A Comparison of the Kinetics of Nucleated Cells and CD34+ Cells in Neonatal Peripheral Blood and Cord Blood

Joong-Pyo Kim, Young-Ho Lee, Young-Ah Lee, and Young-Dae Kim

1Department of Pediatrics, Moonhwa Hospital, Busan, Korea; 2Department of Pediatrics & Cell Therapy Research Center, Hanyang University College of Medicine, Seoul, Korea; 3Department of Pediatrics, Dong-A University College of Medicine, Busan, Korea; 4Department of Pediatrics, Inje University Baik College of Medicine, Seoul, Korea

Correspondence and reprint requests: Young-Ho Lee, Hanyang University College of Medicine, Department of Pediatrics, #17, Haengdang-Dong, Seongdong-Ku, Seoul 133-791, Korea (e-mail: cord@hanyang.ac.kr).

Received July 19, 2006; accepted December 12, 2006

ABSTRACT
We investigated the kinetics of nucleated cells and CD34+ cells to determine the differences between cord blood (CB) and neonatal peripheral blood (NB). The correlation coefficient between white blood cell (WBC) counts and neutrophil counts was statistically significant for CB and NB at 0, 24, and 48 hours postnatally. There were no differences in the absolute counts of lymphocytes, CD3+ cells, and CD19+ cells; however, the CD16/56+ cell counts for CB were significantly decreased 48 hours postnatally (P = .011). The number of GM-CFU counts (P = .02) and CD34+ cells (P = .049) as well as CD34+CXCR4+ cells (P = .002), CD34+CD49d+ cells (P = .001), and CD34+CD44+ cells (P = .001) in CB were significantly increased over those of NB at delivery, and the numbers decreased gradually until 48 hours after delivery. In conclusion, the change in WBC count several days after delivery were closely associated with a temporary increase in neutrophils, and NK cells significantly decreased over time postdelivery. In addition, CD34+ cells were more prevalent in CB than in NB, and gradually decreased after birth. Such a change might be directly related to the kinetic changes of CXCR4 or CD49d and CD44 expression on CD34+ cells in CB and NB.

© 2007 American Society for Blood and Marrow Transplantation

KEY WORDS
Cord blood ● CD34+ ● Cytokine receptors ● Adhesion molecules

INTRODUCTION
In adults, peripheral blood cell counts remain relatively stable; however, the hematologic values of neonatal peripheral blood (NB) show a characteristic series of changes during the neonatal period after birth. Neutrophilic white blood cells (WBCs) tend to increase immediately after birth, and then decrease with time; lymphocytes also tend to decrease over the first 3 days after birth, and then increase; therefore, the normal values for newborns differ from those of children and adults [1]. However, the reason for the initial changes in newborns has not been explained.

Cord blood (CB) has recently been used as a source for donor cells for stem cell transplantation [2-6]. Many studies have reported on the qualitative and quantitative differences in stem cells from CB compared to NB and the bone marrow. It has been found that stem cells from CB are more immature and plentiful compared to those in NB [7,8]. However, there have been no studies yet to explain the reason why the stem cells are comparatively more immature and plentiful in CB than in peripheral blood of children and adults.

Theoretically, CB should have similar cellular components to NB immediately after birth, because CB itself is circulating fetal blood during fetal life; however, in a previous study [9], we observed that there were fewer CD34+ cells immediately after birth in NB than in CB. Although we suggested that CD34+ cells in the NB might decrease because of apoptosis or homing after transition from fetal circulation to neonatal circulation, we may be limited in our ability to explain the kinetic changes between CB and NB because we only compared the CB with NB collected immediately after birth.
We assumed that the difference in kinetics of CB and the NB is caused by apoptotic changes or differences in expression levels of chemokine receptors (CR) or cell adhesion molecules (CAM) on CD34\(^+\) cells. Therefore, we studied the differences in the kinetics of nucleated cells and CD34\(^+\)/H11001 cells between CB and NB collected immediately after birth as well as at defined time intervals.

**SUBJECTS AND METHODS**

**Subjects**

The subjects for this study were 14 normal full-term babies; their average gestational age was 38.74 ± 0.91 weeks (37.00–39.86 weeks), and the average weight was 3,070.91 ± 331.03 g (2,460–3,350 g). Prior to delivery, written consent was obtained from pregnant women who were hospitalized for normal vaginal delivery or Cesarean delivery, and CB and NB were collected. We acquired consents from the mothers, who were acquainted with our hospital personnel, ensuring no undue coercion by the staff involved in the treatment. This study was approved by institutional review boards of Dong-A University Hospital.

**Collection of CB and NB**

The umbilical cord was cut immediately after delivery. The umbilical vein was punctured with a syringe containing acid-citrate-dextrose and 5 mL of CB were collected with the placenta still in the uterus. After routine management of the newborn immediately after delivery (within the first 10 minutes after delivery, NB-0), 5 mL of NB were collected through the jugular vein of the newborn. Then 5 mL of NB were collected 24 (NB-24) and 48 hours postnatally (NB-48) for each participating newborn.

**Cell Counting and Immunophenotype Analysis**

Hemoglobin, WBC with fractionation, and platelet counts were measured from CB and NB samples

---

Table 1. Cell Counts in Cord Blood and Peripheral Blood of Newborns at 0, 24, and 48 Hours after Delivery

<table>
<thead>
<tr>
<th></th>
<th>CB</th>
<th>NB-0</th>
<th>NB-24</th>
<th>NB-48</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>15.71 ± 1.26</td>
<td>16.39 ± 1.17</td>
<td>14.67 ± 2.13</td>
<td>15.67 ± 1.33</td>
<td>NS</td>
</tr>
<tr>
<td>Platelets (×10^9/mL)</td>
<td>319.56 ± 78.63</td>
<td>320.18 ± 54.08</td>
<td>286.30 ± 92.74</td>
<td>334.00 ± 73.74</td>
<td>NS</td>
</tr>
<tr>
<td>WBC (×10^9/mL)</td>
<td>13.66 ± 4.01</td>
<td>18.57 ± 6.90</td>
<td>19.31 ± 2.72</td>
<td>13.62 ± 2.15</td>
<td>.042*</td>
</tr>
<tr>
<td>Neutrophil (×10^9/mL)</td>
<td>8.48 ± 2.75</td>
<td>12.93 ± 6.86</td>
<td>14.03 ± 2.58</td>
<td>9.14 ± 1.29</td>
<td>.047*</td>
</tr>
<tr>
<td>Lymphocyte (×10^9/mL)</td>
<td>4.35 ± 1.58</td>
<td>4.08 ± 0.88</td>
<td>3.71 ± 1.09</td>
<td>2.82 ± 0.67</td>
<td>NS</td>
</tr>
<tr>
<td>Monocyte (×10^9/mL)</td>
<td>0.98 ± 0.28</td>
<td>1.17 ± 0.48</td>
<td>0.91 ± 0.28</td>
<td>0.91 ± 0.27</td>
<td>NS</td>
</tr>
<tr>
<td>Eosinophil (×10^9/mL)</td>
<td>0.44 ± 0.37</td>
<td>0.34 ± 0.16</td>
<td>0.39 ± 0.17</td>
<td>0.54 ± 0.28</td>
<td>NS</td>
</tr>
<tr>
<td>Basophil (×10^9/mL)</td>
<td>0.14 ± 0.09</td>
<td>0.16 ± 0.09</td>
<td>0.10 ± 0.04</td>
<td>0.08 ± 0.02</td>
<td>NS</td>
</tr>
</tbody>
</table>

CB indicates cord blood; NB, neonatal peripheral blood; WBC, white blood cells; NS, not significant. *P values of .05 or less based on Mann-Whitney U test and they indicate that the WBC and neutrophil counts are significantly different between CB and NB-0.

---

**Figure 1.** Kinetics of blood cell counts in cord blood and peripheral blood of newborns at 0 (NB-0), 24 (NB-24), and 48 (NB-48) hours after delivery. The WBC counts as well as neutrophil counts of CB were lower than those of the NB at delivery, and increased until 24 hours then decreased to 48 hours. The correlation coefficients between WBC counts and neutrophil counts were statistically significant (r = .89 at CB, .92 at NB-0, .95 at NB-24, and .95 at NB-48).
using the Sysmex K-800 (Sysmex corporation, Kobe, Japan) automated cell counter. For flow cytometry, mononuclear cells were isolated from each 4.5 mL of CB and NB by the density gradient method with 10% pentastarch (Jeil Pharm, Seoul, Korea). The isolated mononuclear cells were stained with corresponding monoclonal antibodies for 45 minutes, washed with phosphate-buffered saline 3 times, fixed with 1% paraformaldehyde, and then analyzed with Lysys II software (Becton Dickinson, San Jose, CA). FACSort (Becton Dickinson) was used for flow cytometry, and dual-color flow cytometry was performed for CD34, CD34/CXCR4, CD34/CD49d, and CD34/CD44 to determine the kinetic changes of CR and CAM on CD34⁺ cells. Flow cytometry was also performed for CD3, CD19, and CD16/56 to study the kinetic features of lymphocytes. All antibodies were purchased from Becton Dickinson.

Clonogenic Assays

To analyze granulocyte macrophage-colony forming unit (GM-CFU), 4 × 10⁵ cells/mL of isolated nucleated cells were added to methylcellulose media (Stem Cell Technologies Inc., Vancouver, BC), and cultivated under conditions of 37°C and 5% CO₂ for 14 days. Granulocyte-macrophage colonies of more than 50 cells were scored using an inverted microscope.

Apoptotic Analysis

2 × 10⁵ cells/mL were treated with 1× binding Buffer (BD Bioscience Pharminogen™, San Jose, CA) and Annexin V-FITC kit (BD Bioscience Pharminogen™) for the purpose of apoptotic analysis. They were incubated at room temperature without light for 15 minutes, and red blood cells (RBCs) were removed using a lysis solution. They were washed with PBS and then analyzed with FACSort (Becton Dikinson).

Statistics

The Mann-Whitney U test was used to compare the mean values between the 2 nonparametric groups of CB and NB immediately after birth and to analyze the statistical differences in WBC with fractionation and CD34⁺ cells between the 2 groups. The nonparametric Spearman correlation test was used to analyze the correlation between WBC and fractionation. Repeated-measure ANOVA was used to determine whether other variables showed a statistical difference in changes over time; a P < .05 was considered statistically significant.

Figure 2. Kinetics of lymphocytes in cord blood and peripheral blood of newborns at 0 (NB-0), 24 (NB-24), and 48 (NB-48) hours after delivery. There were no differences in CD3⁺ cell and CD19⁺ cell counts between CB and NB at 0, 24, and 48 hours postnatally; however, the CD16/56⁺ cell counts significantly decreased in the CB at 48 hours postnatally.
RESULTS

Cell Counts of CB and NB

There was no difference in hemoglobin and platelet counts for CB and NB immediately after birth; however, WBC counts were significantly higher in NB immediately after birth than in CB ($P = .04$). In addition, WBC counts increased up to $19.31 \pm 2.72 \times 10^6$/mL until 24 hours postnatally; but then decreased by 48 hours postnatally. This change in WBC counts was found regardless of the numerical change in lymphocytes, monocytes, eosinophils, and basophils. However, the change in WBC counts correlated with the numerical difference in neutrophils between CB ($r = .89$) and NB immediately after birth ($r = .92$), at 24 hours postnatally ($r = .95$), and at 48 hours postnatally ($r = .95$) (Table 1 and Figure 1).

Change in Lymphocyte Counts and Lymphocyte Subsets

There was no difference in absolute lymphocyte counts between CB and NB immediately after birth. The lymphocyte counts tended to decrease somewhat over time, but these changes were not statistically significant (Table 1). There were also no differences in CD3+$^+$ or CD19+$^+$ cell counts of CB and the NB with time lapse; however, CD16/56+$^+$ cells tended to gradually decrease from CB to NB after birth ($P = .011$) (Table 2 and Figure 2).

Chemokine Receptor and Cell Adhesion Molecules

In the flow cytometric analysis for CR and CAM on CD34+$^+$ cells from CB and the NB, CD34+$^+$ cells were significantly less prevalent in the NB immediately after birth than in the CB ($P = .049$) and tended to decrease over time ($P = .044$). The expression level of CXCR4 ($P = .002$), CD49d ($P = .001$), and CD44 ($P = .001$) on CD34+$^+$ cells was lower in the NB than CB, and decreased rapidly at 24 hours postnatally (Table 3 and Figure 3).

Clonogenic Assays

Granulocyte macrophage-colony forming unit counts were highest in CB and tended to decrease over time immediately after birth ($P = .02$) (Table 4 and Figure 4).

Apoptotic Analysis

The flow cytometric analysis for cell death in CB and the NB showed similar apoptotic levels in CB and NB immediately after birth, 24 hours postnatally,

<table>
<thead>
<tr>
<th>Cell Subset</th>
<th>CB</th>
<th>NB-0</th>
<th>NB-24</th>
<th>NB-48</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34+$^+$</td>
<td>2.23 $\pm$ 0.98</td>
<td>1.82 $\pm$ 0.94</td>
<td>1.37 $\pm$ 1.13</td>
<td>0.90 $\pm$ 0.26</td>
<td>.049*</td>
</tr>
<tr>
<td>CD34+$^{CXCR4}$</td>
<td>1.78 $\pm$ 0.88</td>
<td>1.46 $\pm$ 0.86</td>
<td>0.74 $\pm$ 0.42</td>
<td>0.64 $\pm$ 0.30</td>
<td>.002</td>
</tr>
<tr>
<td>CD34+$^{CD49d}$</td>
<td>2.11 $\pm$ 0.89</td>
<td>1.87 $\pm$ 1.03</td>
<td>0.87 $\pm$ 0.47</td>
<td>0.72 $\pm$ 0.25</td>
<td>.001</td>
</tr>
<tr>
<td>CD34+$^{CD44}$</td>
<td>2.17 $\pm$ 0.90</td>
<td>1.77 $\pm$ 0.96</td>
<td>0.46 $\pm$ 0.47</td>
<td>0.74 $\pm$ 0.27</td>
<td>.001</td>
</tr>
</tbody>
</table>

CB indicates cord blood; NB, neonatal peripheral blood.

* $P$ values of .05 or less based on Mann-Whitney $U$ test and they indicate that the CD34+$^+$ cell counts are significantly different between CB and NB-0.

Figure 3. Kinetics of CD34+$^+$ cell subsets in cord blood and peripheral blood of newborns at 0 (NB-0), 24 (NB-24), and 48 (NB-48) hours after delivery. The number of CD34+$^+$ cells as well as CD34+$^{CXCR4}$+$^+$ cells, CD34+$^{CD49d}$+$^+$ cells and CD34+$^{CD44}$+$^+$ cells in CB were significantly higher than those in the NB at delivery, which decreased gradually until 48 hours after delivery.
DISCUSSION

Many researchers have reported on changes in the various components of WBCs in the NB [1,10]. According to previous reports, WBC counts at birth are generally as high as 9.0–30.0 \times 10^6/mL, and neutrophils have accounted for most of the WBC; the number of monocytes, eosinophils, and basophils have been reported to be very low, with no significant changes identified over the first 2 weeks postnatally. Significant changes in lymphocytes have been demonstrated over the first 2 weeks postnatally; at the time of birth, the absolute lymphocyte count is on the order of 5.6 \pm 1.0 \times 10^6/mL, begins to decrease over the first 3 days of life, and then it increases again up to 6.0 \times 10^6/mL at 10 days postnatally. In this study, changes in the WBC counts or fractionation also showed results consistent with these changes. The WBC counts of the NB immediately after birth significantly increased compared to that of CB, and then tended to decrease at 48 hours postnatally. The changes observed in WBC counts were consistent with the findings that the concentration of granulocyte colony-stimulating factors in the blood increases from stressful events, such as relative hypoxia at the time of birth [11], the number of neutrophils also increases, followed by a decrease 48 hours after birth resulting from the short life span of neutrophils.

We found that, over time, lymphocyte counts were somewhat decreased, although no changes in T or B lymphocytes were observed. Also, natural killer (NK) cells started to rapidly decrease 24 hours after birth. It is known that NK cells in CB have significant clinical implications [12] for stem cell transplantation using CB. That is, NK cells in CB induce γ-interferon or tumor necrosis factor-α and promote generation of granulocyte/macrophage-colony stimulating factor (GM-CSF) or interleukin (IL)-3, contributing to the reduction in frequency of graft-versus-host disease. Thus, further study is required to determine the clinical impact of NK cell counts in the NB and CB and their gradual decrease in time after delivery.

It has been reported that CD34+ cells in the NB begin to gradually decrease after birth, resulting in reduced numbers in the adult. According to a study of CD34+ cells by Li et al. [13] on normal full-term babies, CD34+ cells rapidly decrease immediately after birth and continue to decrease, up to 30%, over time 2 to 48 hours postnatally. However, as samples were not collected from the same individual, and samples taken immediately after birth were omitted, this study may not accurately reflect the kinetics.

By contrast, considering the blood circulation between fetus and placenta, the CD34+ cells in NB immediately after birth could be the same as those in CB, both qualitatively and quantitatively; however,
based on the results in this study, CD34⁺ cell counts in CB are greater than those in the NB immediately after birth. The influence of cytokines secreted from the placental tissues may be an important factor in these results. Keelan et al. [14] reported that IL-6, IL-8, and IL-1β, all cytokines causing inflammation, are involved in delivery and are secreted by the placenta, and Kauma et al. [15] reported that stem cell factor (SCF) is expressed in placental tissues, having an effect on movement, proliferation, and survival of hematopoietic cells. SCF and its receptor, Kit, control proliferation and survival of the initial hematopoietic cells. Kauma et al. [15] found that SCF and Kit were expressed in the uterine endometrium and placental tissues, and that SCF mRNA was increased 4- to 8-fold, in placental tissues. SCF activates molecules such as CD49d on the surface of CD34⁺ cells, which make CD34⁺ cells adhere to matrix cells [16-18].

Coulomb-L’Hermine et al. [19] reported that the stromal cell-derived factor (SDF)-1 plays a key role in moving stem cells secreted from placental cells. As its receptor, CXCR4, is expressed on the surface of CD34⁺ cells [20], CD34⁺ cells have been found to firmly combine with the placental tissues. These studies may explain our results demonstrating that the number of CD34⁺ cells and CD34⁺CXCR4⁺ cells in CB was greater than in the NB immediately after birth.

The expression level of CAMs such as CD49d or CD44 on CD34⁺ cells in CB was higher than that in the NB immediately after birth. This finding might be closely related to the finding that SCF secreted from the placenta could activate CD34⁺CD49d⁺ cells and adhere to fibronectin [18], and that CD49d had reduced expression in activated CD34⁺ cells from mobilized peripheral blood compared to bone marrow [21-26]. That is to say, CD34⁺CD49d⁺ cells or CD34⁺CD44⁺ cells were plentiful in CB compared to the NB, by adhesion to the inner wall of placental blood vessels under the influence of cytokines secreted from the placenta.

The finding that cells expressing the CXCR4 receptor or CD49d and CD44 in the NB gradually decrease over time after birth might not only result from cell death but also from homing of CD34⁺ cells circulating in the NB immediately after birth. That is, some of the CD34⁺CXCR4⁺ cells could home to the bone marrow under the influence of SDF-1 secreted from the neonatal bone marrow stromal cells. Also, CD34⁺ cells home to the neonatal bone marrow through CAM on the surface of CD34⁺ cells, such as CD49d and CD44. CD49d, a type of CAM expressed on the surface of CD34⁺ cells, is characterized by its combination with vascular CAM-1 or fibronectin, of extracellular matrix protein such as β1 integrin expressed in most mononuclear cells. The fact that cir-

---

**Table 5. Apoptosis Analysis in Cord Blood and Peripheral Blood of Newborns at 0, 24, and 48 Hours after Delivery**

<table>
<thead>
<tr>
<th>Cell Count (%)</th>
<th>CB</th>
<th>NB-0</th>
<th>NB-24</th>
<th>NB-48</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live</td>
<td>80.26 ± 13.11</td>
<td>82.73 ± 11.76</td>
<td>79.61 ± 7.41</td>
<td>81.59 ± 10.11</td>
<td>NS</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>2.72 ± 2.53</td>
<td>2.68 ± 2.30</td>
<td>3.00 ± 2.71</td>
<td>2.18 ± 1.35</td>
<td>NS</td>
</tr>
<tr>
<td>Necrosis</td>
<td>15.52 ± 11.45</td>
<td>13.45 ± 9.42</td>
<td>15.67 ± 5.57</td>
<td>15.33 ± 8.52</td>
<td>NS</td>
</tr>
</tbody>
</table>

CB indicates cord blood; NB, neonatal peripheral blood; NS, not significant.

---

**Figure 5.** Kinetics of apoptotic cells and necrotic cells in cord blood and peripheral blood of newborns at 0 (NB-0), 24 (NB-24), and 48 (NB-48) hours after delivery. The difference in incidence of apoptosis was not statistically significant in the comparison between CB and the NB at 0, 24, and 48 hours postnatally.
culating CD34+ cells express less CD49d, in comparison to CD34+ cells in the bone marrow, proves that it plays an important role in movement from the bone marrow or homing of CD34+ cells [26,27]. In addition, Zanjani et al. [28] emphasized that CD49d plays a key role in homing of stem cells based on the finding that engraftment into the bone marrow was significantly reduced at 24 and 48 hours after transplantation when stem cells were treated with CD49d antibodies and transplanted into animals. Another CAM that influences hematopoiesis is CD44. The ligands of CD44, hyaluronic acid, and fibronectin, are secreted from stromal cells and may make CD44-expressing stem cells adhere to the bone marrow matrix. This is supported by the experimental finding that when CD44 antibodies are treated with stem cells to be supported by the experimental finding that when stem cells adhere to the bone marrow matrix. This is significantly reduced at 24 and 48 hours after transplantation when stem cells were treated with CD49d antibodies and transplanted into animals. Another CAM that influences hematopoiesis is CD44. The ligands of CD44, hyaluronic acid, and fibronectin, are secreted from stromal cells and may make CD44-expressing stem cells adhere to the bone marrow matrix. This is significantly reduced at 24 and 48 hours after transplantation when stem cells were treated with CD49d antibodies and transplanted into animals. Another CAM that influences hematopoiesis is CD44. The ligands of CD44, hyaluronic acid, and fibronectin, are secreted from stromal cells and may make CD44-expressing stem cells adhere to the bone marrow matrix. This is significantly reduced at 24 and 48 hours after transplantation when stem cells were treated with CD49d antibodies and transplanted into animals. Another CAM that influences hematopoiesis is CD44. The ligands of CD44, hyaluronic acid, and fibronectin, are secreted from stromal cells and may make CD44-expressing stem cells adhere to the bone marrow matrix. This is significantly reduced at 24 and 48 hours after transplantation when stem cells were treated with CD49d antibodies and transplanted into animals. Another CAM that influences hematopoiesis is CD44. The ligands of CD44, hyaluronic acid, and fibronectin, are secreted from stromal cells.

In addition, we found that the number of GM-CFU in CB was greater than that in the NB immediately after birth, and it significantly decreases in NB over time; this may be related to the decrease in CD34+ cells and their subsets over time as shown from the study by Lee et al [33].

In conclusion, changes in the WBC counts for several days after delivery in the newborn are closely related to a temporary increase in neutrophils and a significant decrease in NK cells over time after delivery. In addition, CD34+ cells are more prevalent in CB than in NB and gradually decrease after birth; such a change might be directly related to the kinetic changes of CXCR4 or CD49d and CD44 expression on CD34+ cells in CB and NB.

ACKNOWLEDGMENTS

This work was supported in part by a grant from Seoul R&D Program (10548).

REFERENCES


