Journal of Transplantation Technologies & Research

Wang, J Transplant Technol Res 2013, 3:2
DOI: 10.4172/2161-0991.1000e120

Editorial Open Access

# Pre-Clinical Transplantation Safety of Hematopoietic Stem/Progenitor Cell Product Expanded from Human Umbilical Cord Blood

**Jinfu Wang***

*Institute of Cell and Development Biology, Zhejiang University, Hangzhou, Zhejiang 310058, P.R. China*

Umbilical cord blood transplantation (HCBT) has been used in treatment of hematologic malignancies, aplastic anemia, hemoglobinopathies and severe combined immunodeficiency [1]. In comparison with bone marrow and peripheral blood, the umbilical cord blood (UCB) has the advantage of convenient collection, immature cellular immunity and no strict matching in transplantation [2]. Thus, hematopoietic/progenitor stem cells (HS/PCs) from UCB have potential clinical value. However, the HS/PCs in a stock of UCB are low in quantity, which limits its clinical application. Therefore, HS/PCs from UCB should be *ex vivo* expanded to meet the needs for clinical transplantation.

As an *ex vivo* expanded cell product for clinical transplantation, its pre-clinical safety should be paid a great attention before clinical transplantation. Any careless during culture may result in the contamination in the expanded cell product, including contamination of bacterial, fungi, mycoplasma and exogenous virus [3]. Bacterial contamination is one of the major risks associated with blood product transfusion and may cause severe transplantation- related infections [4]. This notion is supported by reports from the French Hemovigilance System demonstrating that between 1994 and 1998. Transplantation associated with bacterial infection was responsible for 22% of fatal cases associated with transplantation [5]. Drug Administrations have defined that there should be no contamination of exogenous factors in cellular products [6-8]. Therefore, we should examine carefully the cellular products to reduce the possibility of contamination before clinical transplantation.

In my lab, human bone marrow-derived mesenchymal stem cells (BM-MSCs) were used as a feeder layer to support the expansion of HS/PCs from UCB in a free-serum culture system. The proliferation potential of HS/PCs was analyzed. The expanded HS/PCs were suspended in the normal saline with 1% human albumin to prepare the HS/PC product. The contamination of bacteria, fungi and mycoplasmas, the infection of exogenous virus, the concentration of bacterial endotoxin, and the SCF residual in the expanded HS/PC product were determined. Finally, cells from the HS/PC product, in company with or without BM-MSCs, were transplanted into the irradiated NOD/SCID mice to determine the *in vivo* engraftment potential.

The results showed that co-culture with BM-MSCs can promote the expansion of HS/PCs. The expansion levels of HS/PCs from UCB in two co-culture systems with BM-MSCs as the feeder layer were higher than that in the culture system without BM-MSCs, which demonstrated that BM-MSCs supported the expansion of HS/PCs. For the proliferation potential of HS/PCs, we analyzed only the expansion level of cells suspended in the medium, although there had been the evidence of HS/PC-derived cobblestone-forming hematopoietic cells beneath BM-MSC layer [9]. However, the expansion levels of TNCs, $CD34^+$ cells and colony-forming cells suspended in the medium were enough to demonstrate the capacity of BM-MSCs supporting the *ex vivo* expansion of HS/PCs from UCB. In addition, we used BM-MSCs as a feeder layer to co-culture MNCs from UCB in a free-serum culture system supplemented with three cytokines. The result showed that the difference of expansion potentials of TNCs, $CD34^+$ cells and colony-forming cells between two co-culture systems with and without serum was no significance. However, the expansion folds of TNCs, $CD34^+$ and colony-forming cells in the free-serum co-culture system were higher than those in the culture system without BM-MSCs. We inferred that BM-MSCs supported the *ex vivo* expansion of HS/PCs, especially the *ex vivo* expansion of hematopoietic stem cells (HSCs). Therefore, the free-serum co-culture system with BM-MSCs can be recommended to culture HS/PCs from UCB for clinical transplantation.

The multipotential ability of BM-MSCs, their easy isolation and culture, as well as their high *ex vivo* expansive potential make these cells an attractive therapeutic tool [10,11]. Co-transplantation of BM-MSCs is capable of enhancing engraftment of HS/PCs in a fetal sheep model [12,13], which suggests that co-transplantation of BM-MSCs and HS/PCs from human UCB results in acceleration of rapid, middle and long-engraftments of HS/PCs. Our experiments of transplantation in NOD/SCID mice indicated the reconstituting ability of expanded HS/PCs in the irradiated mice. The co-transplantation of expanded HS/PCs with BM-MSCs from the free-serum co-culture system promoted the engraftment of HS/PCs in recipients in comparison to the transplantation of only expanded HS/PCs. It is thought that BM-MSCs enhance engraftment and support hematopoiesis by mechanisms that may not require homing of BM-MSCs to the bone marrow and might be mediated by the release of cytokines that promote either the homing or proliferation of HS/PCs [14]. It is well known that stroma derived factor (SDF)-1 has significant importance on the homing of HS/PCs to their niche in the bone marrow. It was demonstrated that SDF-1 effects the recruitment of $CD34^+$ cells to the marrow in a NOD/SCID model of human hematopoiesis [14]. Thus, BM-MSCs have a role in homing by both inducing the expression of SDF-1 via secretion of stroma cell factor (SCF), and directly by secretion of SDF-1. Primary BM-MSCs derived from adult sources promote the engraftment of UCB-derived $CD34^+$ cells to a similar degree as culture-expanded BM-MSCs, indicating that the biological properties of primary BM-MSCs are preserved during expansion, as is the potential safety profile of culture-expanded cells for therapeutic application [15]. In the present study, no genetic alteration in cultured BM-MSCs occurred after 2-3 passages of culture. Therefore, BM-MSCs from co-culture system could be considered as a cell source for co-transplantation. However, the cultured BM-MSCs should also be tested the contamination of exogenous factors before clinical co-transplantation. Of cause, BM-MSCs harvested from the free-serum co-culture system may include HS/PC-derived cobblestone-forming hematopoietic cells beneath BM-MSC layer [9]. The role of these cells in engraftment of expanded HS/PCs is remained for further study.

***Corresponding author:** Jinfu Wang, Institute of Cell and Development Biology, College of Life Science, Zi Jin Gang Campus, Zhejiang University, Hangzhou, Zhejiang 310058, P.R. China, E-mail: tmiloh@phoenixchildrens.com

**Received** August 09, 2013; **Accepted** August 09, 2013; **Published** August 12, 2013

**Citation:** Wang J (2013) Pre-Clinical Transplantation Safety of Hematopoietic Stem/Progenitor Cell Product Expanded from Human Umbilical Cord Blood. J Transplant Technol Res 3: e120. doi:10.4172/2161-0991.1000e120

A series of studies indicates that BM-MSCs escape recognition of alloreactive cells, and are immune privileged [16]. BM-MSCs generally express only HLA class I. Despite a few studies that had demonstrated that BM-MSCs could be induced by IFN-γ to up-regulate the expression of HLA class II, neither the BM-MSCs that expressed HLA class I only, nor the BM-MSCs that expressed both HLA class I and class II induced by IFN-γ, showed immunogenic potential. Furthermore, both cell types could inhibit T cell immune responses, and the up-regulation of HLA class II by IFN-γ did not elicit a proliferative response of T cells [16,17]. BM-MSCs have been shown both *in vivo* [18,19] and *in vitro* [20,21] to suppress T cell activation. Additionally, BM-MSCs have been shown to suppress the proliferation of activated T cells induced by allo-antigens in the mixed lymphocyte reaction (MLR) [21] and induced by mitogens such as phytohemagglutinin [22], or concanavalin A [16], as well as the activation of T cells by CD3 and CD28 antibody stimulation [23]. Several studies have shown similar effects when using BM-MSCs that are autologenic or allogenic to the responder cells, indicating a genetically unrestricted suppression [14]. Having low immunogenicity, BM-MSCs, no requirement of HLA matching, should be considered for co-transplantation with HS/PCs in therapeutic application.

Patients of hematologic malignancies must go through flushing dose of radiotherapy or chemotherapy pretreatment before HSCT to eliminate cancer cells that may result in the side effect of killing the bone marrow cells and inhibiting the activity of immune cells. Therefore, the contamination in the expanded cellular product may result in the serious post-transplantation infection in recipients. Bacterial endotoxin is caused by Gram-negative bacterium. Endotoxin is exogenous pyrogen that can activate heterophil granulocyte to release a kind of endogenous pyrogen which will cause fever by acting up on body temperature regulating center [24]. Due to the possibility of xenogeneic pathogen contamination during isolation and culture of cells, the expanded cellular product must be rigorously controlled before transplantation to make sure that the cellular products are asepsis and free of exogenous matters that may jeopardize the patients. The expanded HS/PC product in the free-serum co-culture system was tested for the possibility of contamination of exogenous pathogens and matters according to the PC and China Biological Regulations. The results showed that there was no any contamination or infection of exogenous pathogens or matters in the expanded HS/PC products. In the culture system, three cytokines were added to the medium. TPO and G-CSF used in the culture system were commercial products approved for clinical application. Because SCF used in the culture system was only for lab application, the content of SCF residual in the expanded HS/PC product was determined. The results showed that the contents of endotoxin and SCF residual in the expanded HS/PC products were lower than the limiting levels. These safety standards will be applied in the preparation of HS/PC products for clinical transplantation.

## References

1. Gluckman E (2000) Current status of umbilical cord blood hematopoietic stem cell transplantation. Exp Hematol 28: 1197-1205.
2. Kurtzberg J, Laughlin M, Graham ML, Smith C, Olson JF, et al. (1996) Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. N Engl J Med 335: 157-166.
3. Goldman M, Sher G, Blajchman M (2000) Bacterial contamination of cellular blood products: the Canadian perspective. Transfus Sci 23: 17-19.
4. Ribault S, Faucon A, Grave L, Nannini P, Faure IB (2005) Detection of bacteria in red blood cell concentrates by the Scansystem method. J Clin Microbiol 43: 2251-2255.
5. Andreu G, Morel P, Forestier F, Debeir J, Rebibo D, et al. (2002) Hemovigilance network in France: organization and analysis of immediate transfusion incident reports from 1994 to 1998. Transfusion 42: 1356-1364.
6. [No authors listed] (1998) WHO Expert Committee On Biological Standardization. World Health Organ Tech Rep Ser 878: i-vi, 1-101.
7. http://www.fda.gov/downloads/biologicsbloodvaccines/safetyavailability/ucm162863.pdf
8. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/OtherRecommendationsforManufacturers/UCM153182.pdf
9. Xie CG, Wang JF, Xiang Y, Jia BB, Qiu LY, et al. (2005) Marrow mesenchymal stem cells transduced with TPO/FL genes as support for ex vivo expansion of hematopoietic stem/progenitor cells. Cell Mol Life Sci 62: 2495-2507.
10. Fibbe WE, Noort WA (2003) Mesenchymal stem cells and hematopoietic stem cell transplantation. Ann N Y Acad Sci 996: 235-244.
11. Minguell JJ, Erices A, Conget P (2001) Mesenchymal stem cells. Exp Biol Med (Maywood) 226: 507-520.
12. Almeida-Porada G, Flake AW, Glimp HA, Zanjani ED (1999) Cotransplantation of stroma results in enhancement of engraftment and early expression of donor hematopoietic stem cells in utero. Exp Hematol 27: 1569-1575.
13. Almeida-Porada G, Porada CD, Tran N, Zanjani ED (2000) Cotransplantation of human stromal cell progenitors into preimmune fetal sheep results in early appearance of human donor cells in circulation and boosts cell levels in bone marrow at later time points after transplantation. Blood 95: 3620-3627.
14. Anklesaria P, Kase K, Glowacki J, Holland CA, Sakakeeny MA, et al. (1987) Engraftment of a clonal bone marrow stromal cell line in vivo stimulates hematopoietic recovery from total body irradiation. Proc Natl Acad Sci U S A 84: 7681-7685.
15. in 't Anker PS, Noort WA, Kruisselbrink AB, Scherjon SA, Beekhuizen W, et al. (2003) Nonexpanded primary lung and bone marrow-derived mesenchymal cells promote the engraftment of umbilical cord blood-derived CD34(+) cells in NOD/SCID mice. Exp Hematol 31: 881-889.
16. Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringdén O (2003) HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. Exp Hematol 31: 890-896.
17. Klyushnenkova E, Mosca JD, Zernetkina V, Majumdar MK, Beggs KJ, et al. (2005) T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression. J Biomed Sci 12: 47-57.
18. Kim DW, Chung YJ, Kim TG, Kim YL, Oh IH (2004) Cotransplantation of third-party mesenchymal stromal cells can alleviate single-donor predominance and increase engraftment from double cord transplantation. Blood 103: 1941-1948.
19. Le Blanc K, Rasmusson I, Sundberg B, Götherström C, Hassan M, et al. (2004) Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 363: 1439-1441.
20. DiNicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, et al.(2004) Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 103: 3313-3319.
21. Sudres M, Norol F, Trenado A, Grégoire S, Charlotte F, et al. (2006) Bone marrow mesenchymal stem cells suppress lymphocyte proliferation in vitro but fail to prevent graft-versus-host disease in mice. J Immunol 176: 7761-7767.
22. Li CD, Zhang WY, Li HL, Jiang XX, Zhang Y, et al. (2005) Mesenchymal stem cells derived from human placenta suppress allogeneic umbilical cord blood lymphocyte proliferation. Cell Res 15: 539-547.
23. Aggarwal S, Pittenger MF (2005) Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105: 1815-1822.
24. National Pharmacopoeia Community (2005) Pharmacopoeia of the People's Republic of China (the Third Section) Appendix, Edition, 360-368.