Detection of Maternal Progenitor Cells in Human Umbilical Cord Blood by Single-Colony Karyotyping

To the Editor:

Fetal blood collected immediately after delivery has been shown to contain hematopoietic progenitor cells at similar or higher frequency than those in bone marrow (BM). Therefore, umbilical cord blood (UCB) has been used as a source of hematopoietic progenitors for clinical transplantation and is proving to be an acceptable potential alternative to BM. However, concerns have been raised about contamination of UCB samples with maternal cells, which might lead to problems of graft-versus-host reactivity. To address this question, UCB samples have been evaluated for presence of maternal cells through polymerase chain reaction and fluorescent in situ hybridization (FISH) techniques. Recently, Hall et al have used FISH to detect the presence of maternal cells in unfractionated male UCB (n = 49) as well as in the CD8+ (n = 39) and CD34+ (n = 27) subpopulations, reporting a contamination of maternal cells in 15%, 13%, and 4% of fractions, respectively.

Because fetal progenitor cells have been identified in maternal peripheral blood during pregnancy due to the higher stimuli on the hematopoietic system, we have used single-colony karyotyping to
contain a homogeneous population of donor cells. Although more than five metaphases were studied to assign the karyotype to each colony. Our data show that maternal hematopoietic progenitor cells should at least provide a note of caution in assuming that UCB samples screened male UCB samples for the presence of maternal hematopoietic progenitor cells. Cyto genetic analysis was performed on a single colony obtained from unseparated UCB (n = 6) and on mononuclear (n = 4) and CD34+ (n = 3) preparations after 14 days in short-term culture assay (Table 1). Mononuclear cells (MNC) were obtained, as previously described, using a two-step separation over poligeline (Emagel; Behringwerke, Marburg, Germany) and ficoll/Hypaque (d = 1.077 g/mL; Sigma Chemical Co, St Louis, MO), whereas CD34+ cells were obtained by immunoadsorption to polystyrene cell separation devices (MicroCELLector; Applied Immune Sciences Inc, Menlo Park, CA). Unseparated, MNC, and CD34+ cells were plated in methylcellulose and stimulated with interleukin-3, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, erythropoietin, and stem cell factor at optimal concentrations, as detailed elsewhere. Single-colony karyotyping was performed as previously described. Briefly, colcemid (1 μg/mL; 100 μL/dish) was added to cultures 3 to 4 hours before the end of the incubation period. Single-well isolated colonies were picked up under inverted microscope and transferred into a 96-well tissue culture plate containing 40 μL of 0.075 mol/L KCl and dispersed by gentle pipetting. After incubation (25 minutes at 37°C), the entire content of each well was transferred onto a microscope slide pretreated with poly-L-lysine (0.1% wt/vol; Sigma Chemical Co) and incubated in a moist environment. Methanol/ glacial acetic acid (3:1) fixative was gently dropped onto the colony on the slide and allowed to air dry. Metaphases suitable for analysis were sequentially Q- and G-banded according to routine methods; more than five metaphases were studied to assign the karyotype to each colony. Our data show that maternal hematopoietic progenitor cells were identified in 1 of 6 (17%) unseparated UCB samples, but no maternal progenitor cells were detected in any of the separated fractions analysed. Although this high frequency in unseparated UCB might be the result of the small sample size in our study, these results should at least provide a note of caution in assuming that UCB samples contain a homogeneous population of donor cells.

### Table 1. Maternal Cell Detection With Karyotyping of Single UCB Colonies From Unseparated, Mononuclear and CD34+ Cells

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Unseparated</th>
<th>MNC</th>
<th>CD34+</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0/45</td>
<td>0/35</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
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<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>0/54</td>
<td>0/60</td>
<td>ND</td>
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<td>0/21</td>
<td>0/32</td>
</tr>
<tr>
<td>5</td>
<td>0/38</td>
<td>0/42</td>
<td>0/27</td>
</tr>
<tr>
<td>6</td>
<td>0/25</td>
<td>ND</td>
<td>0/15</td>
</tr>
</tbody>
</table>

Values represent the number of maternal CFC found over the total number of CFC analyzed.

Abbreviations: CFC, colony-forming cells; ND, not done.

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Camillo Almici
Carmelo Carlo-Stella
Vittorio Rizzoli
Department of Hematology
BMT Unit
University of Parma
Parma, Italy
John E. Wagner
Department of Pediatrics
Bone Marrow Transplant Program
University of Minnesota
Minneapolis, MN

### REFERENCES

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C Almici, C Carlo-Stella, V Rizzoli and JE Wagner