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Human placenta-derived mesenchymal stem cells suppress T cell proliferation and support the culture expansion of cord blood CD34$^+$ cells: A comparison with human bone marrow-derived mesenchymal stem cells

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ABSTRACT

Human placenta-derived mesenchymal stem cells (hPMSCs) have been shown to possess immunosuppressive effects against T cells and support the expansion of hematopoietic stem/progenitor cells (HSPCs) from umbilical cord blood (UCB). However, the characteristics of hPMSCs compared with human bone marrow-derived mesenchymal stem cells (hBMSCs) are not fully understood. Here, we show that hPMSCs have similar regulatory effects on T cell activation, proliferation and cytokine secretion as hBMSCs and demonstrate that PDL1 and B7H4, negative co-stimulatory molecules, are involved in the T cell immunosuppressive activities of hPMSCs and hBMSCs, respectively. hPMSCs efficiently enhanced the expansion of CD34$^+$ cells from UCB compared with hBMSCs. Furthermore, hPMSCs maintained the expression of adhesion molecules (CD11a, CD44 and CD49e) in CD34$^+$ cells. Similar effects were observed for both hPMSCs and hBMSCs on CD34$^+$ cell chemotaxis and cytokine production, such as SDF-1α, IL-6 and SCF. Therefore, hPMSCs may be an ideal alternative source of hBMSCs for basic research and clinical applications, which may be significant in future efforts to explore the potential clinical utility of hPMSCs.

1. Introduction

A recent study showed that the use of umbilical cord blood (UCB) in hematopoietic stem/progenitor cell (HSPC) therapy reduced the rates of graft-versus-host disease (GVHD) compared with bone marrow (Wagner et al., 1996) and mobilized peripheral blood progenitor cell (Bensinger et al., 2001) transplantation, particularly in pediatric patients. However, one of the major obstacles in the widespread use of UCB in HSPC therapy is the low total nucleated cell and CD34$^+$ cell doses that can be transplanted, which may be associated with markedly delayed neutrophil and platelet engraftment and an elevated risk of graft failure (Schiller et al., 1994). To overcome this problem, several protocols have been developed in the last few years for the in vitro expansion of UCB cells. Human bone marrow-derived mesenchymal stem cells (hBMSCs), one of the components of the hematopoietic microenvironment of specialized cells, have been used for the expansion of UCB cells (Andrade et al., 2010; Delalat et al., 2009). In addition, hBMSCs have the property of multilineage differentiation under specific permissive conditions. Therefore, the therapeutic applications of hBMSCs are expected to be wide ranging, from organ repair to gene therapy.

Further interest in the clinical application of hBMSCs has been raised by a study that showed that hBMSCs can exert profound immunosuppression not only by inhibiting T cell responses to various challenges (Kode et al., 2009) but also by modulating the functions of other immune cells in vitro (Caplan, 2009). In addition to GVHD, other clinical trials have been completed or are currently ongoing on treating various diseases using hBMSCs. However, a critical issue for the clinical transplantation of hBMSCs in various diseases is that their therapeutic use requires large quantities of cells for infusion, which are not available in most cases. The hBMSCs are a rare population (approximately 0.001–0.01%) of adult human bone marrow, and their numbers decrease with increasing donor age (Rao and Mattson, 2001). Several laboratories have successfully isolated multipotent cells from the human term placenta, a commonly discarded tissue after delivery, capable of self-renewal, differentiation into lineages of mesenchymal tissues and immunoregulation (Fukuchi et al., 2004; Yen et al., 2005). All of these properties make human placenta derived-mesenchymal stem cells (hPMSCs) an attractive alternative source of MSCs for basic research and clinical applications. In this paper, the hPMSC T cell immunosuppressive activity and their capacity to expand CD34$^+$ cells from UCB were compared with hBMSCs. We showed that hPMSCs, like

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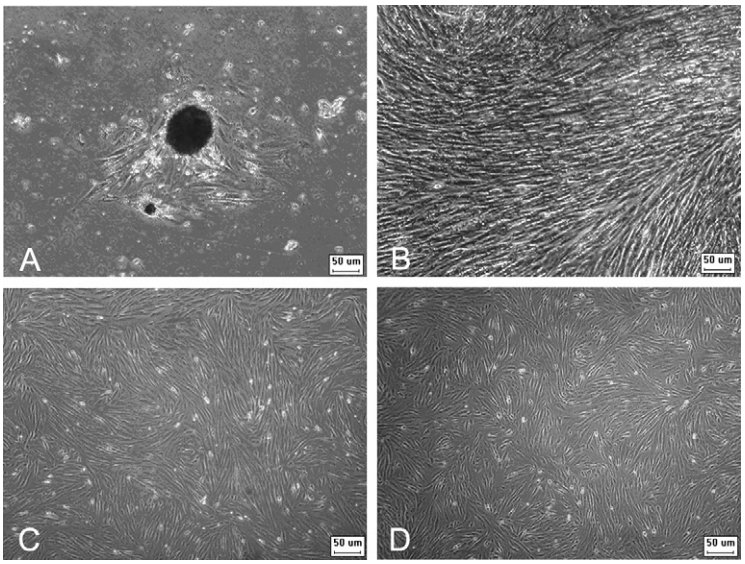

Fig. 1. The morphology of hPMSCs cultured in vitro. The cells isolated from placentas became adherent after 72 h of incubation and formed individual colonies displaying fibroblast-like morphology after 7–10 days (A). The cells showed spiral-like growth after 2 weeks (B), and after three passages, the cells formed a homogenous cell population (C). The morphology of hBMSCs at the fourth passage (D). The original magnification was 100× for all of the figures.

hBMSCs, have potent inhibitory effects on T cells and dramatically expand $CD34^+$ cells from UCB in vitro.

2. Results

2.1. The morphology and phenotype of hPMSCs and hBMSCs

The cells isolated from human term placentas began to form individual cell colonies after 7–10 days of inoculation and displayed fibroblast-like morphology. These adherent cells were readily expanded in vitro through successive cycles of trypsinization, seeding, and culture approximately every 7–8 days for 15 passages without visible morphologic alteration. A homogenous cell population was obtained after 3 passages (Fig. 1A–C). The morphology of hBMSCs in this study was consistent with our previous report (Luan et al., 2009; Xue et al., 2010) (Fig. 1D).

An immunophenotyping assay revealed that these cells exhibited many cell surface makers common to hBMSCs, including CD44, CD29, CD105, and CD166, but were negative for CD14, CD34, CD45 (Li et al., 2009), and the T cell activation surface molecules CD80, CD86, HLA-DR, CD40, CD40L and FasL. Interestingly, hPMSCs highly expressed, a negative co-stimulatory molecules, PDL1 ($57.23 \pm 6.27\%$), but almost did not detect the expression of B7H4 ($3.98 \pm 2.1\%$), the other negative co-stimulatory molecule. hBMSCs highly expressed B7H4 and did not express PDL1 (Xue et al., 2010). In addition, hPMSCs differentiated into lineage-specific osteogenic, adipogenic and neural cells under induction conditions (data not shown).

2.2. Comparison of the immunoregulatory properties of hPMSCs and hBMSCs on T cells

The proliferation of T cells activated by different stimulators, including the CD3 mAb combined with the CD28 mAb, PHA and allogeneic PBMC, was significantly inhibited in the presence of hPMSCs and hBMSCs compared with the positive control group (Fig. 2A, $p < 0.01$). Moreover, there were no significant differences between the inhibitory effects of hPMSCs and hBMSCs on T cell proliferation under different culture conditions.

The ELISA results suggested that both hPMSCs and hBMSCs suppressed the secretion of IFN-γ from T cells induced by different

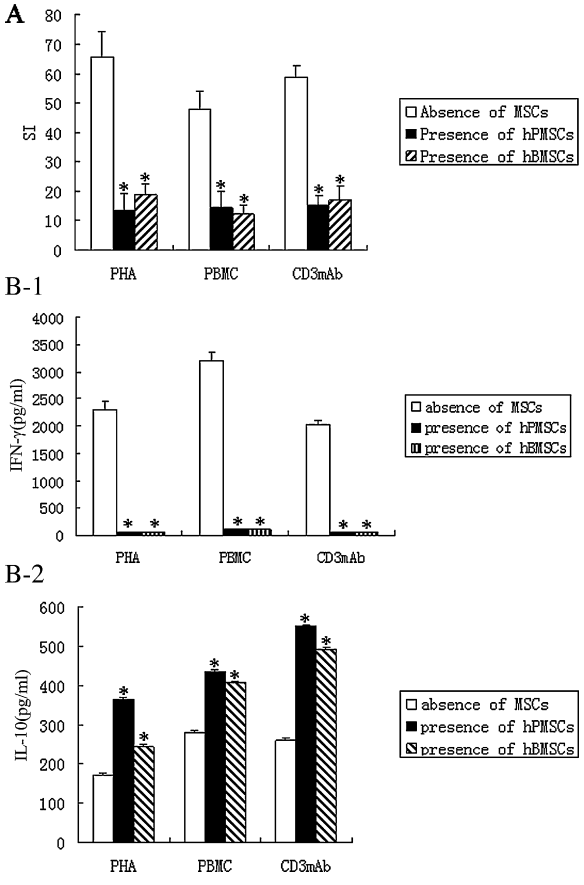

Fig. 2. The immunoregulatory effects of hPMSCs on T cell proliferation and cytokine secretion compared with hBMSCs. hPMSCs, like hBMSCs, strongly inhibited T cell proliferation (A) and the secretion of IFN-γ from T cells (B-1). The level of IL-10 in T cells was elevated in the presence of hPMSCs (B-2). T cells were stimulated with different stimulators in the presence of hPMSCs (■)/hBMSCs (▨) or the absence of hPMSCs and hBMSCs (□). T cell proliferation and cytokine levels were measured as described in the materials and methods. The results are the average of three identically designed experiments (*$p < 0.01$).