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Interferon-γ and tumor necrosis factor-α promote the ability of human placenta–derived mesenchymal stromal cells to express programmed death ligand-2 and induce the differentiation of CD4$^+$interleukin-10$^+$ and CD8$^+$interleukin-10$^+$Treg subsets

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Abstract

Background aims. Mesenchymal stromal cells (MSCs) and regulatory T cells (Treg) have been successfully used in treating autoimmune diseases accompanied by abundant inflammatory cytokines such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α. Therefore, this work investigated the effects of IFN-γ and TNF-α on the ability of human placenta-derived mesenchymal stromal cells (hPMSCs) on inducing the differentiation of CD4$^+$interleukin (IL)-10$^+$and CD8$^+$IL-10$^+$Treg subsets. *Methods.* Human PMSCs were co-cultured with T cells in the presence or absence of a trans-well system or anti-programmed death ligand-2 (PDL2) monoclonal antibody (mAb), respectively. CD4$^+$IL-10$^+$and CD8$^+$IL-10$^+$Treg subsets, as well as the levels of IL-10 in the supernatants, were detected on this basis. Examinations were conducted to explore the impact of IFN-γ and TNF-α on the expression of PDL2 in hPMSCs. In this process, flow cytometry, Western blot and reverse-transcriptase–polymerase chain reaction were used. *Results.* CD4$^+$IL-10$^+$and CD8$^+$IL-10$^+$Treg subsets from T cells either non-activated or activated by use of phytohaemagglutinin (PHA) or CD3/CD28mAb significantly increased in the presence of hPMSCs. However, these levels markedly decreased after blocking the expression of PDL2 in hPMSCs. IL-10 followed the same pattern. Furthermore, the percentages of CD4$^+$IL-10$^+$ and CD8$^+$IL-10$^+$T cells also sharply declined under the trans-well system, whereas the percentages as well as the expression of PDL2 in hPMSCs oppositely raised after hPMSCs pre-stimulated by IFN-γ and TNF-α. IFN-γ could promote the expression of PDL2 partly through the JAK/STAT signaling pathway. *Conclusions.* IFN-γ and TNF-α could promote the ability of hPMSCs in inducing the differentiation of CD4$^+$IL-10$^+$and CD8$^+$IL-10$^+$Treg subsets and enhance the expression of PDL2 in hPMSCs. These would benefit the application of hPMSCs in clinical trials.

Key Words: *human placenta, IFN-γ, mesenchymal stromal cells, PDL2, TNF-α, Treg subsets*

Introduction

The mechanism of therapeutic effects of mesenchymal stromal cells (MSCs) is mediated partly through its immuno-regulatory properties, which make MSCs a new approach for preventing or treating autoimmune diseases such as multiple sclerosis (MS) [1–3]. The main immuno-regulatory property of MSCs is the regulation of various immune cell functions, such as inhibiting the proliferation of T cells and B cells, suppressing the functions of natural killer cells (NK) and affecting the maturation and differentiation of dendritic cells (DCs) [4–7]. The immunosuppressive ability of MSCs on T cells has been demonstrated in many aspects, such as inhibiting the proliferation of T

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cells and the secretion of interferon (IFN)-γ, as well as inducing regulatory T cells (Treg) such as CD4$^+$CD25$^+$FOXP3$^+$Treg [4,8,9]. Human placenta-derived mesenchymal stromal cells (hPMSCs) have been regarded as an ideal source for cell-based therapy because they are accessible and abundant from the placenta. In addition, we have shown that the immunosuppressive ability of hPMSCs was stronger than that of human bone marrow–derived mesenchymal stromal cells (hBMSCs) in our *in vitro* studies [10]. Meanwhile, in *in vivo* studies, it was demonstrated that transplantation of hPMSCs could reduce the symptoms of many autoimmune diseases, for instance, experimental autoimmune encephalomyelitis (EAE), a common animal model of MS [11]. Moreover, Mansilla *et al.* [12] found that induction of CD4$^+$CD25$^+$FOXP3$^+$Treg could reduce the severity of autoimmune diseases such as EAE. The CD4$^+$interleukin (IL)-10$^+$T-cell subset, a new Treg subset that is involved in the negative regulation in T-cell response, was found in a murine study [13]. However, the CD8$^+$IL-10$^+$T-cell subset has not been thoroughly explored. Moreover, it is not clear whether hPMSCs could induce the differentiation of CD4$^+$IL-10$^+$and CD8$^+$IL-10$^+$Treg subsets and in turn alleviate the severity of autoimmune diseases.

IL-10 is an important anti-inflammatory cytokine that plays a crucial role in maintaining immune tolerance and treating autoimmune diseases [14]. In our previous study, we demonstrated that hPMSCs could augment the secretion of IL-10 from activated T cells partly through programmed death ligand-1 (PDL1) [10]. PDL1 and programmed death ligand-2 (PDL2) are the members of B7 family, and the engagement of their receptor, programmed death 1 (PD-1), negatively regulates immune response [15]. In addition, we showed that PDL2 was highly expressed in hPMSCs and promoted hPMSC immunosuppressive ability on activated T cells [16]. Nevertheless, whether PDL2 was also involved in the secretion of IL-10 from the activated T cells regulated by hPMSCs is not clear. Type1 regulatory T cells (Tr1) have emerged as a key player in the prevention of autoimmunity. They produce high levels of the immunosuppressive cytokine IL-10 and confer protection against many autoimmune diseases. Thus, it is meaningful to detect whether PDL2 participates in the differentiation of CD4$^+$IL-10$^+$and CD8$^+$IL-10$^+$Treg subsets, two brand-new breakings of Treg, from the activated T cells induced by hPMSCs.

It was proven that IFN-γ and tumor necrosis factor (TNF)-α could enhance the immunosuppressive ability of MSCs [17]. The expression of chemokines and adhesive molecules in MSCs were upregulated by IFN-γ and TNF-α, resulting in more T cells adhering to MSCs [18,19] and consequently enhancing the immunosuppressive ability of MSCs. It was clear that MSCs could significantly induce the differentiation of the CD4$^+$CD25$^+$Foxp3$^+$Treg subpopulation and in turn enhance the immunosuppressive ability of itself. However, the effect of IFN-γ and TNF-α on the differentiation of CD4$^+$IL-10$^+$and CD8$^+$IL-10$^+$Treg subsets induced by hPMSCs, as well as the expression of PDL2 in hPMSCs remains to be elucidated.

In the present study, we investigated the possible mechanism of hPMSC immunosuppressive ability under the inflammatory conditions fabricated by IFN-γ and TNF-α. Our results showed that hPMSCs could induce T-cell differentiating into CD4$^+$IL-10$^+$and CD8$^+$IL-10$^+$Treg subsets mainly by cell-cell contact, regardless of whether T cells are activated by different stimulators, and these effects were enhanced by IFN-γ and TNF-α alone or in combination. Moreover, PDL2 crucially affected the differentiation of CD4$^+$IL-10$^+$and CD8$^+$IL-10$^+$Treg subsets and the secretion of IL-10 induced by hPMSCs from T cells. Besides, IFN-γ, synergizing with TNF-α, could promote the expression of PDL2 in hPMSCs, possibly through the JAK/STAT signaling pathway.

Methods

Isolation of primary hPMSCs

Human PMSCs were isolated as described in our previous study [16]. Briefly, human term placentas were obtained from unrelated donors who signed the informed consents. The placentas were carefully dissected, washed with phosphate-buffered saline, mechanically minced and digested with 0.1% collagenase IV (Gibco) for 30 min at 37°C. The digested tissue was filtered through a 100-μm nylon membrane to remove undigested fragments. Cells were collected and centrifuged at 524*g* for 10 min; they were re-suspended in Dulbecco's modified Eagle's medium–low glucose (Gibco) supplemented with 10% fetal bovine serum (FBS) (Gibco), 100 U/mL penicillin G and 100 U/mL streptomycin sulfate and were cultured at 37°C in a humidified atmosphere with 5% CO_2. The medium was replaced one to two times every week. Cells were identified through morphology under a light microscope and showed positive surface staining for CD44, CD29, CD166 and CD105 but negative surface staining for CD34, CD14 and CD45. The cells were used in the experiments after three passages [16].