INTRODUCTION

Cord blood (CB) stem cells are increasingly being used as a source of hematopoietic stem cells transplantation (HSCT). Although there are several advantages, such as a lower incidence of graft-versus-host disease (GVHD) or viral infections in cord blood stem cell transplantation (CBSCT) as compared to bone marrow transplantation (BMT), slower engraftment speed and the limitation of the cell dose are still obstacles (1-5). Recent studies regarding the homing mechanism following HSCT have revealed that homing-associated cell adhesion molecules (H-CAMs) and chemokine receptors on the CD34+ cells play very important roles for engraftment (6, 7). Until recently, most studies on H-CAMs have been commonly performed by using purified CD34+ cells, and the quantity of these cells is expressed as percentages of positive cells or as the antigen density on the CD34+ cells (8-11).

Although most of the CAMs including CD49d, CD44 and CXCR4, are present on primitive hematopoietic cells, they are also found on another nucleated cells (NCs) including monocytes and lymphocytes (12-15). Therefore, not only CD34+ cells but also the other NCs expressing H-CAMs and chemokine receptors could be implicated in the engraftment process and the proliferation of hematopoietic stem cells. We investigated the differences of H-CAM and cell cycle status on the NCs in cord blood (CB), bone marrow (BM), and mobilized peripheral blood (PB). The proportions of CXCR4+ cells within the NC populations were greater in CB than in PB or BM ($p=0.0493$), although the proportions of CXCR4+, CD44+, and CD49d+ cells within the CB CD34+ cell populations were same within BM or PB. A lower proportion of CD34+CD49d+ cells within the CD34+ cell populations was more noted in CB than in PB or BM ($p=0.0085$). There were no differences in cell cycle status between CB and BM or PB. Our results suggest that the migrating potential of CB would be enhanced with increased CXCR4 expression on the NCs, but the adhesion potential of CB CD34+ cells would be less than that of PB and BM. These findings may help explain why the lower cell dose is required and engraftment is delayed in cord blood stem cell transplantation.

**Key Words**: Cell Adhesion Molecules; Nucleated Cells; Erythrocytes; Bone Marrow; Peripheral Blood; Fetal Blood
MATERIALS AND METHODS

Isolation of nucleated cells from 3 different sources of stem cells

Eight BM samples were obtained from normal healthy donors for related BMT, and these cells were cryopreserved after a red cell depletion process by density gradient separation with 10% pentastarch (Jeil Pharm, Seoul, Korea), and the cells were then analysed after thawing. Ten PB samples were obtained from the apheresed products of acute myelogenous leukemia patients, which were collected after mobilization chemotherapy for the PBSC harvest.

Thirteen CB samples were collected into transfer bags containing acid citrate dextrose (ACD) from the umbilical cord vein immediately after a full-term vaginal delivery, and the red cells were depleted by the same method as was used with the BM.

Phenotype analysis

Dual-color flow cytometry of CD34/CXCR4, CD34/CD49d, CD34/44 for the isolated nucleated cells was performed using FACSort (Becton Dickinson, San Jose, CA, U.S.A.). The cells were stained with the corresponding monoclonal antibodies for 45 min. After incubation, the cells were then washed three times in phosphate-buffered saline (PBS), fixed in 1% paraformaldehyde, and finally analysed by using Lysys II software (Becton Dickinson, San Jose, CA, U.S.A.).

Cell cycle analysis

The cells were washed two times with PBS by centrifugation for 3 min at 4°C, then they were fixed with 50% ethanol for 30 min at room temperature. Cells were suspended in PBS containing RNase (1 mg/mL, 20 μL) and propidium iodide (10 mg/mL, 2.5 μL), and they were incubated for 30 min at room temperature. The cell cycle was analysed with Multicycle using Coulter EPICS XL flow cytometer (Beckman Coulter, FL, U.S.A.).

Statistical analysis

Student’s t-test and the Wilcoxon rank sum test were performed between CB and BM, and also on the differences between the CB and PB. The SAS window version 6.12 was used for our analysis.

RESULTS

CXCR4, CD49d, and CD44 expression on the NCs

The proportions of CXCR4+ cells in the NCs were significantly higher in CB (82.76±5.89%) than in BM (62.31±15.64%) or PB (76.35±23.7) (p=0.0493). However, the percentages of CD49d+ and CD44+ cells in CB were not statistically different from BM or PB. (Table 1, Fig. 1)

The percentage of CD34+CXCR4+, CD34+CD49d, and CD34+CD44+ cells within the NC populations and within the CD34+ cell populations

Although the CD34+CXCR4+ and CD34+CD44+ cells within the NC populations in CB were not statistically different from PB or BM, the CD34+CD49d+ cells were significantly lower in CB (0.91±0.49%) than in BM (2.38±1.19%) or PB (4.23±3.14%) (p=0.0085) (Table 2). However, in the CB CD34+ cell populations, the proportions of CD34+CXCR4+, CD34+CD49d+, and CD34+CD44+ cells were not significantly different from BM or PB (Table 3).

Table 1. The proportions of CXCR4+, CD49d+, and CD44+ cells within the NC populations

|       | CB (n=13) | BM (n=8) | PB (n=10) |
|-------|-----------|----------|-----------|
| CXCR4+ cells (%) | 82.76±5.89 | 62.31±15.64* | 76.35±23.7 |
| CD49d+ cells (%) | 94.20±7.18 | 83.48±13.86 | 86.49±10.44 |
| CD44+ cells (%) | 98.61±1.3 | 98.65±1.14 | 98.51±1.73 |

Values given represent a mean±the standard deviation. CB, cord blood; BM, bone marrow; PB, peripheral blood; *: significant difference between CB and BM; †: significant difference between CB and PB.

Table 2. The proportions of CD34+ cells expressing CXCR4, CD49d, and CD44 within the NC populations

|       | CB (n=13) | BM (n=8) | PB (n=10) |
|-------|-----------|----------|-----------|
| CD34+CXCR4+ cells (%) | 0.95±0.49 | 2.09±0.83 | 3.46±3.37 |
| CD34+CD49d+ cells (%) | 0.91±0.49 | 2.38±1.19* | 4.23±3.14* |
| CD34+CD44+ cells (%) | 1.52±0.64 | 2.06±0.97 | 5.17±3.66 |

Values given represent a mean±the standard deviation. CB, cord blood; BM, bone marrow; PB, peripheral blood; *: significant difference between CB and BM; †: significant difference between CB and PB.

Table 3. The proportions of CD34+ cells expressing CXCR4, CD49d, and CD44 within the CD34+ cell populations

|       | CB (n=13) | BM (n=8) | PB (n=10) |
|-------|-----------|----------|-----------|
| CD34+CXCR4+ cells (%) | 94.11±7.11 | 82.54±12.93 | 74.81±30.71 |
| CD34+CD49d+ cells (%) | 97.90±2.38 | 98.46±2.56 | 91.29±10.42 |
| CD34+CD44+ cells (%) | 98.24±1.51 | 99.00±1.54 | 98.66±1.66 |

Values given represent a mean±the standard deviation. CB, cord blood; BM, bone marrow; PB, peripheral blood.
Cell cycle status

Most of NCs of CB (87.83 ± 6.71%), BM (87.62 ± 5.01%), and PB (88.38 ± 32%) were in G0/G1 phase and they were not statistically different. The cell fractions in S/G2/M phase showed no significant differences between those fractions in CB (12.17 ± 6.75%) and BM (12.4 ± 5.07%) or PB (11.6 ± 1.35%) (Fig. 2).
DISCUSSION

In hematopoietic stem cell transplantations, CB offers substantial advantages when compared to BM or PB. There is the rapid availability of cells and the less stringent requirements for HLA identity between the donor and recipient because of the lower risk of acute and chronic graft versus host disease. The major disadvantage of CBSCT is the delayed recovery of neutrophils and platelets, which could increase the risk of life-threatening infections and bleedings (4, 5). Although the engraftment following CBSCT is strongly correlated with infused nucleated cell numbers (2), very little is known about why the engraftment speed is slower in CBSCT than in BMT or PBSCT.

To rescue the patient’s hematopoietic systems after myeloablative chemotherapeutic or radiotherapy, the intravenously transplanted hematopoietic stem cells (HSCs) have to migrate to the bone marrow’s microenvironment for their ultimate proliferation and differentiation. In this homing process, various adhesion molecules present on both HSC and endothelial cells are involved (6, 7). The CD34+ cells exhibit other adhesion receptors they are involved in the multistep process of HSCT: homing in to the BM, adhesion to the BM microenvironment and finally, the stem cell differentiation. CXCR4 is a dominantly expressed chemokine receptor on primitive human blood cells within all stages of human development (21). Stromal cell-derived factor-1 (SDF-1), which is produced by stromal cells in various organs, is a powerful chemoattractant for its receptor CXCR4. The SDF-1/CXCR-4 interaction is clinically relevant during embryonic development, hematopoiesis and the migration of CD34+ stem cells. We revealed that although the proportions of CD34+CXCR4+ cells within the NC or CD34+ cell populations were not statistically different in BM, PB and CB, the proportions of CXCR4+ cells in NC populations were significantly higher in CB than in BM or PB. These results suggest that NCs, except CD34+ cells expressing CXCR4, were more abundant in CB than in BM or PB.

The integrins, such as VLA-4 or VLA-5, they mediate the adhesion of HSC to extracellular matrix proteins. The mobilization of BM CD34+ cells into the PB is followed by a decrease of VLA-4 and VLA-5 expression (22, 23), and the low frequency of CD49d+ on PB CD34+ cells has been also reported by many authors (24-27). In our study, we observed the same percentage of CD49d+ cells within the CB CD34+ cell populations in the BM, PB and CB. However, CD34+CD49d+ cells within the NC populations in the CB were significantly lower than in BM or PB.

In conclusion, our results suggest that the migrating potential of CB would be enhanced with increased CXCR4 expression on the NCs. However, the adhesion potential of CB CD34+ cells would be less than that of PB and BM because of the lower expression of CD34+CD49d+ cells within the NC populations. These findings may help explain why the
lower CB cell dose is required and engraftment is delayed in CBSCT.

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