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Allogeneic Transplantation of Mesenchymal Stem Cells from Human Umbilical Cord Blood

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The cord blood serves as a vehicle for the transportation of oxygen and nutrients to the fetus. In the past, the human cord blood has generally been discarded after birth. However, numerous studies have described the regenerative ability of the cord blood cells in various incurable diseases. The umbilical cord blood (UCB)-derived stem cells are obtained through non-invasive methods that are not harmful to both the mother and the fetus. Furthermore, the cord blood stem cells are more immature than the adult stem cells and expand readily *in vitro*. The mesenchymal stem cells (MSCs) have the capacity to differentiate *in vitro* into various mesodermal (bone, cartilage, tendon, muscle, and adipose), endodermal (hepatocyte), and ectodermal (neurons) tissues. This review describes the immunological properties of the human UCB-MSCs to assess their potential usefulness in the allogeneic transplantation for the regenerative medicine.

Key words: *allogeneic transplantation, cord blood, immune rejection, mesenchymal stem cell, regenerative medicine*

Background

Intensive researches on the evaluation of the stem cells for their therapeutic potentials are underway worldwide. Although the use of embryonic and fetal-derived stem cells may be a viable approach in developing new treatments for incurable diseases [Ourednik *et al.*, 2002; Studer *et al.*, 1998], ethical and moral concerns, as well as the limited availability of stem cells have prompted the search for alternative stem cell sources.

Bone marrow (BM) is the main source of adult stem cells, which have already been used in various preclinical studies [Chen *et al.*, 2004; Chopp *et al.*, 2000]. However, the number of stem cells in the BM and their capacity to differentiate into multi-lineage decline with age [Mueller *et al.*, 2001]. Therefore, the human umbilical cord blood (hUCB) is often used as an alternative source of stem or progenitor cells such as hematopoietic stem cells (HSCs) and MSCs [Broxmeyer *et al.*, 1989]. The cord blood-derived stem cells have a practical advantage over the BM-derived stem cells in that they can be obtained using non-invasive methods that do not harm both the mother and the fetus [Laughlin *et al.*, 2001]. Furthermore, the cord blood stem cells are more immature than the other adult stem cells and expand readily *in vitro* [Kern *et al.*, 2006]. Given these features along with their potent differentiation potential [Yang *et al.*, 2004], more than three thousands cases of hUCB transplantation have been performed worldwide on the patients suffering from various hematologic and genetic disorders [Gluckman 2000; Kato *et al.*, 2000; Laughlin 2001; Laughlin *et al.*, 2001; Rubinstein *et al.*, 1998]. The cord blood-derived MSCs are also known to be an attractive source for the cellular and the gene transfer therapies. In one case, a female patient with a chronic spinal cord injury, when

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Abbreviations: BM, Bone marrow; ENA, epithelial neutrophil-activating protein; FGF, fibroblast growth factor; GCP, granulocyte chemotactic protein; GM-CSF, granulocyte-macrophage colony-stimulating factor; GRO, growth-related oncogene; GVHD, graft versus host disease; HLA, human leukocyte antigen; hMSCs, human MSCs; HSCs, hematopoietic stem cells; hUCB, human UCB; IFN-γ, Interferon-γ; IGFBP, insulin-like growth factor binding protein; IL, interleukin; IP, interferon-inducible protein; LIF, leukemia inhibitory factor; MCP, monocyte chemotactic protein; MHC, major histocompatibility complex type; MIF, macrophage migration inhibitory factor; MIP, macrophage inflammatory protein MLR, mixed lymphocyte reaction; MSCs, Mesenchymal stem cells; PARC, pulmonary and activation-regulated chemokine; PIGF, phosphatidylinositol glycan complementation class F; TGF, transforming growth factor; TIMP, tissue inhibitors of matrix metalloproteinases; TNF, tumor necrosis factor; UCB, Umbilical cord blood; VEGF, vascular endothelial growth factor

given the hUCB-derived multipotent stem cells, showed functional and morphological improvements [Kang *et al.*, 2005]. These cells were also useful for the treatment of Buerger's disease [Kim *et al.*, 2006].

MSCs are multipotent cells found in the fetal liver, BM, adipose tissue, and cord blood [Campagnoli *et al.*, 2001; Erices *et al.*, 2000], and have the capacity to differentiate *in vitro* into several mesodermal (bone, cartilage, tendon, muscle, and adipose), endodermal (hepatocyte), and ectodermal (neurons) tissues [Jeong *et al.*, 2004; Yang *et al.*, 2004]. Classically, MSCs are defined as being able to adhere to the plastic, expressing CD29, CD73, CD44, CD90, CD105, and HLA-AB (MHC class I antigens), but not expressing the hematopoietic cell markers CD34, CD45, and HLA-DR (MHC class II antigens) [Yang *et al.*, 2004]. MSCs constitutively secrete a large number of cytokines and promote the expansion and differentiation of HSCs [Jang *et al.*, 2006]. The therapeutic efficacies of MSCs on the bone, joint, and neuronal diseases have recently been reported [De Bari *et al.*, 2003; Newcomb *et al.*, 2006; Swanger *et al.*, 2005]. Furthermore, MSCs are not immunogenic. Namely, they do not induce allogeneic lymphocytes to proliferate *in vitro* [Gotherstrom *et al.*, 2004]. Indeed, MSCs appear to suppress these allogeneic proliferative responses. For example, in *in vivo* trials, the co-infusion of MSCs as a third party delayed the donor cell rejection, even when the immunosuppressant drugs were not used [Devine *et al.*, 2001]. These characteristics make MSCs potent candidates for the development of allogeneic cell-based therapeutic strategies.

The aim of this paper is to explain the immunological properties of hUCB-MSCs to assess their potential usefulness in the allogeneic transplantation for the regenerative medicine. In particular, I propose a conceptual framework in which the administration of hUCB-MSCs may be possible with no or minimal immune suppression in the recipient. This evaluation will lead to the acceleration of clinical application and a wide-spread utilization of hUCB-MSCs for the therapy of incurable diseases.

Basic characteristics of hUCB-MSCs: what is the merit on clinical application?

The cord blood serves as a vehicle for the transportation of oxygen and nutrients to the fetus. In the past, the human cord blood has usually been discarded after birth. However, instead of the BM stem cells, recent studies have shown that the cord blood could be an alternative source of the adult stem cells, which can be used as valuable materials for transplantation in hematological disease and tumor. A high impact on the clinical exploitation might be related to the abundance and expansion capacity of MSCs. UCB-MSCs have a relatively low isolation efficacy of a maximum of 63% [Bieback *et al.*, 2004]. Despite the low frequency of UCB-MSCs, the expansion potential was the highest compared with the other sources of MSCs [Kern *et al.*, 2006]. Given these features along with their potent differentiation potential [Yang *et al.*, 2004], hUCB-MSCs are an attractive source for the cellular and the gene transfer therapies.

Kern *et al.* compared the basic MSC-characteristics of MSCs from BM, cord blood, and adipose tissue [Kern *et al.*, 2006]. These MSCs derived from three sources exhibited typical MSC-characteristics, a fibroblastoid morphology and the expression of typical set of the surface markers, although the expressions of CD90, CD105, and CD106 were slightly different. In addition, they exhibited the capability to differentiate into multi-lineage. In particular, UCB-MSCs have been proven to differentiate not only into the mesodermal layer cells such as osteocytes, chondrocytes, adipocytes, and myoblast, but also into the germ layer cells such as hepatocytes and neuronal cells [Bicknese *et al.*, 2002; Erices *et al.*, 2000; Gang *et al.*, 2004; Goodwin *et al.*, 2001; Lee *et al.*, 2004b; Rosada *et al.*, 2003].

Interestingly, hUCB-MSCs are less sensitive towards the some lineage, such as adipogenic and neurogenic lineage differentiations [Chang *et al.*, 2006]. My colleague and I have found that different hUCB-MSCs have different ability to differentiate into the neurogenic lineage as well as the adipogenic lineage. Bieback *et al.* [2004] reported that the hUCB-MSC-like cells showed difficulty in differentiating into adipocytes, requiring specific culture conditions to differentiate into an adipogenic lineage. Campagnoli *et al.* [2001] and Goodwin *et al.* [2001] suggested that the clonal cell isolation and a large number of the cell doublings may have caused the altered responsiveness in the MSC-like cells towards the adipogenic induction. Moreover, the varied capacity to undergo the adipogenic differentiation may be explained by the fact that the cord blood cells more closely resemble the primitive cells, because the adipocytes may increase with aging to either excessively occupy or support the hematopoiesis [Gimble *et al.*, 1996].

The reason for the varied capacity to undergo neurogenic differentiation has not yet been reported. In a previous study, I noted that the number of cells per square centimeter as well as the source of the cord blood affected the differentiating capacity of MSCs. In most hUCB-MSCs, the cell density of 2500 to 3000/cm^2 resulted in the reproducible neurogenic differentiation. Interestingly, recent studies have indicated that MSCs derived from BM using simple chemicals do not represent neurogenic differentiation [Lu *et al.*, 2004]. That is because the

morphological changes and the increase in the neuronal markers of these MSCs are likely due to the cellular toxicity. So far, the neurogenic differentiation of MSCs is under debate.

Clinical application of hUCB-MSCs

A rising number of public and private cord blood banks store huge quantities of cord blood units, which in the past have been considered as a waste product of reproduction, and make possible an HLA match for the autologous or allogeneic transplantation. Allogeneic BM transplantation can be used to cure a variety of diseases including haematological malignancies, BM failure syndrome, immunodeficiencies, and some incurable diseases [Bensinger *et al.*, 1997; Will, 1999]. The use of allogeneic BM transplantation is limited by the need for adequate tissue matching of the host and the donor cells to reduce the risk of rejection and the severity of graft versus host disease (GVHD). Therefore, the need for new stem cell sources, which require less rigorous HLA matches, has been increasing for transplantation into the patients who do not have conventional donors. Thus, the cord blood derived stem cell has now emerged as an attractive source of stem cells for research and clinical application in treating a variety of diseases [Will, 1999].

The utilization of the cord blood stem cells starts with the treatment of pediatric hematological malignancies. For example, 'Eurocord Registry' has reported the results of 65 allogeneic UCB-stem cell transplantations. The median age and weight of the patients respectively were 9 years old and 30 kg: 41 patients with acute or chronic leukemia 7 patients had an HLA identical donor at HLA-A,-B and -DRB1; 43 had one antigen mismatch; 11 had two mismatches; and 2 had three HLA differences. These findings indicate that the HLA differences did not affect the survival of the patients. Moreover, the author concluded that the unrelated cord blood transplantation is a feasible and potent therapeutic method to cure hematological malignancies when compare to the results of the unrelated BM transplantation.

MSCs are one of the major populations of the stem or the progenitor cell contained in the cord blood mononuclear cell. The specific characteristics of UCB-MSCs, including their extensive proliferative potential and the ability to differentiate into various cell types, make them an attractive candidate as functional materials in the regenerative medicine. This is especially evident in such fields as cellular biology and gene therapy, resulting in a considerable increase in the number of clinical trials based on the use of MSCs.

MSCs have been proposed to be an excellent potential tool for gene therapies. Recent reports have demonstrated that human MSCs can engraft and differentiate when transplanted into the experimental animals [Devine *et al.*, 2001; Shake *et al.*, 2002; Toma *et al.*, 2002] and play a role in the medical cure [Eckes *et al.*, 2000; Kinner *et al.*, 2002a; Kinner *et al.*, 2002b; Mangeot *et al.*, 2002; Skalli *et al.*, 1989]. In the animal studies, hMSCs were successfully transplated into the fetal sheep and persisted in multiple tissues for as long as 13 months [Mackenzie *et al.*, 2001]. These cells seem to have unique immunologic characteristics that permit the persistence in a xenogeneic environment. These properties suggest the potential usefulness of MSCs in the cell- and gene-based therapies [Hutcheson *et al.*, 2000; Millington-Ward *et al.*, 2002]. Therefore, MSCs have recently been investigated for their potential as vehicles for the systemic delivery of the therapeutic proteins *in vivo* after the gene transfer [Lee *et al.*, 2004a]. In a mouse model, the genetically modified MSCs grafted into an ectopic site showed about 74% stable gene transfer efficiency [Drize *et al.*, 1992]. Furthermore, there have been clinical studies in humans using MSCs transfected with the viral vectors containing the gene of the coagulation factor VII or IX for the treatment of haemophilia [Chuah *et al.*, 2000]. However, the majority of the gene therapy studies involving MSCs were performed by BM-derived MSCs. So far, only one study has been reported on the UCB-derived MSCs [Lu *et al.*, 2005], in which the lentiviral vectors appeared to be effective in delivering and expressing the transgenes in the UCB-derived MSCs.

Interests are currently rising on the use of hUCB-MSCs in the transplantation for the myocardium regeneration after the myocardial infarction. MSCs were shown to be able to differentiate into cardiomyocytes *in vitro* [Heng *et al.*, 2004]. Nishiyama *et al.* [2007] demonstrated the cardiomyogenic potential of hUCB-MSCs. They cocultured hUCB-MSCs with the fetal murine cardiomyocytes. After 5 days culture, hUCB-MSCs contracted rhythmically and synchronously, suggesting the presence of an electrical communication between the hUCB-MSCs. Moreover, these cells were positively stained for the cardiomyocyte specific markers, such as cardiac troponin-I and connexin 43, using the immunocytochemical method. Cardiac troponin-I-positive cardiomyocytes accounted for $45 \pm 3\%$ of the total hUCB-MSCs. These results indicated that the half of the hUCB-MSCs was successfully transdifferentiated into the cardiomyocytes *in vitro*. Prompted by the *in vitro* studies, scientists performed *in vivo* experiments. In the *in vivo* studies on the heart ischemic animal models, the BM-derived MSCs differentiated into the cardiomyocytes [Dai *et al.*, 2005; Shake *et al.*, 2002], thereby improving the function of the

left ventricle [Silva *et al.*, 2005]. However, less information is available on the potential therapeutic capabilities of MSCs from human origin. In the rat acute myocardial infarction models, the transplantation of hMSCs from the healthy subjects across the xenogeneic barriers with or without immune suppression improved the function of the left ventricle [Grauss *et al.*, 2007; Grinnemo *et al.*, 2006]. Moreover, in the clinical trial on myocardial infarction, Chen *et al.* [2004] found that the intracoronary infusion of autologous BM-MSCs resulted in an improved left ventricle function. Grauss *et al.* [2007] demonstrated the feasibility of using the ischemic heart disease patient-derived hMSCs in the cell-based therapy for the acute myocardial infarction.

Besides gene therapy and myocardial infarction, the tissue repair capacity of MSCs was also reported in various organs, such as kidney [Herrera *et al.*, 2004], muscle [De Bari *et al.*, 2003], lung [Ortiz *et al.*, 2003], and brain [Chopp *et al.*, 2002; Chopp *et al.*, 2000]. MSCs were also found to promote angiogenesis and were used in the skin wound healing.

Immune evasion mechanisms of hUCB-MSCs

The main theoretical limitation hampering the clinical transplantation of hUCB-MSCs would be the graft rejection due to the immunological barrier imposed by the MHC mismatching. Indeed, the commercially practicable exploitation of the regenerative medicine may well depend on the capacity to use the allogeneic MSC on a large scale. Interestingly, previous studies have shown that MSCs from other tissues, namely BM [Aggarwal *et al.*, 2005; Chang *et al.*, 2006; Gotherstrom *et al.*, 2004], adipose tissue [Puissant *et al.*, 2005], and fetal lung tissue [Gotherstrom *et al.*, 2004], inhibit the lymphocyte proliferation (mixed lymphocyte reaction: MLR) and the inflammatory cytokine production. The immune evasion properties of these cells were not lost upon their differentiation. A previous study has shown that BM-MSCs that had differentiated into the osteocytes continued to be unable to induce the proliferation of the allogeneic T cell, regardless of whether they were treated with IFN-γ or not [Klyushnenkova *et al.*, 2005]. Moreover, MSCs that had differentiated into the osteocytes or the adipocytes were as effective immunosuppressors as the undifferentiated MSCs when added to a two-way MLR [Klyushnenkova *et al.*, 2005]. In my previous results, chondrogenic or neurogenic differentiated hUCB-MSCs also did not elicit an immunologic response when added to the allogeneic peripheral blood mononuclear cells (unpublished data). In the *in vivo* studies, when the allogeneic MSCs were transplanted, these cells differentiated in a site-specific manner into chondrocytes, adipocytes, myocytes, and cardiomyocytes, and persisted for 13 months [Liechty *et al.*, 2000]. Taken together, the published results described above suggest that MSCs can be successfully transplanted even when they are MHC-mismatched. However, the theoretical background of the immune privilege mechanisms has not been clearly demonstrated yet.

Here, I provide the rationale to explaining the immune suppressive activities of hUCB-MSCs using two different mechanisms based on my research experiences, because no report is yet available on the immunological properties of hUCB-MSCs.

Hypoimmunogenic properties of hUCB-MSCs depend on cell surface phenotype

Human cord blood derived MSCs were positive for the MSC relative cell surface markers and MHC class I [Gang *et al.*, 2004; Lee *et al.*, 2004b; Yang *et al.*, 2004]. MHC class I molecules are found on almost every nucleated cell of the body and are loaded with proteins generated in the cytosol. As viruses infect a cell by entering its cytoplasm, this cytosolic MHC class I-dependent pathway of the antigen presentation is the primary way to expose a virus-infected cell to the T cells. In general, MHC class I molecules interact exclusively with the $CD8^+$ T cells. The fate of the virus-infected cell is apoptosis, in most cases initiated by the $CD8^+$ T cells, effectively reducing the risk of infecting the neighboring cells. The cells expressing MHC molecules can stimulate the T cells directly if they have co-stimulatory molecules or indirectly by the cross presentation on the professional antigen presenting cells. However, the MHC class I-positive MSCs have been shown not to provoke the immune problems, although their mechanisms are not clear.

A general feature of MSCs, regardless of the original source, is that they express low or zero level of MHC class II [Gotherstrom *et al.*, 2004; Yang *et al.*, 2004]. The MHC class II molecules are well known to play important roles in the antigen presentation and the allogeneic response. Therefore, the lack of MHC class II is considered as a main mechanism for the low immunogenecity of MSCs. It is also important to note that MSCs lack the cell surface expression of the T cell co-stimulatory molecules, because, in the absence of costimulation, MHC class II reactive T cell can result in anergy that would contribute to the tolerance. Klyushnenkova *et al.* showed that although IFN-γ pretreatment elevated the HLA class I and II expressions on the BM-MSCs, these cells continued to be unable to provoke the proliferative response of the

alloreactive lymphocytes [Klyushnenkova *et al.*, 2005]. MSCs were treated with IFN-γ to represent a bad case, in which these cells are implanted into the sites of inflammation. Similarly, my colleagues and I found that IFN-γ increased the expression of MHC molecules in hUCB-MSCs as well. MSCs were, however, still unable to provoke an allogeneic response (unpublished data). Thus, hUCB-MSCs and other MSCs failed to act as allogeneic response-inducing antigen-presenting cells despite carrying the MHC molecules.

Cytokine and inflammatory soluble factors involve to immune suppressive effect of hUCB-MSCs

In the previous studies, BM-MSCs significantly inhibited the alloreactivity, even when they were separated from the responder lymphocytes by a transwell membrane [Di Nicola *et al.*, 2002; Djouad *et al.*, 2003]. Notably, these studies found that the degree of inhibition achieved in the transwell chamber experiments was less than that observed in the non-separated situation. My own studies demonstrated that hUCB-MSCs also suppressed the lymphocyte alloreactivity *via* a soluble factor(s), because the MSCs continued to suppress MLR across the transwell membrane and the degree of inhibition achieved in the transwell chamber experiments was similar to that of the non-separated situation. Thus, these results suggest that the soluble factors secreted from hUCB-MSCs suppress the allogeneic immune responses.

The soluble factors from hUCB-MSCs that inhibit the lymphocyte alloreactivity may be produced spontaneously or only when the MSCs are exposed to the allogeneic lymphocytes. I have found that when MLR was performed in the presence of the hUCB-MSC culture supernatants, the alloreactive T lymphocyte proliferation was not suppressed; however, when the conditioned hUCB-MSC/MLR medium was used, the allogeneic lymphocyte proliferation was inhibited, albeit slightly (8-12% reduction in proliferation). This low inhibition effect could have been caused by using the supernatants previously frozen and thawed. Moreover, the suppressive soluble factors would have been diluted by the fresh medium used in the MLR assay. Nevertheless, these results suggest that hUCB-MSCs produce their suppressive soluble factor(s) only when the allogeneic lymphocytes are in their vicinity.

Liu *et al.* [2005] suggested that several cytokines and growth factors, including epithelial neutrophil-activating protein (ENA)-78, granulocyte-macrophage colony-stimulating factor (GM-CSF), growth-related oncogene (GRO), interleukin (IL)-1β, IL-6, IL-8, monocyte chemotactic protein (MCP)-1, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF)-4, FGF-7, FGF-9, granulocyte chemotactic protein (GCP)-2, insulin-like growth factor-binding protein (IGFBP)-1, IGFBP-2, IGFBP-3, IGFBP-4, IP-10, leukemia inhibitory factor (LIF), macrophage migration inhibitory factor (MIF), macrophage inflammatory protein (MIP)-3a, osteoprotegerin, pulmonary and activation-regulated chemokine (PARC), phosphatidylinositol glycan complementation class F (PIGF), transforming growth factor (TGF)-β2, TGF-β3, tissue inhibitors of matrix metalloproteinases (TIMP)-1, and TIMP-2, were secreted by hUCB-MSCs, whereas IL-4, IL-5, IL-7, IL-13, TGF-β1, tumor necrosis factor (TNF)-α, and TNF-β were not expressed under the normal growth conditions. The cytokine profile of hUCB-MSCs is very similar to that of BM-MSCs. IL-6, IL-8, MCP-1, RANTES, GRO-α, IFN-γ, IL-1α, TGF-β, GM-CSF, angiogenin, and oncostatin M were constitutively expressed, whereas the macrophage inflammatory protein (MIP)-1β, IL-2, IL-4, IL-10, IL-12, and IL-13 were not expressed by the BM-MSCs [Potian *et al.*, 2003]. The characterization of the cytokines produced by MSC is still rudimentary and is again hampered by the diversity of the cells and the culture systems. Moreover, the involvement of these cytokines as a major mechanism for the suppression activities of UCB-MSCs has not yet been elucidated.

The anti-inflammatory cytokines such as TGF-β and IL-10 have been thought to mediate the immunosuppressive effects of MSCs [Rasmusson *et al.*, 2003]. However, in my results, hUCB-MSCs did not elevate the IL-10 levels in the MLR culture supernatants, which were undetectable under both suppressed and unsuppressed conditions. These observations are consistent with those of the previous studies on the adipose tissue and BM-MSCs, in which undetectable levels of IL-10 were reported in the supernatants of these cells [Puissant *et al.*, 2005]. Furthermore, I found similar amounts of TGF-β (0.6~0.8 ng/mL) in MLR supernatants that had been processed with or without hUCB-MSCs. These observations agree with those of the previous report by Gotherstrom *et al.* [2004]. Thus, based on these findings, I can suggest that TGF-β and IL-10 are not involved in the immuno-suppressive effect of hUCB-MSCs on the allogeneic responses.

IFN-γ and IL-2 are considered to play important roles in the allogeneic immune responses and T-lymphocyte proliferation. IFN-γ increases the expression levels of the cell-surface HLA I and II class molecules and reinforces the T lymphocyte activities, together with lymphotoxins produced by lymphocytes (CD4 or CD8 positive cells) [Takaoka *et al.*, 2006]. IL-2 is also necessary for the

growth, differentiation, and survival of the antigen-selected cytotoxic T cells and supports the long-term T-cell proliferation [Beadling *et al.*, 1993; Beadling *et al.*, 2002; Robb *et al.*, 1981; Stern *et al.*, 1986]. We have found that the levels of IFN-γ and IL-2 in the supernatants of hUCB-MSC-suppressed MLR cultures were reduced by almost 30% relative to the unsuppressed MLR cultures. Other proinflammatory cytokines such as TNF-α, IL-1, and IL-6 are also considered as strong immune stimulators. Several studies have suggested that MSCs suppress the T-lymphocyte proliferation by reducing their TNF-α production [Aggarwal *et al.*, 2005; Beyth *et al.*, 2005; Klyushnenkova *et al.*, 2005]. I also founded that the TNF-α levels in the MLR supernatants were greatly reduced when the MLR had been performed in the presence of hUCB-MSCs. However, IL-1α and IL-1β were not involved in the mechanism by which hUCB-MSCs suppress the lymphocyte alloreactivity, because they were not detected in the hUCB-MSC-suppressed MLR cultures.

Conclusion

In summary, the usability of hUCB-MSCs depends on overcoming the allogeneic barriers. The use of MSCs without pretreatment of the immune suppressor on the recipients as regenerative medicines would be a great progress for the clinical application of the stem cell therapeutics. This progress can only be achieved by understanding the unique immunological properties of hUCB-MSCs, developing the cell expansion capacity, and identifying the safety of the recipient after the MSCs transplantation. Although the clinical applications of hUCB-MSCs are still in early stages at the present time, hUCB-MSCs may be applied to incurable diseases as a cell therapy tool in the near future.

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