Rapid Communication

Thy-1 Expression Is Linked to Functional Properties of Primitive Hematopoietic Progenitor Cells From Human Umbilical Cord Blood

By Hector Mayani and Peter M. Lansdorp

We have previously shown that the most primitive human hematopoietic cells are included within a cell subpopulation expressing high levels of CD34 and low or undetectable levels of CD45RA and CD71. In this study, cord blood cells with this phenotype were sorted and further separated based on their expression of the Thy-1 antigen. The proliferation and differentiation of the purified cell fractions in response to a mixture of hematopoietic cytokines was analyzed in serum- and stroma-free liquid cultures. Thy-1+ cells (25% of CD34+ CD45RA+ CD71+ cells) were particularly enriched for high proliferative potential colony-forming cells (HPP-CFC; up to 45% of the clonogenic cells), whereas Thy-1− cells were enriched for multipotential colony-forming cells (CFU-MIX; up to 46% of the clonogenic cells). When both subpopulations were cultured in serum-free liquid cultures supplemented with a cytokine mixture that included steel factor, interleukin-6 (IL-6), granulocyte-macrophage colony-stimulating factor (GM-CSF)/IL-3 fusion protein, M-CSF, G-CSF, and erythropoietin, Thy-1+ cells showed a much higher numerical expansion of CD34+ cells (30,000-fold) and colony-forming cells (4,700-fold) than was observed in cultures initiated with Thy-1− cells (900-fold increase in CD34+ cell numbers and 241-fold increase in CFC numbers). Cells coexpressing CD34 and Thy-1 were only transiently expanded (up to 29-fold) and were not detected after day 22 of culture. When CD34+ CD45RA+ CD71+ Thy-1+ cells were cultured, either in semisolid or liquid cultures, in the presence of anti-Thy-1 antibody, a significant reduction in progenitor cell numbers (particularly HPP-CFC) was observed. In contrast, CD34+ CD45RA− CD71− Thy-1− cells were not affected by anti-Thy-1. The results of this study indicate that Thy-1 is expressed on primitive cord blood progenitors with the highest in vitro proliferative potential, and further suggest that Thy-1 is involved in hematopoietic cell development, possibly by mediating a negative signal that results in inhibition of primitive cell proliferation.

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tured in the presence of 5E10 (anti-Thy-1 antibody), a significant reduction in progenitor cell number was observed only in cultures of Thy-1+ cells, which suggests that Thy-1 may be involved in the regulation of hematopoietic cell proliferation.

MATERIALS AND METHODS

Cell separation. Cord blood cells, collected according to institutional guidelines, were obtained during normal full-term deliveries. Low-density cells (<1.077 g/mL) were isolated using Ficoll-Paque (Pharmacia LKB, Uppsala, Sweden), washed twice in phosphate-buffered saline (PBS), resuspended in Hank's HEPES-buffered salt solution containing 30% fetal calf serum (FCS) and 7.5% dimethyl sulfoxide (DMSO), aliquoted, and frozen until used. Vials of frozen cells were rapidly thawed and slowly diluted with Iscove's medium containing 30% FCS and 0.1 mg/mL DNA-se (type II-S, D4513; Sigma Chemical Co, St Louis, MO). Cells were then washed twice and resuspended in Hank's HEPES-buffered salt solution containing 2% FCS and 0.1% sodium azide (HFN) for subsequent staining.

Cells were stained as previously described. Briefly, cells (107/mL) were incubated simultaneously with monoclonal antibodies (MoAbs) specific for CD34 (8G12)16 labeled with cyanine 5-succinimidyl ester (Cy5), CD71 (OKT9)22 labeled with fluorescein isothiocyanate (FITC), and CD45RA (8d2)22 labeled with phycoerythrin (RPE), at 20, 1, and 4 pg/mL, respectively, for 30 minutes at 4°C. Controls consisted of single-stained suspensions and three-color staining with anti-trinitrophenyl (TNP)-RPE instead of 8d2-RPE. Cells were then washed twice and resuspended in HFN containing 2 pg/mL propidium iodide (PI) before sorting. Cells were sorted on a FACStar Plus (Becton Dickinson, San Jose, CA) equipped with a 5-W argon and a 30-nm helium neon laser. Specific fluorescence of FITC, RPE, PI, and Cy5 excited at 488 nm (0.4 W) and 633 (30 mW) nm, as well as forward and orthogonal light scatter signals, were used to establish sort windows. Cells were separated into fractions expressing low or undetectable levels of any of the antigens (ie, CD71+) and cells expressing intermediate or high levels of antigen (ie, CD71+). Once separated, all the CD34+ CD45RA+ CD71+ cells were incubated with anti-Thy-1 MoAb (5E10)22 labeled with RPE at 5 pg/mL for 30 minutes at 4°C. Cells were then resorted on a FACStar Plus.

Cell culture. Purified CD34+ CD45RA+ CD71+ cells, expressing intermediate/high (CD34+ CD45RA+ CD71+ Thy-1+) or low/undetectable (CD34+ CD45RA+ CD71+ Thy-1-) levels of Thy-1, were cultured in serum-free medium consisting of Iscove's modified Dulbecco's medium (IMDM) supplemented with bovine serum albumin (BSA) (2%), insulin (10 pg/mL), transferrin (200 pg/mL), 2-mercaptoethanol (10-5 mol/L), low-density lipoprotein (40 pg/mL), and penicillin-streptomycin (10 U and 50 pg/mL, respectively). The medium was supplemented with the following recombinant hematopoietic cytokines: mast cell growth factor or steel factor (MGF, 50 ng/mL), interleukin-6 (IL-6; 10 ng/mL), IL-3 (20 ng/mL), erythropoietin (Epo; 3 U/mL), granulocyte-macrophage colony-stimulating factor (GM-CSF)/IL-3 fusion protein (FP; 20 ng/mL), M-CSF (10 ng/mL), and G-CSF (21 ng/mL). MGF, IL-3, IL-6, and FP were kindly provided by Dr D.E. Williams (Immunex, Seattle, WA). Epo and G-CSF were provided by colleagues in the Terry Fox Laboratory. M-CSF was a gift from Genetics Institute (Cambridge, MA).

Cells were cultured in 24-well tissue culture plates (Nunc, Kamstrup, Denmark) at 37°C, 5% CO2, at a density of 1,000 cells/well. At weekly intervals, cells were harvested from the wells, washed, counted in a hemocytometer using trypan blue, stained with MoAbs (as described above), and analyzed and resorted (CD34+ cells) on a FACStar Plus.

Clonogenic assays. To determine the numbers of myeloid (CFU-C), erythroid (BFU-E and CFU-E), multipotent (CFU-MIX), and high proliferative potential (HPP-CFC)24 progenitors within the cells harvested from the liquid cultures, a fraction of such cells was plated in semisolid cultures, as described previously, at a concentration of 5,000 cells/mL. The cultures were supplemented with agar-leukocyte conditioned medium (10% vol/vol; Media Preparation Service, Terry Fox Laboratory), Epo (3 U/mL), MGF (50 ng/mL), and FP (20 ng/mL).

RESULTS

Frequency of cell subpopulations. As shown in Fig 1, and in agreement with our previous findings, low side scatter, CD34+ cells represent a distinct, small (0.8% to 2.9%; range from 12 separate experiments, each one corresponding to a different cord blood) subpopulation of umbilical cord blood mononuclear cells (Fig 1A). Among gated CD34+ cells, cells expressing low/undetectable levels of both CD45RA and CD71 (CD34+ CD45RA+ CD71+) cells corresponded to 8% to 32% (range from 12 experiments) of the CD34+ cell population (Fig 1B). CD34+ CD45RA+ CD71+ cells were further subdivided into cells expressing intermediate/high (Thy-1+) or low/undetectable (Thy-1-) levels of Thy-1 (Fig 1C). The majority (68% to 79%; range from five experiments) of CD34+ CD45RA+ CD71+ cells expressed low/undetectable levels of Thy-1, whereas only 21% to 32% expressed intermediate/high levels of this cell surface molecule (Fig 1C). Thus, CD34+ CD45RA+ CD71+ Thy-1+ cells comprised approximately 5% of all CD34+ cells in cord blood, and 0.07% of all mononuclear cells.

Apart from small subpopulations of CD34+ CD45RA+ CD71+ Thy-1+ cells (3% to 6% of all CD34+ cells) and CD34- CD45RA+ CD71+ Thy-1+ cells (0.7% to 2.1% of all CD34- cells), the CD34+ Thy-1+ cells used in this study represented all Thy-1+ cells from umbilical cord blood. Because the purpose of the present study was to further separate the subpopulation of CD34+ CD45RA+ CD71+ cells, the characterization of the Thy-1+ cells outside this subpopulation was not pursued.

Composition of CD34+ CD45RA+ CD71+ cell subpopulations. The functional properties of cord blood-derived CD34+ CD45RA+ CD71+ cells sorted on the basis of Thy-1 expression were initially studied in colony assays. The previously described high content of cells giving rise to colonies containing multiple cell lineages (CFU-MIX) was confirmed in the present study. Among CD34+ CD45RA+ CD71+ cells, significant differences in the proportion of various hematopoietic progenitors between the cells expressing intermediate/high and low/undetectable levels of Thy-1 were observed. CD34+ CD45RA+ CD71+ Thy-1+ cells were particularly enriched for CFU-MIX (43% of all clonogenic cells; Table 1); they also contained a higher frequency of CFU-C (1.6-fold) and BFU-E (1.4-fold) than those cells expressing intermediate/high Thy-1 levels (Table 1). In contrast, CD34+ CD45RA+ CD71+ Thy-1+ cells contained a significantly higher frequency (42% of all clonogenic cells in this population) of HPP-CFC compared with CD34+ CD45RA+ CD71+ Thy-1+ cells (7%; Table 1). It is noteworthy that all the colonies derived from HPP-CFC with the CD34+ CD45RA+ CD71+ Thy-1+ phenotype had a diameter
of 1 to 2.5 mm, whereas 50% to 70% of the HPP-CFC present in the CD34+ CD45RA−CD71+ Thy-1+ cell subpopulation gave rise to colonies with a diameter of >2.5 mm.

CD34+ cell expansion. As reported previously, among the different CD34+ cell subpopulations, those expressing low/undetectable levels of CD45RA and CD71 possess the highest CD34+ cell-expansion potential. In this study, we assessed the expansion potential of both CD34+ CD45RA−CD71+ Thy-1+ and CD34+ CD45RA−CD71+ Thy-1− cells by culturing them at 1,000 cells/well for up to 40 days. The cultures were supplemented with MGF, IL-6, FP, M-CSF, and Epo, a cytokine mixture that has proven to be very effective for proliferation and maturation of primitive hematopoietic progenitors. As shown in Fig 2A, we found that the Thy-1− cells had a much higher CD34+ cell expansion potential than the Thy-1+ cells. This was observed throughout the entire culture period. These results were in keeping with the fact that CD34+ CD45RA−CD71+ Thy-1+ cells are enriched for HPP-CFC (Table 1), which have been reported to have a very high replating and proliferative potential. CD34+ CD45RA−CD71+ Thy-1− cells also showed an impressive CD34+ cell expansion; however, as mentioned above, it was significantly lower than that observed with Thy-1+ cells (Fig 2A).

Thy-1+ cell expansion. In cultures initiated with CD34+ CD45RA−CD71+ Thy-1+ cells, we observed that the vast majority of the CD34+ cells expanded in culture expressed low/undetectable levels of Thy-1. In fact, at day 8, only 11% of the CD34+ cells present in culture were Thy-1+. This proportion decreased gradually until no CD34+ Thy-1+ cells were detected by day 29. However, in terms of absolute numbers, a significant increase (up to 29-fold) was observed in the levels of CD34+ Thy-1+ cells during the first 3 weeks of culture (Fig 2B). It is noteworthy that at day 8, a small fraction (1.4%) of the CD34+ cells developed in culture were Thy-1− (not shown), which indicated that this antigen remained expressed or was re-expressed, although only transiently, on a small proportion of more mature cells that were not further characterized in this study. CD34+ Thy-1− cells were practically absent from day 16 of culture.

Clonogenic cell expansion. The production of CFC was assessed on days 8 and 29 of culture. In terms of total clonogenic cell numbers, CD34+ CD45RA−CD71+ Thy-1+ cells showed the highest expansion observed during the first week of culture (2.4-fold higher than CD34+ CD45RA−CD71+ Thy-1− cells). Regardless of the subpopulation used to initiate the culture, the progenitors committed to the erythroid lineage (CFU-E and BFU-E) were the most numerous at day 8 (Table 2). In cultures of Thy-1+ cells, the mean increments observed in progenitor cell numbers were as follows: CFU-C, 83-fold; BFU-E, 73-fold; CFU-MIX, 22-fold; and HPP-CFC, 31-fold. On the other hand, in cultures of Thy-1− cells, the mean increments were as follows: CFU-C, 157-fold; BFU-E, 161-fold; CFU-MIX, 40-fold; and HPP-CFC, 40-fold (Table 2). Similar to day 0 cells, approximately 65% of the HPP-CFC produced in cultures of Thy-1+ cells gave rise to colonies with a diameter of greater than 2.5 mm; on the other hand, all of the HPP-CFC developed in cultures of Thy-1− cells formed colonies with a diameter of 1 to 2.5 mm.

After 29 days of culture, we were unable to detect erythroid as well as multipotential progenitors. At this point, only CFU-C and HPP-CFC were observed (Table 2). In cultures initiated with CD34+ CD45RA−CD71+ Thy-1− cells, CFU-C numbers increased 381-fold compared with the

Table 1. Progenitor Cell Content in CD34+ CD45RA−CD71+ Cell Subpopulations Expressing Intermediate/High or Low/Undetectable Levels of Thy-1

<table>
<thead>
<tr>
<th>Cell Fraction</th>
<th>Cloning Efficiency (%)</th>
<th>Relative Proportion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CFU-C</td>
<td>BFU-E</td>
</tr>
<tr>
<td>Thy-1+</td>
<td>17 ± 5</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>Thy-1−</td>
<td>29 ± 4</td>
<td>24 ± 5</td>
</tr>
</tbody>
</table>

Results represent mean ± SD from six separate experiments. Each experiment corresponded to a different cord blood. Cells were plated at a concentration of 100 cells per dish (1 mL). Colonies were scored on days 14 (CFU-C, BFU-E, and CFU-MIX) and 24 (HPP-CFC).
PROLIFERATION AND MATURATION OF Thy-1+ CORD BLOOD CELLS

For days 16 through 40 the total number of CD34+ cells was extrapolated from the number obtained at the previous harvest (eg, total CD34+ cell number at day 16 equals total CD34+ cell number at day 8 times the number of CD34+ cells in culture at day 16 divided by 1,000). The number of CD34+ Thy-1+ cells was calculated in a similar manner. CD34+ Thy-1+ cells were not detected in cultures initiated with CD34+ CD45RA-CD71-Thy-1- cells. Results shown correspond to mean ± SD from three separate experiments.

Table 2. Total Number of Progenitor Cells in Cultures Initiated With CD34+ CD45RA- CD71-Thy-1+ Subpopulations Expressing Intermediate/High or Low/Undetectable Levels of Thy-1

<table>
<thead>
<tr>
<th>Cell Fraction</th>
<th>Progenitor</th>
<th>Day 0</th>
<th>Day 8</th>
<th>Day 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thy-1*</td>
<td>CFU-C</td>
<td>31 ± 10</td>
<td>2,585 ± 977</td>
<td>986,852 ± 371,059</td>
</tr>
<tr>
<td></td>
<td>CFU-E</td>
<td>BD</td>
<td>6,900 ± 2,870</td>
<td>BD</td>
</tr>
<tr>
<td></td>
<td>BFU-E</td>
<td>42 ± 11</td>
<td>3,064 ± 1,247</td>
<td>BD</td>
</tr>
<tr>
<td></td>
<td>CFU-MIX</td>
<td>62 ± 12</td>
<td>1,370 ± 430</td>
<td>BD</td>
</tr>
<tr>
<td></td>
<td>HPP-CFC</td>
<td>86 ± 9</td>
<td>2,645 ± 988</td>
<td>56,077 ± 17,759</td>
</tr>
<tr>
<td>Thy-1*</td>
<td>CFU-C</td>
<td>58 ± 12</td>
<td>9,126 ± 3,704</td>
<td>51,687 ± 23,348</td>
</tr>
<tr>
<td></td>
<td>CFU-E</td>
<td>BD</td>
<td>14,575 ± 4,600</td>
<td>BD</td>
</tr>
<tr>
<td></td>
<td>BFU-E</td>
<td>67 ± 10</td>
<td>10,786 ± 3,225</td>
<td>BD</td>
</tr>
<tr>
<td></td>
<td>CFU-MIX</td>
<td>105 ± 19</td>
<td>4,267 ± 1,839</td>
<td>BD</td>
</tr>
<tr>
<td></td>
<td>HPP-CFC</td>
<td>20 ± 8</td>
<td>791 ± 301</td>
<td>8,675 ± 3,901</td>
</tr>
</tbody>
</table>

Results represent mean ± SD from three separate experiments. Each experiment corresponded to a different cord blood. Progenitor cell numbers were calculated based on the total cell number at each time point of cultures initiated with 1,000 purified cells and the number of progenitors observed in semisolid cultures.

Proliferation and maturation of Thy-1+ cord blood cells

Fig 2. Production of CD34+ cells (A) and CD34+ Thy-1+ cells (B) in serum-free liquid cultures initiated with CD34+ CD45RA- CD71-Thy-1+ cells (B) or CD34+ CD45RA-CD71-Thy-1- cells (C). Cultures—supplemented with MGF, IL-6, FP, M-CSF, G-CSF, and Epo—were initiated with 1,000 cells of the indicated phenotype. On the indicated days, CD34+ cells with a low side scatter were resorted and used to initiate subcultures at 1,000 cells/culture. CD34+ cell numbers were calculated from the percentage of CD34+ cells times the total cell number at each time point. For days 16 through 40 the total number of CD34+ cells was extrapolated from the number obtained at the previous harvest (eg, total CD34+ cell number at day 16 equals total CD34+ cell number at day 8 times the number of CD34+ cells in culture at day 16 divided by 1,000). The number of CD34+ Thy-1+ cells was calculated in a similar manner. CD34+ Thy-1+ cells were not detected in cultures initiated with CD34+ CD45RA- CD71-Thy-1- cells. Results shown correspond to mean ± SD from three separate experiments.

Numbers observed at day 8, and 31,833-fold compared with day 0. HPP-CFC numbers were increased 21-fold compared with day 8, and 652-fold compared with the numbers observed at day 0 (Table 2). In cultures initiated with CD34+ CD45RA-CD71-Thy-1+ cells, CFU-C levels increased sixfold compared with day 8, and 891-fold compared with day 0. On the other hand, HPP-CFC numbers were increased 11-fold compared with day 8, and 433-fold compared with day 0 (Table 2). Interestingly, at this time point all the HPP-CFCs observed in cultures of either Thy-1+ or Thy-1- cells gave rise to colonies with a diameter of 1 to 2.5 mm.

Total cell expansion. To assess the total cell production of the two CD34+ CD45RA-CD71-Thy-1+ cell subpopulations, we quantitated the total cell number produced in culture and calculated the average number of cells produced per CD34+ cell. As shown in Table 3, during the first 3 weeks of culture the highest total cell expansion was observed in the CD34+ CD45RA-CD71-Thy-1- cell subpopulation (1.6- to 3.8-fold higher than the expansion observed in Thy-1+ cells). However, at days 29 and 40, the total cell numbers observed in both subpopulations were practically the same (Table 3). These results indicate a continuous decrease in the cellular output by cells expressing low/undetectable levels of Thy-1, in contrast to a relatively constant cell production of Thy-1+ cells.

After the first week, the majority of the cells produced in culture showed an erythroid phenotype (ie, high levels of CD71 and low levels of CD45RA; Fig 3), which correlated with the high proportion of erythroid progenitors observed in semisolid cultures (Table 2). In contrast, at day 29 practically all of the cells present expressed intermediate or high levels of CD45RA, and intermediate levels of CD71, a phenotype consistent with the myeloid lineage (Fig 3). Once again, this correlated with the fact that at this time point, practically all of the progenitors observed in semisolid cultures were CFU-C and HPP-CFC (Table 2).

Anti-Thy-1 antibody inhibits the growth of Thy-1+ primitive cells. To further investigate the possible role of Thy-1 in hematopoietic cell development, we cultured both CD34+ CD45RA-CD71-Thy-1+ and CD34+ CD45RA-CD71-Thy-1- cells in the presence of anti-Thy-1— at 5 μg/mL—
Table 3. Total Cell Production per CD34+ Cell in Cultures Initiated With CD34+ CD45RA- CD71+ Cell Subpopulations Expressing Intermediate/High or Low/Undetectable Levels of Thy-1

<table>
<thead>
<tr>
<th>Cell Fraction</th>
<th>Total Cell No. Produced per CD34+ Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 8</td>
</tr>
<tr>
<td>Thy-1+</td>
<td>766 ± 409</td>
</tr>
<tr>
<td>Thy-1-</td>
<td>2,151 ± 1,366</td>
</tr>
</tbody>
</table>

Results represent mean ± SD from three separate experiments. Each experiment corresponded to a different cord blood. Total cell production per CD34+ cell was calculated based on the total cell number at each time point of cultures initiated with 1,000 purified cells and the number of CD34+ cells used to initiate the subcultures. For example, numbers at day 16 were calculated based on the total cell number in culture at day 16 and the input number of CD34+ cells (1,000 CD34+ cells) used to initiate the subcultures on day 8.

in semisolid and liquid cultures. Addition of anti-Thy-1 to semisolid cultures of CD34+ CD45RA- CD71+ Thy-1+ cells resulted in a significant reduction in the number of hematopoietic colonies produced (Table 4). Among them, HPP-CFC, particularly those giving rise to colonies of greater than 2.5 mm in diameter, were the most affected. In contrast, no significant effects were observed in semisolid cultures of CD34+ CD45RA- CD71+ Thy-1- cells (Table 4). Incubation of total CD34+ CD45RA- CD71+ cells with anti-Thy-1 antibody for 2 hours before culture did not inhibit the formation of HPP-CFC. However, culture of such cells in the presence of anti-Thy-1 resulted in a dose-dependent inhibition of HPP-CFC, suggesting that the continuous or prolonged presence of this antibody during culture is required for inhibition of colony formation (results not shown).

In a different set of experiments, CD34+ CD45RA- CD71+ Thy-1+ and CD34+ CD45RA- CD71+ Thy-1- cells were cultured in liquid cultures for 8 days, in the absence or in the presence of anti-Thy-1, after which the total numbers of nucleated, CD34+, and CFC were determined. Addition of anti-Thy-1 to cultures of CD34+ CD45RA- CD71+ Thy-1+ cells resulted in a slight decrease in the total cell number (16% inhibition) and in the CD34+ cell number (10% inhibition), compared with control cultures. However, in terms of CFC numbers, a more significant inhibitory effect was observed (Table 5). In keeping with the results obtained in semisolid cultures, addition of anti-Thy-1 resulted in the preferential inhibition of HPP-CFC (Table 5). In contrast, in cultures of CD34+ CD45RA- CD71+ Thy-1- cells, anti-Thy-1 had no significant effect on the numbers of either total nucleated, CD34+ (not shown), or CFC (Table 5).

DISCUSSION

In the human system, primitive hematopoietic progenitors are comprised within a cell subpopulation expressing high levels of CD34 and low/undetectable levels of CD45RA and CD71 (CD34+ CD45RA- CD71-). However, this subpopulation is still very heterogeneous and includes, in umbilical cord blood, multipotent progenitors (CFU-MIX; up to 42% of the progenitor cells), as well as committed

![Fig 3. Phenotypic characterization of hematopoietic cells produced in serum-free cultures initiated with purified CD34+ CD45RA- CD71- Thy-1+ cells (top panels) or CD34+ CD45RA- CD71+ Thy-1- cells (bottom panels). All dot plots are from a representative experiment (n = 3).](www.bloodjournal.org)
myeloid (CFU-C) and erythroid (BFU-E) progenitors. Because the Thy-1 antigen has been shown to be expressed on primitive cells capable of establishing human hematopoiesis in vivo in SCID mice, and in vitro in long-term marrow cultures, we used this antigen to further subdivide the subpopulation of cord blood-derived CD34+ CD45RA+ CD71+ cells.

The majority (70% to 80%) of CD34+ CD45RA+ CD71+ cells expressed low/undetectable levels of Thy-1, whereas only 20% to 30% expressed intermediate/high Thy-1 levels. CD34+ CD45RA+ CD71+ Thy-1+ cells corresponded to only 0.07% of all cord blood mononuclear cells, and these cells were found to be enriched for primitive progenitors, including HPP-CFC (up to 45% of all clonogenic cells in this fraction) and CFU-MIX (up to 29%). Previous studies by Lu et al have shown the presence of HPP-CFC in human cord blood. These investigators showed that such progenitors can be subdivided, based on the size of the colonies produced, in relatively more mature (progenitors giving rise to colonies of 1 to 2.5 mm in diameter) and immature (colonies > 2.5 mm in diameter) HPP-CFC. We also observed significant differences in colony size between Thy-1+ and Thy-1- HPP-CFC, with the majority of Thy-1+ HPP-CFC giving rise to large (>2.5 mm in diameter) colonies, whereas all of the Thy-1- HPP-CFC produced small (1 to 2.5 mm in diameter) colonies. These differences in colony size suggest that Thy-1+ HPP-CFC could be more immature than their Thy-1- counterparts.

In keeping with their high content of HPP-CFC, CD34+ CD45RA+ CD71+ Thy-1+ cells showed a very high CD34+ and CFU expansion capacity, that was much higher than that of CD34+ CD45RA+ CD71- Thy-1- cells, when cultured in similar cytokine-supplemented cultures. Throughout this study we observed a direct correlation between long-term hematopoiesis in vitro and the presence of HPP-CFC, and not CFU-MIX, which suggested that in this experimental system long-term hematopoiesis is sustained by a population that includes, or produces, HPP-CFC. This was further supported by the fact that after 3 weeks in culture there was a complete shift toward myelopoiesis, ie, only myeloid progenitors (CFU-C and HPP-CFC) were detected, and all the cells observed showed a nonerythroid phenotype (high levels of CD45RA and intermediate levels of CD71). These results support the notion that HPP-CFC possess a higher proliferative potential than CFU-MIX, and that, in our system, the latter have a very limited, if any, replating capacity. However, it is noteworthy that recent reports indicate that cord blood-derived CFU-MIX have some degree of replating ability, which can be increased by the addition of cord blood plasma.

The presence of long-term culture initiating-cells (LTC-IC) before and after culture, was not assessed in the present study. However, we have previously shown that such progenitors are included within the cell population expressing high levels of CD34 and low/undetectable levels of CD45RA and CD71, and the cell population coexpressing

### Table 4. Effect of 5E10 (anti-Thy-1) on Progenitor Cell Growth in Semisolid Cultures of Cord Blood CD34+ CD45RA+ CD71+ Cells

<table>
<thead>
<tr>
<th>Cell Fraction</th>
<th>Antibody</th>
<th>CFU-C</th>
<th>BFU-E</th>
<th>CFU-MIX</th>
<th>HPP-CFC I</th>
<th>HPP-CFC II</th>
<th>Total CFC</th>
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<tbody>
<tr>
<td>Thy-1*</td>
<td>—</td>
<td>3; 3</td>
<td>3; 4</td>
<td>5; 5</td>
<td>5; 4</td>
<td>3; 4</td>
<td>19; 20</td>
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<tr>
<td>5E10</td>
<td>—</td>
<td>3; 2</td>
<td>3; 3</td>
<td>3; 4</td>
<td>BD; BD</td>
<td>3; 2</td>
<td>11; 11</td>
</tr>
<tr>
<td>Control MoAb</td>
<td>—</td>
<td>3; 3</td>
<td>3; 3</td>
<td>5; 6</td>
<td>4; 4</td>
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</tbody>
</table>

Results represent mean ± SD from two to four separate experiments. One hundred CD34+ CD45RA+ CD71+ cells, expressing intermediate/high (Thy-1+) or low/undetectable (Thy-1-) levels of Thy-1, were cultured in semisolid medium supplemented with a mixture of hematopoietic cytokines in the presence or absence of antibodies. After 8 days of cultures, a fraction of the nucleated cells produced in semisolid cultures was plated in semisolid medium to determine the number of CFC.

Abbreviations: BD, below detection; HPP-CFC I >2.5 mm; HPP-CFC II 1–2.5 mm.
CD34 and Thy-1. Thus, it seems very likely that LTC-IC are present at significant levels within the CD34⁺ CD45RA⁺ CD71⁺ Thy-1⁺ cells. Indeed, Moore has recently reported that cord blood-derived LTC-IC can be expanded 18- to 20-fold over a period of 2 to 3 weeks in liquid cultures supplemented with IL-1, IL-3, MGF, and Epo. This correlates very well with the 19- and 15-fold expansion of CD34⁺ Thy-1⁺ cells observed at weeks 2 and 3 in this study.

The CD34⁺ and clonogenic cell expansion observed in cultures of CD34⁺ CD45RA⁺ CD71⁺ Thy-1⁺ cells is, to our knowledge, the highest reported to date for BM- or cord blood-derived progenitors. In view of the recent interest in the in vitro expansion of primitive progenitors for stem cell transplantation, our results indicate that CD34⁺ CD45RA⁺ CD71⁺ Thy-1⁺ cells derived from cord blood represent an excellent starting population.

Finally, we started to address a possible role of Thy-1 in hematopoietic cell development. When both subpopulations of CD34⁺ CD45RA⁺ CD71⁺ cells were cultured in the presence of anti-Thy-1 antibody, a significant decrease in CFC numbers was observed only in cultures of Thy-1⁺ cells. Interestingly, primitive progenitors—particularly HPP-CFC—were the most affected by addition of anti-Thy-1. This suggests that Thy-1 is involved in hematopoietic cell growth and is capable of mediating a negative signal that results in inhibition of proliferation of primitive hematopoietic progenitors (ie, HPP-CFC). Recently, other investigators have shown that Thy-1 expression on a neural cell line (NG115) results in inhibition of neurite outgrowth when cultured on astrocytes, but not on other cellular substrata, suggesting that in that particular system Thy-1 inhibits neurite outgrowth by interacting with a molecule(s) expressed by mature astrocytes. In separate studies, Nakashima et al have shown that Thy-1, in synergy with the CD3/TcR complex, induces a negative signal in T lymphocytes that results in growth inhibition and DNA fragmentation. These findings have to be reconciled with numerous studies showing that cross-linking of Thy-1 on lymphocytes results in activation and cell proliferation. Our results support the notion that Thy-1 is a cell-surface molecule involved in inhibition of immature hematopoietic cell proliferation. The physiologic relevance of this observation and the mechanisms involved remain to be determined.

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Thy-1 expression is linked to functional properties of primitive hematopoietic progenitor cells from human umbilical cord blood

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