Hematopoietic Stem Cells from Cord Blood were Expanded Ex Vivo in Batch and Perfusion System

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Abstract

Hematopoietic stem cells (HSCs) have two charicteristics : Self-renewering and differentiating into various blood cells. Many kinds of growth factors play key roles in proliferation and differentiation of HSCs.[1), 2)] Demand of successful expansion of HSCs ex vivo has increased not only for a part of stem cell researches but also for clinical applications. In this study, we used human cord blood to isolate HSCs, which is collected after informed consent. Mononuclear cells (MNCs) were separated by using Ficoll-paque and $CD34^+$ cells were isolated by an immunomagnetic separation method using MidiMACS (Miltenyi Biotec, Germany). Various Cytokines such as EPO, GM-CSF, SCF, and IL-3 were used without serum supplementation. Conventional well plate and modified well plate for perfusion operation were used to figure out the effect of perfusion cultures. Expansion of HSCs was sensitively dependent on the concentration of cytokines. In respect of cell expansion, batch culture in multiwell plate showed better performance than perfusion culture, while perfusion culture resulted in higher expansion ratio in colony forming units than batch culture. Expanded cells showed changes in immunophenotypic characterization. Populations of $CD34^+$, $CD33^-CD38^-$, and $CD45^+$ cells were decreased, whereas the $CD235a^+$ cells were proliferated. Total cell expansion reached to 1,500 fold and 50 fold in 28 days culture and CFU-GM expansion was about 1,000 fold and 13 fold when $CD34^+$ cells and MNCs were inoculated, respectively. These highly effective expansion techniques seem to be used to realize cell therapy using HSCs.

References

1. Daniel R. Marshak, Richard L. Gardner, David Gottlieb, "Stem Cell Biology", CSHL Press, Cold Spring Harbor, New York, 2001, pp 289~306.
2. Roland Mertelsmann, Friedhelm H, "Hematopoietic Growth Factors in Clinical Applications", Marcel Dekker, Inc., New York, 1995, pp 227~239.