Hematopoietic Stem Cells

Comparison of efficiency of *ex vivo* expansion of whole blood, mononuclear cells and purified CD34⁺ cells from human umbilical cord blood

We used a dual-chamber culture system separated by a dialysis membrane to test the efficiency of expansion of whole cord blood and cell fractions. We found that expansion of progenitor cells was more efficient from whole blood than from purified CD34⁺ or partially purified mononuclear cell fractions.

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Although several effective methods of *ex vivo* expansion of cord blood (CB) progenitor cells have been developed, their clinical application remains very limited.[1,2] One important reason is the concern that *ex vivo* manipulation might, in some way, diminish the safety of the expanded cells for clinical application.[3] On this background, we have attempted to develop an effective and economic way of expanding progenitor cells while considerably restricting *ex vivo* manipulation.

One of the easiest ways of restricting *ex vivo* manipulation would be to culture the whole blood (WB) itself, without purifying the cells, in a closed culture system. However, because of the large volume and high cell density, WB cultures require large amounts of media as well as growth factors. In this regard, we tested the efficiency of a dual-chamber culture system separated by a dialysis membrane to reduce the requirement for growth factors.

Six samples of umbilical CB were harvested from babies born at term. The mothers gave informed consent to the study which had been approved by the Institutional Review Board of Ewha Women's University Hospital. The heparinized CB samples were divided into three identical parts; one part was used as WB itself; another was fractionated to provide mononuclear cells (MNC); the third was purified to form a CD34⁺ cell fraction. The purification procedure was carried out as previously described.[4] WB aliquots were cultured in sterile disposable dialysis tubes (Spectra/Pro; Sigma, St. Louis, MO, USA; MW cut-off, 10,000) containing growth factors, which were immersed in culture flasks containing serum-free essential media (SFEM; Stem Cell Technologies Inc., Vancouver, BC, Canada), and agitated on a low speed shaker in a humidified incubator at 37°C in 5% CO_2. MNC and purified CD34⁺ cells were expanded in a conventional stroma-free liquid culture system in SFEM as described previously.[5] The growth factors used in expansion were thrombopoietin (50 ng/mL; Kirin Brewery, Maebashi, Japan) and flt3-ligand (50 ng/mL; Chemicon, Temecula, CA, USA). Data are represented as mean±SD of three to six separate experiments.

At the beginning of the culture, the nucleated cell count in each CB unit was different and ranged from 0.8 to 1.5×10^7 cells/mL. The proportions of MNC and purified CD34⁺ cells were also different in each CB sample. In order to standardize the absolute counts into relative numbers for quantitative comparison, the absolute cell count in each WB sample was set to 100. In this way, the relative numbers of MNC and CD34⁺ fraction were 32.5±12.2 and 0.4±0.2, respectively

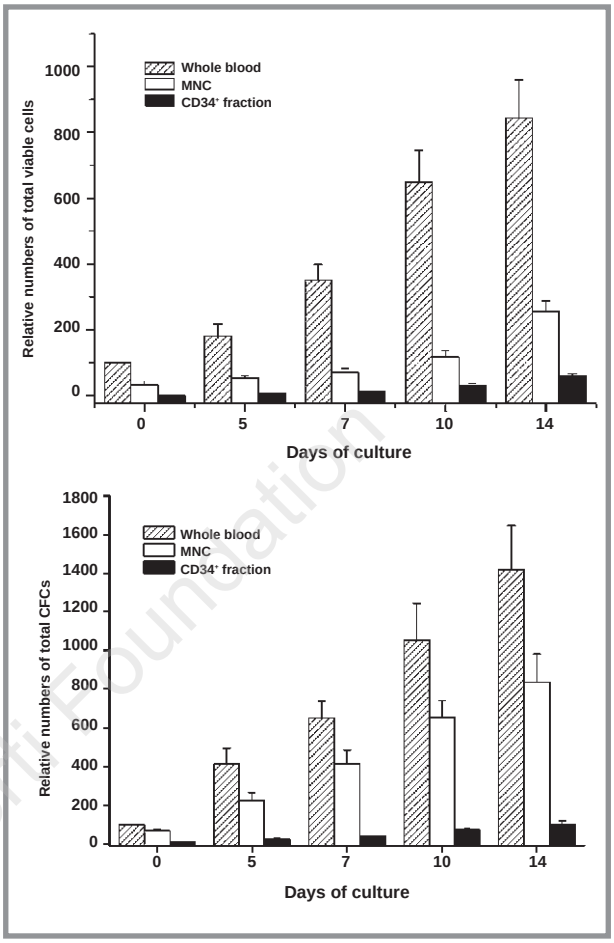

Figure 1. Evolution of the numbers of total viable nucleated cells (A) and total colony-forming cells (CFC) (B) during *ex vivo* expansion. Whole blood (WB) samples were cultured in a two-chamber culture system while mononuclear cells (MNC) and purified CD34⁺ fractions were cultured in a conventional stroma-free liquid culture system. The growth factors used in expansion were thrombopoietin (50 ng/mL) and flt3-ligand (50 ng/mL). Data represent mean±SD of three to six separate experiments and each number is a standardized value as the number of total nucleated cells (A) or total CFC (B) in cord blood at the beginning was set at 100.

(Figure 1A). After *ex vivo* expansion for 14 days, the nucleated cell count increased 8.5±1.2 fold in WB, 7.9 ±1.0 fold in MNC, and 143.8±22.3 fold in CD34⁺ fractions. However, in terms of total cell count, the WB counts were 3.3±0.5-fold and 14.7±2.0-fold higher than those of the MNC and purified CD34⁺ fractions, respectively.

Colony-forming assays using a methylcellulose-based commercial kit (Methocult™ GF H4534; Stem Cell Technologies) showed that the total colony-forming cell (CFC) count in each CB unit was different and ranged from 3.4 to

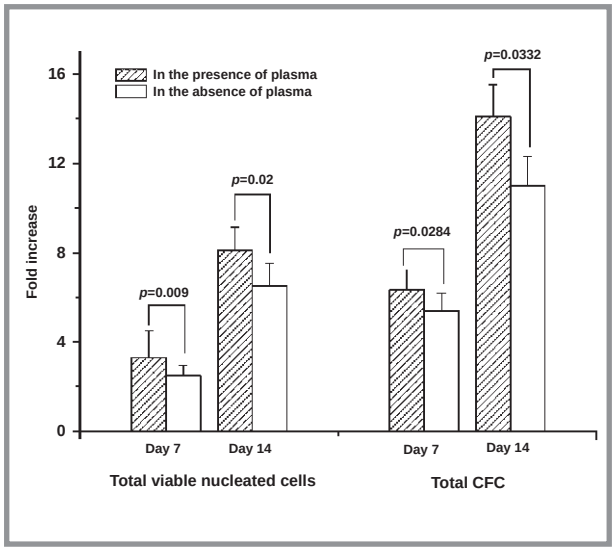

Figure 2. Comparison of the efficiency of expansion of whole blood cord cells in the presence and in the absence of plasma. Whole bloods (WB) samples were cultured in a two-chamber culture system using thrombopoietin (50 ng/mL) and flt3-ligand (50 ng/mL). In order to culture the WB cells in the absence of plasma, the plasma was removed and substituted by medium after centrifugation of WB. Data represent the mean±SD of three separate experiments. Statistical analysis was performed by a 2-tailed Student's t test.

4.6 (3.9±0.6) $\times 10^3$ CFCs/mL. Standardization of these counts showed 67.0±9.3% and 10.8±2.6% of CFC remained in the MNC and $CD34^+$ fractions, respectively, after purification and before expansion (Figure 1B). After *ex vivo* expansion for 14 days, the total CFC count increased 14.2±2.3 fold in WB, 12.5±2.2 fold in MNC, and 9.4±1.5 fold in $CD34^+$ fractions. So, the efficiency of expansion of total CFC was highest in WB, not only in terms of total count but also in terms of fold-increase. We had expected at the beginning that WB would produce higher absolute cell counts than would purified cells as cell purification inevitably causes a certain loss of cells. However the results showed that WB samples were superior to purified $CD34^+$ cells not only in terms of absolute counts but also in terms of fold increase of total CFC. These results suggest that WB may constitute a favorable microenvironment for *ex vivo* expansion. To address this issue, we compared the efficiency of expansion in the presence and in the absence of plasma. The results showed that the efficiency of expansion, in terms of the fold increase of total viable cells and total CFC, was significantly higher in the presence of plasma (Figure 2). This result suggests that plasma is a microenvironmental factor that favors *ex vivo* expansion. In conclusion, *ex vivo* expansion of whole CB in dialysis tubes could be an efficient method with potential clinical applications.

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