

PDL₁Ig gene-loaded BMSCs Induce liver transplantation immune tolerance

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Abstract. – OBJECTIVE: We investigated the effects of BMSCs, which was modified by programmed death ligand-1 immunoglobulin (PDL₁Ig) gene, on the immunological rejection of orthotopic liver transplantation (OLT) in rats.

MATERIALS AND METHODS: Rat BMSCs were cultured and modified by recombinant adenovirus pAdEasy-1/PDL₁Ig for 72h. The total protein was extracted, and the protein expression of PDL₁Ig after transfection was detected by Western blotting. Mixed lymphocyte reaction was applied to detect the inhibitory effect of BMSCs, including pre-transfection and post-transfection, on the cell activity of T lymphocytes in peripheral blood. The male Wistar rats were used as donors, and the male Sprague-Dawley (SD) rats were used as recipients. The improved cuff method was computed in OLT to establish the rat orthotopic liver transplantation model of acute rejection. The rats were randomly divided into 4 groups, including a control group (10 pairs), BMSCs treatment group (10 pairs), BMSCs/GFP (green fluorescent protein) treatment group (10 pairs), and BMSCs/PDL₁Ig treatment group (10 pairs). 5 rats in each group were randomly collected and euthanized at day 7 after the operation. The peripheral blood was gathered to detect levels of 3 types of cytokines, including interferon gamma (IFN- γ), interleukin-4 (IL-4), and IL-2. In addition, the liver function of rats was checked, and the pathological changes of liver transplantation were observed under the optical microscope. The remaining five rats in every group were used for measuring the survival situation and survival time.

RESULTS: After BMSCs were modified by a recombinant adenovirus pAdEasy-1/PDL₁Ig for 72h, the expression of PDL₁Ig in the BMSCs was detected. The inhibitory effects of BMSCs/PDL₁Ig on the cell activity of lymphocytes were stronger than that of BMSCs/GFP. The levels of IL-4 in BMSCs/PDL₁Ig group were significantly higher than those in the other 3 groups, and the levels of IFN- γ and IL-2 were significantly decreased ($p < 0.05$). The liver function of 4 groups was measured at day 7 after transplantation. Our results showed that the liver function in BMSCs/PDL₁Ig group was most significantly improved, and the levels of ALT, AST, and TBil al-

most recovered to normal. The differences were statistically significant compared to the control group, BMSCs treatment group, and BMSCs/GFP treatment group. Results of pathological examination of liver tissue showed that the control group underwent severe rejection of liver transplantation. The BMSCs treatment group and BMSCs/GFP treatment group also rejected the liver transplantation, but the degree was lighter when compared to the control group. In addition, there was almost no rejection of liver transplantation in the BMSCs/PDL₁Ig group. The recipient survival time of most rats was more than 100d, which was significantly longer than the other 3 groups ($p < 0.05$).

CONCLUSIONS: PDL₁Ig-modified BMSCs can inhibit the rejection of liver transplantation in rats, induce the formation of the immune tolerance of liver transplantation, and the effect was more significant than that of BMSCs alone.

Key Words

Programmed death ligand-1, Immunoglobulin, Adenovirus, Bone marrow mesenchymal stem cells, Immune tolerance of liver transplantation.

Introduction

Bone marrow mesenchymal stem cells (BMSCs) are a type of stem cells with various types of function, including multi-differential potentialities and immunomodulatory effects, which could effectively prolong the survival time of grafts. Programmed death ligand-1 immunoglobulin is one of the cell surface proteins on many types of cells, including inflammatory cells, malignant tumor cells, cells of parenchymal organs (such as heart, placenta and lung), antigen presenting cell (APC), and so on, and it plays an important role in the immune negative regulation. The mechanism may lie in the fact that T lymphocyte activation is inhibited by blocking the second signaling pathway, which is necessary for T lymphocyte activation; then, the immune responses of self-antigens

are effectively inhibited, and it plays an important role in maintaining the central and peripheral immune tolerance. In recent years, there were several studies about organ transplantation immune¹. Due to the fact that second signal pathway contains a number of pathways, and the application of PD-L₁Ig can only block one of them, it cannot guarantee the long-term survival of the graft. Therefore, this experiment combines PDL₁Ig with BMSCs. Studies showed that, without affecting its function and status, BMSCs can stably transfect exogenous gene and express related proteins²⁻⁴. In this study, the recombinant adenovirus pAdEasy-1/PDL₁Ig was made and transfected into BMSCs to discuss its effect on the inhibition of acute rejection reaction of allogeneic transplantation.

Materials and Methods

Animal

Adult male Sprague Dawley (SD) rats (40) and male Wistar rats (40) with a body weight of 250 g were provided by the Experimental Animal Center at our hospital. The related drugs and instruments were provided by our laboratory. The Ethics Committee of our hospital approved the study.

Main Reagents

The ELISA Kit for IL-2, IL-4, and IFN- γ was provided by Wuhan Boshide Biological Engineering Co. Ltd., Wuhan, China. The recombinant adenovirus pAdEasy-1/PDL₁Ig was already completely described in a previous work. The CCK-8 kit of cell viability assay (Roche Co., Basel, Swiss); Western blot kit and fetal bovine serum (Hyclone, Logan, UT, USA), L-DMEM medium and 0.25% trypsin [Dulbecco's Modified Eagles Medium (DMEM) Gibco, Grand Island, NY, USA], flow cytometry (Beckman Coulter, Fullerton, CA, USA), inverted microscope and U-RFLT50 microscope light source system (Olympus, Tokyo, Japan) were obtained commercially.

Main Methods

Isolation, Culture, and Identification of BMSCs

SD rats were selected and euthanized by the cervical dislocation technique 7 days after the operation. The femur and tibia were separated and the medullary cavity of them was repeatedly washed by the LG-DMEM medium until the bone changes from red to white. The shedding cells were put into the culture flask and cultured in the incubator at a constant temperature (37°C and 5% CO₂). The cul-

ture solution was replaced the first time after 24 h; then, the frequency was 1 time/every 3 days. When cell confluence reaches 80%, 0.25% trypsin was added and deciduous cells were cultured until the twentieth generation. After, 0.25% trypsin was added again, centrifuged and the supernatant was abandoned. Precipitated cells were collected, suspended in 10 mL phosphate buffered saline (PBS) and transferred to a 10 mL centrifuge tube and then centrifuged again. The supernatant was discarded, the cells were washed 3 times, and finally, cell sedimentation was collected. An inverted microscope was used to count cells, and cells were diluted to 1×10^7 cells/mL cell suspension with PBS dilution. 100 μ L of the cell suspension and 5 μ L of the cell surface antigen with markers (CD29, CD90, CD45, CD34) were uniformly mixed and added to the flow tube, which was standard and sterile; then, they were placed in the refrigerator (4°C), and incubated for 30 min. Then, cells in the bottom were collected, and the prepared samples were managed by flow cytometry to detect the expression of the antigen.

Recombinant pAdEasy-1/PDL₁Ig Transfected with BMSCs and Detection of the Target Protein

According to the concentration [multiplicity of infection (MOI)=200], the recombinant pAdEasy-1/PDL₁Ig virus was transfected into BMSC; the transfection rate reached 90%. Then, the BMSCs were placed in the incubator (37°C, 5% CO₂). After 72h, cells were washed 2 times with phosphate-buffered saline (PBS) and diluted to a concentration of 4×10^6 cells/mL. The total protein was extracted and the expression of PDL₁Ig in BMSCs was detected by Western blot. The fluorescence microscopy was used to observe green fluorescent protein (GFP).

The Identification of BMSCs After Gene Transfection

After transfection, the surface markers on BMSCs, including CD29, CD90, CD34, and CD45 were measured by flow cytometry. An inductive agent was used *in vitro* to induce osteogenic differentiation and adipogenic differentiation of BMSCs of the twentieth generation. Cells were inoculated into the 6 well plate (1×10^5 /well); then, an inductive agent was added after 24h. The inducers of osteogenic differentiation and adipogenic differentiation of BMSCs respectively were L-DMEM medium (10% fetal bovine serum, 60 μ mol/L indometacin, 5 μ g/mL insulin, 0.5 mmol/L 1-methyl-3-isobutyl-xanthine and 1

μmol/L dexamethasone) and L-DMEM medium (10% fetal bovine serum, 100 nmol/L dexamethasone, 0.2 mmol/L ascorbic acid 10 mmol/L and β-glycerophosphate). Then, cells were cultured with a periodic replacement of the culture solution (2 times/week). 2 weeks later, BMSCs was induced to differentiate into adipocytes and bone cells; haematoxylin-eosin staining was used to detect mineral deposits in bone cells, and red oil staining was applied to detect the neutral lipid droplets in the adipose tissue.

Mixed Lymphocyte Reaction (MLR)

Wistar rat spleen cells were regarded as a stimulator, SD rats T cells were regarded as reaction cells. On one-way mixed lymphocyte experiment, BMSCs were joined into one group, and BMSCs transfected with PDL₁Ig were put into another group; then, the CCK-8 kit was applied to detect the activity of T lymphocytes.

The Establishment and Grouping of Experimental Animal Models

The inbred male Wistar rats (40) and male SD rats (40), with a body weight of 250 g, were selected to establish the rat orthotopic liver transplantation model of acute rejection. The male Wistar rats were used as donors; the SD rats were used as recipients. Improved cuff method was computed in orthotopic liver transplantation OLT. After OLT, these rats were randomly divided into 4 groups, including a control group (10 pairs), BMSCs treatment group (10 pairs), BMSCs/GFP treatment group (10 pairs), and BMSCs/PDL₁Ig treatment group (10 pairs). When liver transplantation occurred, BMSCs were infused through a portal vein into each group. 5 rats in each group were randomly collected and euthanized at day 7 after the operation. The peripheral blood was gathered to detect levels of 3 types of cytokines, including interferon gamma (IFN-γ), interleukin-4 (IL-4), and IL-2. At 9th day after the operation, collected rats were executed their liver tissues were obtained. Then, the liver tissues was embedded with paraffin, made into pathological sections and stained with HE. The pathological changes of liver transplantation were observed under the optical microscope. The remaining five rats in every group were used for measuring the survival situation and survival time.

Statistical Analysis

The SPSS11.0 software (SPSS Inc., Chicago, IL, USA) was applied for statistical analysis. Measurement data of each groups were shown by $\bar{x} \pm s$. A one-way ANOVA and SNK-q test were applied in

the comparison among the 4 groups. Kaplan-Meier survival curves of 4 groups of rats were drawn, and curves were compared using the Log-rank test. $p < 0.05$ was considered statistically significant.

Results

The Culture, Identification and Induction In Vitro of BMSCs and the Osteogenic Differentiation and Adipogenic Differentiation of BMSCs

Even if continuous passaging can purify BMSCs, after the 20th generation, the growth rate of part of BMSCs will gradually slow down and the cell cycle will extend; cells become fibroblast-like shape, wide and flat, some cells are polygonal or fusiform. At the same time, cell debris in the culture supernatant of BMSCs increase, as seen in Figure 1. Flow cytometry was used to compute and detect CD29, CD34, CD45, and CD90 molecules on the surface of BMSCs, which were the twentieth generation. Results showed that there was high expression of CD29 and CD90 on the surface of BMSCs (98.6%, 99.7%), while CD34 and CD45 were low expressed (1.8%, 5.0%), as seen in Figure 2. The inductive agent, which was added *in vitro*, deduced BMSCs osteogenic differentiation. At day 3, BMSCs changed from long fusiform shape to short fusiform shape, and the cell volume increased. At day 7, BMSCs changed from short fusiform shape to polygonal shape, and cytopla-

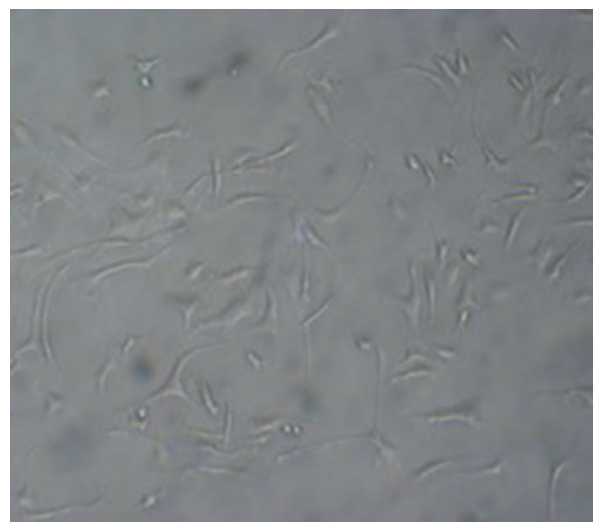


Figure 1. Cultured bone marrow mesenchymal stem cells (×100), looked like fibroblast with broad, flat, and some were polygonal or fusiform shapes.

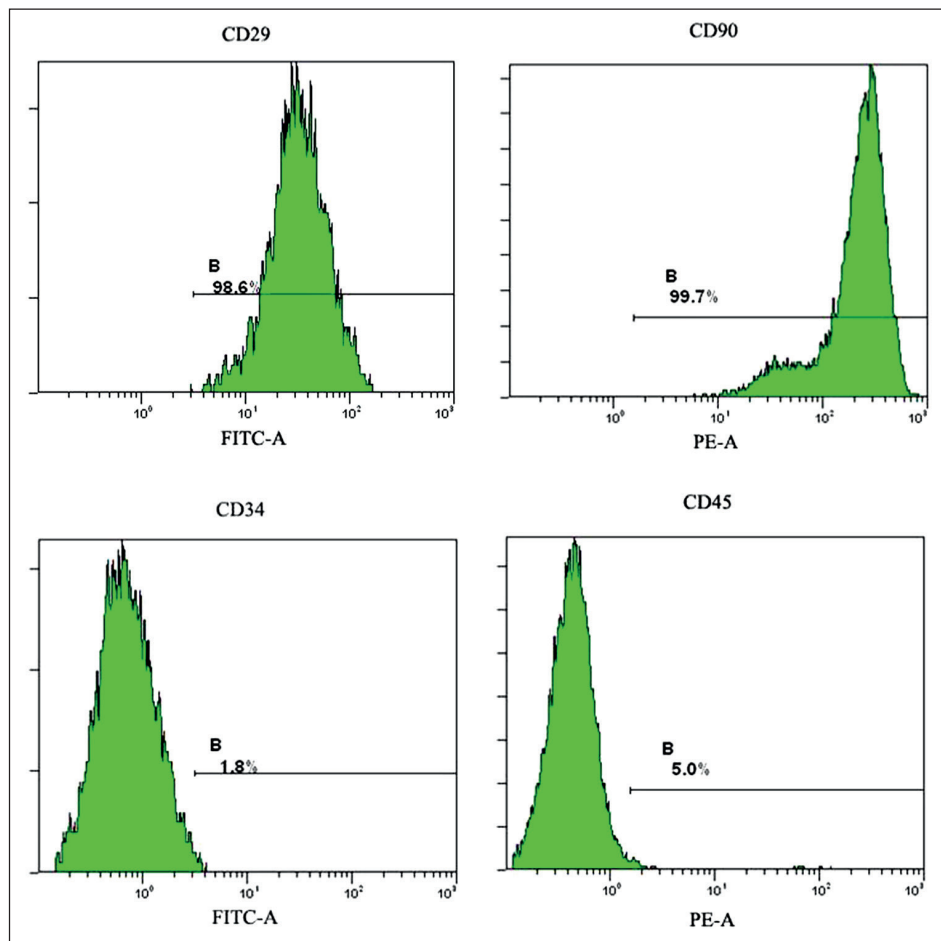


Figure 2. The surface markers of BMSCs were detected by flow cytometry with high expression of CD29, CD90 and low expression of CD34, CD45.

smic granules increased gradually. At day 14, BMSCs showed colony growth, the cytoplasm was filled with particles, and calcium deposition appeared in intercellular space. At the day 21, BMSCs gradually fused from colony growth, cell structure gradually loosened, calcium nodules between cells gradually increased, and through alizarin red staining, the color of calcium nodules were red, which indicated that the BMSCs turned into osteocytes. The inductive agent, added *in vitro*, deduced BMSCs adipogenic differentiation. After 72h, small fat droplets began to appear in cells and after about 2 weeks, the number of small fat droplets increased. The small fat droplets fused, and the shape of BMSCs changed from long fusiform to round or polygonal. The results of red oil staining showed that a large number of deep red lipid deposition appeared in cells, which indicated that BMSCs turned into adipocytes. These studies showed that BMSCs

have the ability of osteogenic differentiation and adipogenic differentiation, as shown in Figure 3.

The Expression of PDL₁Ig Gene in BMSCs After Transfection

After BMSCs were modified by a recombinant pAdEasy-1/PDL₁Ig for 72h, the expression of PDL₁Ig in the BMSCs after transfection was detected through Western blot; the results were shown in Figure 4. Figure 4A represented the lysate after 72h, from pAdEasy-1/PDL₁Ig transfected BMSCs, and the detection of specific band with a molecular weight of 35.5 kD accorded with the molecular weight of a PDL₁Ig protein. Figure 4B represented lysate of BMSCs. Figure 4C represented lysate of BMSCs/GFP; the results showed that PDL₁Ig could be expressed after recombinant pAdEasy-1/PDL₁Ig transfected BMSCs. The green fluorescence after transfection also showed the expression of target gene in BMSCs, as shown in Figure 4.

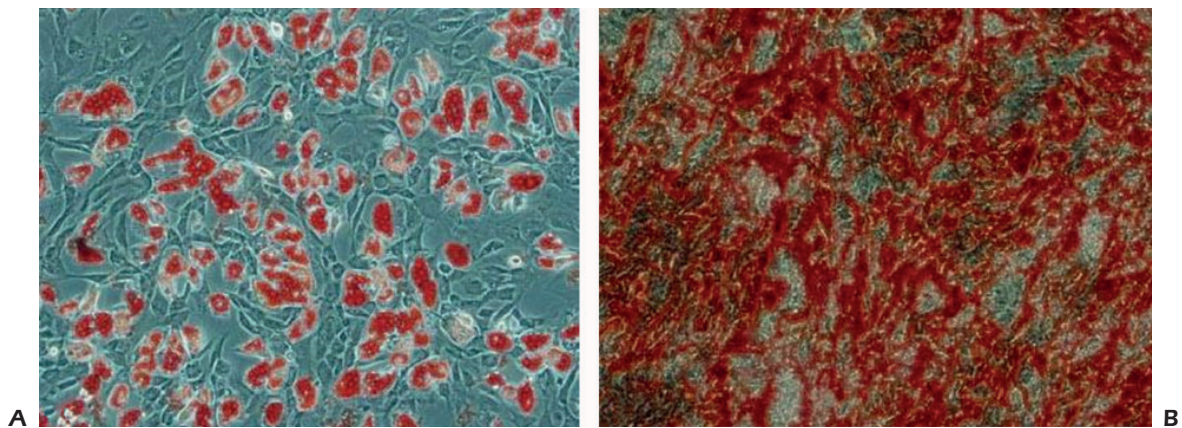


Figure 3. The adipogenic (A) and osteogenic (B) differentiations of the BMSCs ($\times 200$).

The Identification of “Dry” and “Pluripotent” BMSCs After Transfection

Flow cytometry was computed to detect the surface markers of BMSCs after transfection. The results showed that CD29 and CD90 on the surface of BMSCs were highly expressed (97.3%, 99.1%), while CD34 and CD45 were lowly expressed (2.1%, 4.8%), as seen in Figure 5. The induction of osteogenic differentiation and adipogenic differentiation of BMSCs verified that BMSCs, after transfection, were still BMSCs, as seen in Figure 6.

The Comparison of BMSCs in the Inhibition of Lymphocyte Activity in Peripheral Blood Before and After Transfection

After BMSCs were modified by a recombinant pAdEasy-1/PDL₁Ig for 72h; a mixed lymphocyte reaction was used to detect the effects of

the inhibition of lymphocyte activity. The result was 90.5%, and it was 42.3% in BMSCs treatment group. Therefore, BMSCs after transfection can be more effective in inhibiting lymphocyte activity ($p < 0.05$), as seen in Figure 7.

The Comparison of Survival Situation and Survival Time After Liver Transplantation in Rats

After liver transplantation of 4 groups of rats, the survival time was different, and they were as follows: 10 ± 4.1 d (control group), 23.4 ± 4 d (BMSCs group), 23.1 ± 3.9 d (BMSCs/GFP group), and 100 ± 5 d (BMSCs/PDL₁Ig group). The results showed that there was postoperative performance of rats in the BMSCs/PDL₁Ig group, which were better compared to the other 3 groups. At the 7d after liver transplantation, there were no evident abnormalities of activity condition, eating, drinking, and weight gain in rats of the BMSCs/PDL₁Ig group, compared to those before the

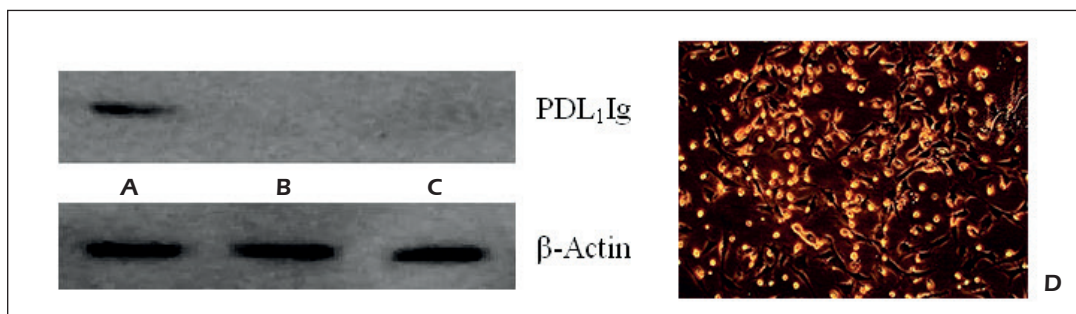


Figure 4. The expression of PDL₁Ig protein in transgenic BMSCs infected with recombinant PDL₁Ig determined by Western blotting and green fluorescent protein observation. **A**, BMSCs transfected with pAdEasy-1/PDL₁Ig for 72 h. **B**, BMSCs blank control. **C**, BMSCs transfected with pAdEasy-1 (empty vector). **D**, Green fluorescent protein indicated that PDL₁Ig was successfully expressed in the BMSCs after transfection ($\times 200$).

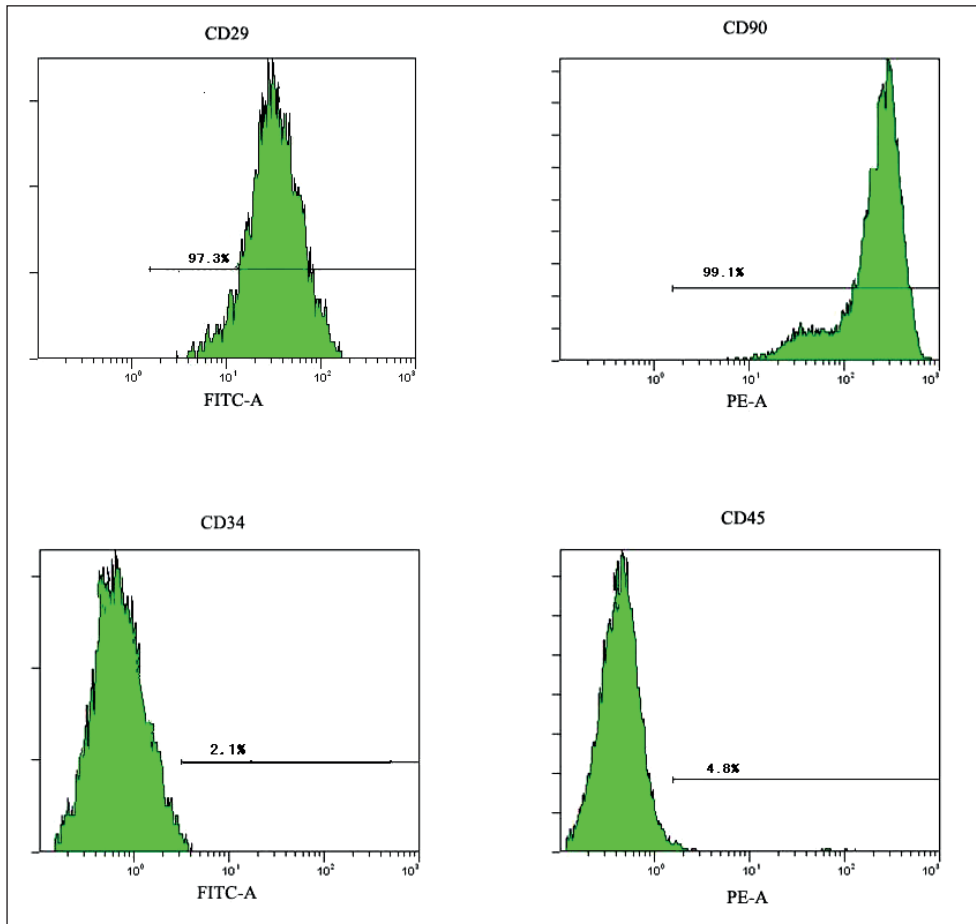


Figure 5. The surface markers of BMSCs/PDL₁Ig were analyzed by flow cytometry with high expression of CD29, CD90 and low expression of CD34, CD45.

surgery. In addition, the survival time of rats in BMSCs/PDL₁Ig group was longer, and most were more than 100d, which was significantly longer than the other 3 groups ($p < 0.05$). At the same time, the survival time of receptors in the BMSCs group and BMSCs/GFP group were longer than

those of the control group ($p < 0.05$). There were no statistically significant difference in the comparison of the survival time of receptors between the BMSCs group and BMSCs/GFP group ($p > 0.05$). The comparison of Kaplan-Meier survival curves was shown in Figure 8.

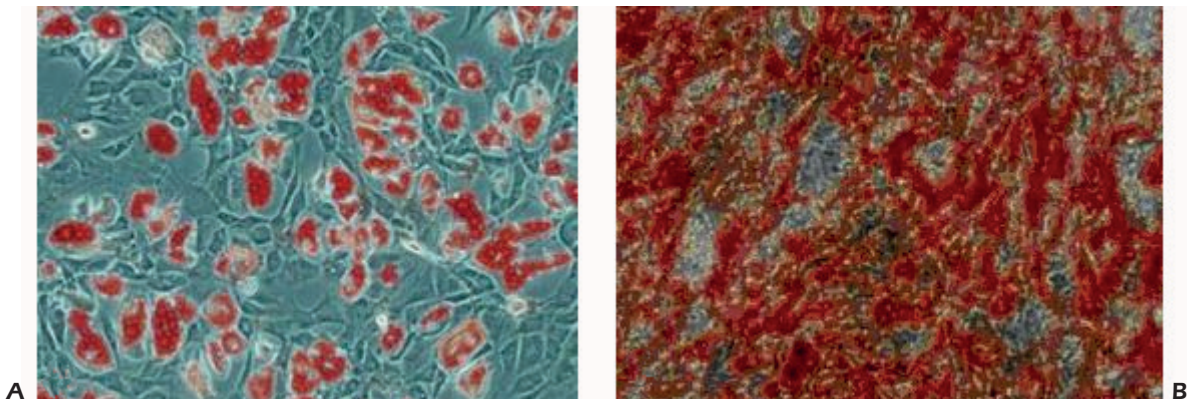


Figure 6. The adipogenic (A) and osteogenic (B) differentiations of the BMSCs after transfected with pAdEasy-1/PDL₁Ig ($\times 200$).

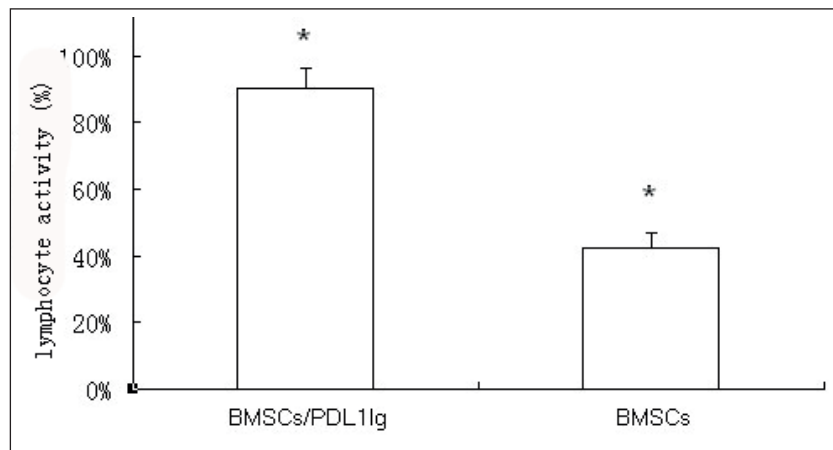


Figure 7. BMSCs inhibited the cell activity of T lymphocytes in peripheral blood by mixed lymphocyte reaction (MLR). The suppression effect of BMSCs/PDL₁Ig on the cell activity of T lymphocytes was 90.5%±5.8% and that of BMSCs was 42.3%±4.7%. (Mean±SD. *n*=10. *p*<0.05 vs BMSCs).

The Comparison of Cytokines Levels in Peripheral Blood After Liver Transplantation in Rats

At day 7, after BMSCs were modified by a recombinant pAdEasy-1/PDL₁Ig, the cytokine levels of IFN- γ , IL-4, and IL-2 in the peripheral blood were detected; the results were showed in Table I. The levels of IL-4 in the BMSCs group and BMSCs/GFP group were significantly higher than those in the control group; differences were statistically significant (*p* < 0.05), but there was no significant difference between the BMSCs group and BMSCs/GFP group (*p* > 0.05). The level of IL-4 in the BMSCs/PDL₁Ig group was signifi-

cantly higher than those in the other 3 groups; differences were statistically significant (*p* < 0.05) compared with the control group (*p* < 0.01). The levels of IL-2 and IFN- γ in the BMSCs group and BMSCs/GFP group were significantly lower than those of the control group (*p* < 0.05), while the levels of IL-2 and IFN- γ in BMSCs group and BMSCs/GFP group were similar; differences were not statistically significant (*p* > 0.05). The level of IL-2 and IFN- γ in BMSCs/PDL₁Ig group was significantly lower than those in the other 3 groups; differences were statistically significant (*p* < 0.05), which was compared with the control group (*p* < 0.01), as seen in Table I.

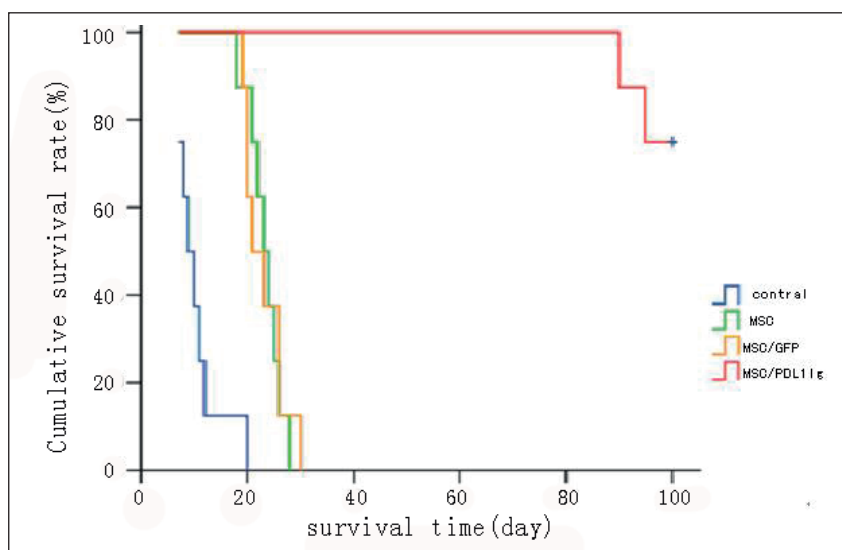


Figure 8. Kaplan-Meier survival curves of the rats in 4 groups. Rats survival time of 4 groups after liver transplantation were 10 ± 4.1d (control group), 23.4 ± 4d (BMSCs group), 23.1 ± 3.9d (BMSCs/GFP group), 100 ± 5d (BMSCs/ PDL₁Ig) respectively.

Table I. Comparison of the levels of cytokines of the rats in 4 groups after liver transplantation (ng/L. Mean±SD. n=5).

Treatment	IL-2	IL-4	IFN-γ
Control	86±9	55±7	83±12
BMSCs	42±7*	143±14*	54±8*
BMSCs/GFP	40±8*	139±17*	59±6*
BMSCs/PDL ₁ Ig	26±4*#	239±15*#	31±6*#

#BMSCs/PDL₁Ig group compared with BMSCs group and BMSCs/GFP group ($p < 0.05$).

*Each group compared with control group ($p < 0.05$).

**BMSCs/PDL₁Ig group compared with control group ($p < 0.01$).

The Comparison of Liver Function After Liver Transplantation in Rats

At day 7, after liver transplantation, the liver function of the 4 groups of rats was detected. The results showed that liver function of rats in the BMSCs group and BMSCs/GFP group improved, which were shown through the significant decrease in levels of total bilirubin and aminotransferase compared with the control group ($p < 0.05$). There was a significant statistical difference in the comparison between the BMSCs/PDL₁Ig group and the control group ($p < 0.01$). There were no significant difference in the comparison between the BMSCs group and the BMSCs/GFP group ($p > 0.05$). The improvements of liver function in 4 groups of rats were compared to each other, and the degree in the BMSCs/PDL₁Ig group had the highest improvement. At day 7, after liver transplantation, the liver function of rats in the BMSCs/PDL₁Ig group were essentially the same as that of the normal rats, and there were significant differences compared with other groups ($p < 0.05$), as seen in Table II.

The Comparison of Pathologic Results After Liver Transplantation in Rats

At day 9, after liver transplantation of the 4 groups of rats, pathological examination of hepatic tissue was conducted and results showed that rejection reaction appeared in all groups. The severity from severe to moderate was as follows: control group, BMSCs group, BMSCs/GFP group, and BMSCs/PDL₁Ig group. Typical and severe rejection reaction appeared in the control group where the manifestation was most serious, such as a liver cell disorder, liver cell degeneration and necrosis, inflammation of bile duct damage, a large number of lymphocytes and eosinophils infiltration in the portal area, inflammatory cells infiltration in central vein, necrosis area appeared in part of liver parenchyma, and so on. The

Table II. The liver function of the recipients after 7 d of liver transplantation (Mean±SD. n=5).

Treatment	TBiL (μmol/L)	ALT (IU/L)	AST (IU/L)
Control	185.3±43.3	383.8±34.8	356.7±61.3
BMSCs	57.1±24.1*	154.3±14.1*	134.6±11.9*
BMSCs/GFP	61.2±7.2*	141.4±9.2*	123.9±13.7*
BMSCs/PDL ₁ Ig	15.6±4.3*#	38.2±4.5*#	30.3±7.5*#

#BMSCs/PDL₁Ig group compared with BMSCs group and BMSCs/GFP group ($p < 0.05$).

*Each group compared with control group ($p < 0.05$).

**BMSCs/PDL₁Ig group compared with control group ($p < 0.01$).

rejection reaction in the BMSCs group and BMSCs/GFP group were more moderate, and were shown by the inflammatory cell infiltration of bile ducts and blood vessels in the minority of the portal area. The rejection reaction of the BMSCs/PDL₁Ig group was the lightest where there was almost no rejection reaction. The liver tissues were similar to that of normal rats; for example, the hepatic lobules were arranged orderly, and only a small amount of neutrophils or lymphocytes in portal space, as seen in Figure 9.

Discussion

Bone marrow mesenchymal stem cells (BMSCs) are a type of stem cells with many functions, containing multi-differential potentialities and immunomodulatory effects. They were derived from the mesoderm in the early development of embryos. Due to the low expression of the MHC-I class molecule, and the non-expression of MHC-II class molecule and second signaling molecules on the surface of BMSCs, BMSCs don't have the ability to stimulate the proliferation and differentiation of T lymphocyte⁶. BMSCs can secrete a variety of cytokines, such as IL-10, hepatocyte growth factor (HGF), transforming growth factor TGF-β1, and so on. They could play a role in a variety of functions, such as the inhibition of the activation of T lymphocytes^{7,8}, induce the differentiation of helper T cells to Th1/Th2 cells, and increase the proportion of Th2 cells, the promotion of the secretion of IL-4, and the reduction of the secretion of IL-2 and IFN-gamma. They could achieve a role in the immune regulation^{9,10}. Through the differentiation to liver cells, the damaged liver function can be repaired. BMSCs can be transfected

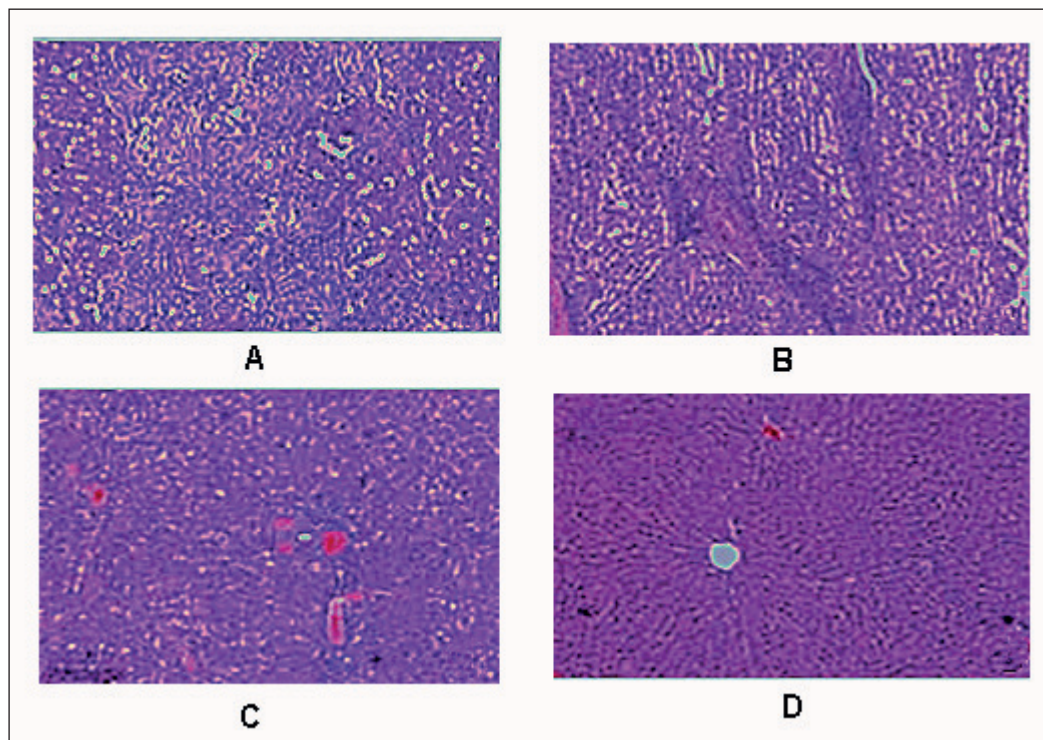


Figure 9. The pathological changes in the liver tissues of the rats in 4 groups on the 9th d after transplantation (HE, $\times 100$). **A**, The rejection of control group was severe and liver cells arranged in disorder, partial degeneration and necrosis. **B-C**, The rejections of BMSCs group and BMSCs/GFP group were similar and mild. **D**, The rejection of BMSCs/PDL₁Ig group was the slightest and almost similar to the normal liver tissue seemingly.

by an exogenous gene and express the exogenous gene without damaging and repairing function, immune regulation function, and differentiation effect of BMSCs. Through the direct contact and the secretion of negative regulation cytokines, BMSCs can inhibit the role of antigen presenting cells (APC), and so on. These functions can reduce the rejection reaction of organ transplantation to a certain extent^{11,12}.

In this study, mixed lymphocyte reaction was computed to detect the vitality of lymphocytes. The results showed that BMSCs have the effect of inhibiting the activity of lymphocytes, and the inhibitory effect of BMSCs, transfected with PD-L1Ig, was stronger. After orthotopic liver transplantation was conducted in rats, the BMSCs had many functions. For example, BMSCs can inhibit the rejection reaction to prolong the survival time; it can reduce the levels of IFN- γ and IL-2 and elevate the levels of IL-4 and alleviate post-operative rejection in rats. Rats in the BMSCs/PDL1Ig group also have the functions above, and these were stronger. Therefore, we confirm that both *in vivo* and *in vitro*, BMSCs can inhibit the occurrence of rejection reaction in allogeneic liv-

er transplantation¹³. The occurrence of rejection reaction in organ transplantation involves multiple pathways, and blocking one of them cannot restrain the rejection effectively. Therefore, we believe that we need to block multiple paths¹⁴. We found that although BMSCs can clearly inhibit rejection, it is also possible to differentiate into tumor tissue, with a certain degree of risk. BMSCs has the role of inhibiting T lymphocytes; however, this effect could disappear through the combination of Toll-like receptor (TLR) on the surface and its ligand. At the same time, BMSCs can express MHC-I class molecules, and even if the MHC molecules of donor and receptor do not match, the rejection reaction could also be triggered through activating CD8⁺T cells, which causes the damage of transplanted organs¹⁵. Therefore, BMSCs alone could not induce transplantation immune tolerance effectively. Lafferty's "double signal" theory indicates that the rejection reaction can be accomplished by the activation of T lymphocytes, which requires the interaction of the first and the second signals. Researches have shown that if the second signal is short, T-lymphocytes could not be activated effectively, and the secretion of IL-2 is sig-

nificantly decreased, which accounts for 1/30 of the secretion of IL-2 when the first signal and the second signal have a combined action¹⁶. Previous studies have shown that the second signal pathway has a number of paths, in which the CD28-B7 pathway is the clearest, which can be effectively blocked by PDL₁Ig to reduce the rejection of organ transplantation⁵. Exogenous genes can be transfected into BMSCs; exogenous protein can be effectively expressed without affecting the function of damage and repair, immunomodulatory effects and differentiation of BMSCs¹⁷. A costimulatory pathway may be blocked and BMSCs can induce immune tolerance.

Conclusions

We suggest that the possible mechanism lies in BMSCs, as exogenous antigen receptors, which can be attacked by the immune system and a part of them may be consumed. Therefore, BMSCs have difficulty in fully playing a role in immune suppression. After a recombinant adenovirus PD-L₁Ig transfected BMSCs, the immunosuppressive effect, through blocking the second signaling pathway, can protect BMSCs from the damage, prolong the survival period, and ultimately create a negative immune response (1+1>2); then, achieve liver transplantation immune tolerance in rats. These results have provided a new idea for clinical liver transplantation immune tolerance.

Conflict of Interest:

The Authors declare that they have no conflict of interests.

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