations of CCL5, CXCL9 and CXCL10 in BALF were markedly elevated at 7 days, but only CXCL9 was significantly increased at 3 days after IPS induction. But simple irradiation did not increase CCL5, CXCL9-10 level. Moreover, CB-MSCs significantly decreased the levels of TNF-α, IFN-γ, CCL5, CXCL9 and CXCL10 in BALF after IPS induction (Fig. 4).

CB-MSCs promoted the expansion of Treg cells in both PB and BALF

We assayed Tregs using labeling of CD4, CD25 and Foxp3 on T cells. Representative flow diagrams of Tregs were shown in Fig. 5A. We found that the percentage of Treg cells (CD4^+CD25^+Foxp3^+^/CD4^+^) in PB (p < 0.05; Fig. 5B) and BALF (p < 0.05; Fig. 5C) was significantly higher in IPS with MSCs group than that of IPS group at 7 days post-transplantation.

CB-MSCs altered CCR5, CXCR3, and CCR7 expression on T cells and their mRNA levels in lung tissues

Representative example of CXCR3, CCR5 and CCR7 expression on T cells in the mice of IPS with MSCs group were showed in Fig. 6A. Compared to the mice in IPS group, those who also received CB-MSC transplantation exhibited significantly decreased CXCR3 and CCR5 expressions (p < 0.01) and significantly increased CCR7 expression (p < 0.01) on CD3^+ T
Fig. 4. CB-MSCs co-transplantation suppressed the production of inflammatory cytokines and chemokines in BALF. Lungs were lavaged on day 1, 3 and 7 after IPS induction and the concentrations of TNF-α, IFN-γ, IL-4, CCL5, CXCL9 and CXCL10 in BALF were analyzed by enzyme-linked immunosorbent assay. Each data represents mean ± SD four to six mice per group. **p < 0.01 with respect to IPS group.

CB-MSCs induced the shift of Th1/Th2 balance toward Th2 polarization in the lung tissue

As shown in Fig. 7, the mRNA level of T-bet in lung tissues of mice was significantly higher in IPS group than IPS+MSCs group at 3 (p < 0.05) and 7 days (p < 0.01) after IPS induction, whereas no significant difference in GATA-3 mRNA level was observed between cells (Fig. 6B). Additionally, we detected the CXCR3, CCR5 and CCR7 mRNA levels in lung tissues by real time RT-PCR. We found that CXCR3 mRNA in each group was clearly increased after irradiation, and this increase was further expanded by allo-HSCT. However, the level of CXCR3 mRNA was significantly decreased when CB-MSC added. Also, CCR5 mRNA in simple irradiation group was no significant increase when compared with normal control group, but it was clearly increased in the IPS group, and CB-MSCs significantly down-regulated the increased level of CCR5 mRNA. As for CCR7, there was no significant difference in mRNA level between the simple irradiation and the normal control group. Although the level of CCR7 mRNA in the mice of IPS group was not significantly increased, CB-MSC markedly upregulated its expression (Fig. 6). These results indicated that CB-MSCs up-regulated CCR7, and down-regulated CCR5 and CXCR3 expression after IPS induction.
IPS and IPS+MSCs groups (p > 0.05). Correspondingly, T-bet/GATA-3 ratio was significantly decreased in IPS+MSCs group when compared with those in IPS group at 3 (p < 0.01) and 7 days (p < 0.01) after IPS induction. These data suggested that CB-MSCs induced a marked
decrease in Th1 type immune responses, leading to a shift of Th1/Th2 balance toward anti-inflammatory Th2 polarization in lung tissues of IPS mice.

**Discussion**

In this study, BALB/c recipient mice transplanted with allogeneic bone marrow plus splenocytes after lethal TBI developed a progressive pulmonary inflammation. The mice from IPS group exhibited the manifestations of severe GVHD and lung injury, which are similar to that of IPS in human allo-HSCT recipients including lung edema, alveolar hemorrhage, leukocyte infiltration, and alveolar wall thickening with severe inflammatory responses. Also, the infiltration of CD4+ and CD8+ T cell in the lung of IPS mice is significantly increased through immunohistochemical analysis. Furthermore, the increased inflammatory cytokines (TNF-α and IFN-γ) and chemokines (CCL5, CXCL9 and CXCL10) levels were observed in the BLAF of IPS-induced mice. Correspondingly, CCR5 and CXCR3 were also significantly increased, either in peripheral blood T cells or lung tissues after IPS induction. Additionally, the
severity of lung injury was correlated with a decrease of Treg percentage and the imbalance of Th1/Th2 immunity in the lung. These findings suggested that distributing imbalance of inflammatory cytokines, Th1-associated chemokines and their receptors might be a primary cause for the development of IPS. These changes promoted donor T cells infiltration into lung, which contributes to the evolution of IPS.

Recently, MSCs has been used to prevent or treat GVHD in some clinical trials [24-26]. It should be noted that MSCs is not spontaneous inhibitor, its immunosuppressive activities are closely related to their environment. MSCs can acquire more pronounced immunosuppressive activity if provided by inflammatory cytokines in the environment [17, 30, 31]. Polchert et al. [31] found that IFN-γ-treated MSCs were more effective in the prevention of GVHD in vivo. Joo et al. [32] reported that intravenously infused MSCs first reach the lungs, then migrate to the gastrointestinal tract, lymph nodes and skin in a mouse model of GVHD. Above findings provide some clues for using MSCs to prevent or treat IPS. That is, (1), MSCs can enter lung tissue and exert a direct effect in the local; (2), the lung injury due to IPS provides a high level inflammatory cytokines in the lung that is necessary to elicit the immunosuppressive effect of MSCs.

In the present study, we have demonstrated the potential of a new therapeutic approach for IPS through the co-transplantation of CB-MSC in a mouse model. Our data showed that CB-MSCs transplantation attenuated the severity of lung injuries and increased survival of IPS mice. In addition, we also confirmed that CB-MSCs co-transplantation significantly reduced the infiltration of CD4+ and CD8+ T cells into the lung of IPS mice. The ability of CB-MSCs to prevent the development of or improve IPS is due to their profound immunomodulatory capacity in vivo, and there are probably at least four potential mechanisms as follows: (1) suppress the release of several inflammatory cytokines and Th1-like chemokines in the lung, block the enlargement of inflammatory response and lower the concentration gradient of chemokines between lung tissue and blood flow, thus reducing donor effector cells recruitment to the lung; (2) promote the expansion of Tregs and cause an obvious increase of Treg ratio in both BALF and PB, induce the immune tolerance of lung tissues against alloantigens; (3) regulate the expression profiles of chemokine receptors, reduce alloreactive T cells migration to the lung and promote them homing to secondary lymphoid tissue through increasing expression of CCR7 on T cells; (4) dampen Th1 cell responses and increase Th2 relative to Th1 activity in the lung by regulating expression of transcription factor T-bet and GATA-3 genes, resulting in the imbalance of Th1/Th2 toward anti-inflammatory Th2 polarization, further mitigating the lung damages caused by Th1-associated immunity.

Tregs are thought to have a critical role in the suppression of immune responses and induction of immune tolerance [33, 34]. In vivo setting, several studies had reported the protective effects of MSCs by directly inducing the generation of Tregs in transplant model and some models of allergic and autoimmune disease [35-37]. In consistent with these studies, we found that CB-MSCs cotransplantation significantly increased Treg percentage, either in the BLAF or PB from IPS mice. These data suggest that expansion of Tregs might be one of the protective mechanisms of CB-MSCs during IPS.

Some published data indicated that CCR5, CXCR3 and CCR7 were closely related to the inflammatory reaction and transplantation immunology [9-11]. CCR5 and CXCR3 mainly mediated effector lymphocytes trafficking towards sites of inflammation, whereas CCR7 was responsible for directing T lymphocytes homing to secondary lymphoid tissue [38]. The interactions between chemokines receptors and their cognate ligands play critical role in the development of IPS [12-13]. The data from our study showed that CB-MSCs co-transplantation significantly down-regulated CCR5 and CXCR3, while up-regulated CCR7 expression after IPS induction, which contributes to the migratory characteristics alternation of donor T cells. It was conceivable that MSCs transplantation regulate not only the migration of T cells but also their activation and differentiation.

The balance between Th1/Th2 is crucial for immunoregulation and its imbalance was involved in the development of GVHD [39]. Some researchers had observed the cytokine
profile shift in Th1/Th2 balance toward the anti-inflammatory phenotype Th2 following allogeneic MSCs administration [40]. Our findings showed that CB-MSCs co-transplantation primarily decreased the mRNA level of T-bet, while not markedly affected GATA-3 mRNA level, and caused a decrease of T-bet/GATA-3 ratio. These data indicated that CB-MSCs could exhibit a protective effect on IPS mice by shifting from a Th1 to Th2 type response.

In conclusion, our results indicated that CB-MSCs co-transplantation increases survival of mice and attenuates the severity of lung injuries in a murine IPS model. The data presented in this manuscript also provide a mechanistic explanation for the prophylactic effect of CB-MSCs in the development of IPS. Although the details of the functional mechanisms of CB-MSCs in clinical usefulness require further investigation, CB-MSCs may offer a novel strategy for prevention and treatment of IPS.

Acknowledgments

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Disclosure Statement

The authors indicate no potential conflicts of interest.

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