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Identification of a Novel Subpopulation of Human Cord Blood CD34−CD133−CD7−CD45+Lineage− Cells Capable of Lymphoid/NK Cell Differentiation After In Vitro Exposure to IL-15

Sergio Rutella, Giuseppina Bonanno, Maria Marone, Daniela de Ritis, Andrea Mariotti, Maria Teresa Voso, Giovanni Scambia, Salvatore Mancuso, Giuseppe Leone, and Luca Pierelli

The hemopoietic stem cell (HSC) compartment encompasses cell subsets with heterogeneous proliferative and developmental potential. Numerous CD34+ cell subsets that might reside at an earlier stage of differentiation than CD34+ HSCs have been described and characterized within human umbilical cord blood (UCB). We identified a novel subpopulation of CD34−CD133−CD7−CD45+ lineage− HSCs contained within human UCB that were endowed with low but measurable extended long-term culture-initiating cell activity. Exposure of CD34−CD133−CD7−CD45+ lineage− HSCs to stem cell factor preserved cell viability and was associated with the following: 1) concordant expression of the stem cell-associated Ags CD34 and CD133, 2) generation of CFU-granulocyte-macrophage, burst-forming unit erythroid, and megakaryocytic aggregates, 3) significant extended long-term culture-initiating cell activity, and 4) up-regulation of mRNA signals for myeloperoxidase. At variance with CD34+ lineage− cells, CD34−CD133−CD7−CD45+ lineage− HSCs maintained with IL-15, but not with IL-2 or IL-7, proliferated vigorously and differentiated into a homogeneous population of CD7−CD45brightCD25+CD44+ lymphoid progenitors with high expression of the T cell-associated transcription factor GATA-3. Although they harbored nonclonally rearranged TCRγ genes, IL-15-primed CD34−CD133−CD7−CD45+ lineage− HSCs failed to achieve full maturation, as manifested in their CD3+ TCRβ−γ− phenotype. Conversely, culture on stromal cells supplemented with IL-15 was associated with the acquisition of phenotypic and functional features of NK cells. Collectively, CD34−CD133−CD7−CD45+ lineage− HSCs from human UCB displayed an exquisite sensitivity to IL-15 and differentiated into lymphoid/NK cells. Whether the transplantation of CD34− lineage− HSCs possessing T/NK cell differentiation potential may impact on immunological reconstitution and control of minimal residual disease after HSC transplantation for autoimmune or malignant diseases remains to be determined. The Journal of Immunology, 2003, 171: 2977–2988.

Hemopoiesis can be defined as a hierarchical process sustained by pluripotent stem cells capable of extensive self-renewal and differentiation (1). An orderly sequence of changes in gene expression occurs, leading to lineage restriction and, ultimately, production of mature blood cells. Hemopoietic stem cells (HSCs) possess enormous clinical relevance, because they can reconstitute both the lymphoid and the myeloid compartment in the recipients of autologous, allogeneic, or xenogeneic transplants (1). Furthermore, HSCs can be targets for ex vivo expansion protocols and for gene therapy strategies.

CD34 Ag is a sialomucin that has been considered to be a hallmark of human transplantable HSCs (2–4); accordingly, experimental and clinical protocols have been designed exclusively for CD34+ subsets. Recently, several investigators have identified very primitive CD34+ lineage− HSCs capable of long-term re-population of nonobese diabetic/SCID mice and fetal sheep (5–9). The isolation of highly purified subsets of HSCs not expressing Ags associated with lineage commitment (or lin− cells) has demonstrated a previously unrecognized heterogeneity within the human HSC compartment (10). In particular, CD133+CD34+CD38− lin− cells isolated from human umbilical cord blood (UCB) possess in vitro growth factor requirements and progenitor capacity equivalent to the CD34+ stem cell fraction (10). Because CD34+ HSCs can be obscured by mature CD34− cells, functional assays have been developed to isolate these HSCs, including Hoechst 33342 dye efflux (11). Using these approaches, a rare side population of primitive CD34+ HSCs has been identified in human UCB; CD34+ lin− side-population cells express CD7 Ag and contain lymphoid progenitors capable of rapid expansion and differentiation into functionally active NK cells, if plated on stroma supplemented with cytokines (11). In the study by Storms et al. (11), CD34−CD7− lin− cells were detected at very low frequency and were excluded from analysis; thus, whether CD34−CD7− lin− cells contain HSCs endowed with progenitor or stem cell activity has not been determined.
The molecular events occurring during lineage restriction involve activation or silencing of genes encoding cytokine receptors, signal transduction molecules, and transcription factors (12–14). In this respect, the isolation of progenitor populations from normal in vivo sources can greatly contribute to the understanding of the process of differentiation and lineage plasticity, by providing access to intermediates that can be characterized by functional assays and molecular approaches (15–17). In the present study, we identified a novel, rare, and primitive subset of human UCB CD34+CD133−CD7−lin progenitors that generate T cell precursors and functionally active NK cells after culture with stromal cells supplemented with the IL-2R γc-signaling cytokine IL-15. The CD34+CD133+CD7−CD45dimlin lymphoid progenitors that we differentiated in vitro harbored rearrangement at the TCRγ locus, expressed the T cell-specific transcription factor GATA-3, and could be hierarchically correlated to the recently described lymphoid-oriented CD34+CD7−lin and to the transitional myeloid CD34+CD133−CD7−lin UCB cells.

Materials and Methods

UCB processing and isolation of CD34+CD133−CD7−lin cells

UCB samples were obtained after full-term cesarean delivery from healthy donors after written informed consent. The local human research committee approved all investigations. UCB samples were collected in sterile bags containing citrate-phosphate dextrose and adenine anticoagulant and were diluted 1/5 in PBS. Low-density mononuclear cells were separated over Ficoll-Hypaque (density, 1.077 g/dl; Pharmacia, Uppsala, Sweden). Mononuclear cells were subsequently subjected to removal of lineage-committed cells by incubation with a mixture of mAb directed against CD2, CD3, CD4, CD8, CD16, CD19, CD24, CD56, CD66b, CD41, and glycoprotein A (CD235a). The manufacturer (StemCell Technologies, Vancouver, BC, Canada) provided all mAb. After incubation, a secondary Ab conjugated to a metal colloid was added, and cells were depleted of lineage marker-positive elements by a magnetic device (StemSep; StemCell Technologies). CD34lin cells were then removed and separately collected from remaining lin cells by binding with PE- or FITC-conjugated anti-CD34 mAb (QBEND/10 clone; Serotec, Oxford, UK), followed by sorting on a FACStarPlus flow cytometer (BD Bioscienes, San Jose, CA). After extensive washings with PBS/1% BSA, isolated CD34lin cells were stained with PE-conjugated anti-CD34 mAb (Miltenyi Biotec, Bergisch-Gladbach, Germany) and FITC-conjugated anti-CD7 mAb (BD Biosciences). CD7+ and CD133+ cells were removed from the CD34lin starting population by sorting on a FACStarPlus cytometer (BD Biosciences). The remaining cells were designated as CD34+CD133+CD7−CD45dimlin cells, in accordance with flow-cytometric analysis of surface marker expression.

Culture of purified CD34+CD133−CD7−CD45dimlin cells

In a preliminary set of experiments, we tested the ability of an array of growth factors to promote differentiation, which was monitored through the determination of propidium iodide, and FACS analysis of light scatter characteristics. The cocultures were then incubated for 24 h to identify megakaryocytic aggregates (Meg-aggregates), as previously described (18). Cloning activity was expressed as cumulative colony efficiency (CFU-GM plus BFU-E plus Meg-aggregates) both in terms of absolute number of colonies per number of seeded cells and in terms of percentage of seeded cells. Extended (12-wk) long-term culture-initiating cell (ELTC-IC) activity was assessed by limiting-dilution analysis (LDA) and 12-wk long-term culture on a murine stromal cell line engineered to produce human G-CSF and IL-3 (M2-10B4 cell line; a gift from Dr. C. Eaves (Terry Fox Laboratory, Vancouver, BC, Canada)) (18, 21). At wk 6, cultures were trypsinized, and cells were seeded on a new irradiated layer of M2-10B4 cells to circumvent cell senescence. Freshly isolated CD34+CD133+CD7−CD45dimlin cells were tested in NK culture assays, which consisted of 21-day stroma-based culture on MS-5 murine cells in α-MEM supplemented with 10% horse serum, in the presence of IL-15 at 50 ng/ml. The cloning population of CD34+CD133+CD7−CD45dimlin cells was seeded at 1 × 105 per well in 24-well plates, where a confluent stromal layer of MS-5 cells had been grown previously.

Limiting-dilution assays

Suspension cultures containing IL-15 were set up with purified CD34+CD133+CD7−CD45dimlin cells in 20 μl of medium in the individual wells of a 60-well Terasaki microwell plate. After 7 days of culture, each well was examined for the presence of viable cells and then assayed individually for the presence of lymphoid progenitors. Wells were scored as positive for IL-15 responsiveness when >20 viable cells could be identified on day 7 and when cells expressed detectable levels of the lymphoid-associated Ag CD7 by flow cytometry. All other wells were scored as negative and usually contained no detectable cells. The frequency of IL-15-responsive cells in the CD34+CD133+CD7−CD45dimlin cells was calculated from the proportion of negative wells based on Poisson statistics, as previously reported (18).

Immunological markers

Aliquots of cells were incubated for 30 min at 4°C with pretitrated saturating dilutions of the following FITC-, PE-, PerCP-, or PE-Cy5 (Tricolor)-conjugated mAb: CD16 (NKPR15 clone, IgG1, IgG1) (BD Biosciences, San Jose, CA), CD19 (4D9 clone, IgG1), CD22 (S-HCL-1 clone, IgG2b), CD34 (8G12 clone, IgG1), CD38 (HBB7 clone, IgG1), CD56 (M33 clone, IgG1), CD45RA (L48 clone, IgG1), CD57 (2A3 clone, IgG1), CD15 (2B5 clone, IgG1), CD44 (L17A clone, IgG1), glycoprotein-A (CD235a; GAR-2 clone, IgG2b), CD132 (IL-2R γ-chain; AG1B4 clone, IgG1), TCRβ (W3T1 clone, IgG1), TCRγ (1F2F clone, IgG1; BD Biosciences), CD94 (HP-3D9 clone, IgG1), CD158a (HP-3E4 clone, IgM), CD158b (CH-L clone, IgG2b; BD Pharmingen, San Diego, CA), CD3 (S4.1 clone, IgG1) (BD Pharmingen), CD4 (S5.1 clone, IgG2a), CD8 (3B5 clone, IgG2a), CD9 (F10-42 clone, IgG1), CD164 (10.1 clone, IgG1), CD65 (VIM2 clone, IgG1), HLA-DR (TU36 clone, IgG2b), C11b (CR3 Bear-1 clone, IgG1), CD133 (TUK1 clone, IgG1), CD45 (HJ30 clone, IgG1), Sca-1 (Ly-6A.2 clone, IgG2a; Caltag Laboratories, Burlingame, CA), CD105 (SN6 clone, IgG1), CD45 (TU36 clone, IgG2b), isolated CD34+CD133+CD7−CD45dimlin cells, in accordance with flow-cytometric analysis of surface marker expression.

CFU assays, long-term culture-initiating cell assays, and lymphoid cell cultures

Colonial-forming cells (CFU-granulocyte-macrophage [CFU-GM] and CFU-erythroid [BFU-E]) were generated from purified UCB cells (CD34+CD133+CD7−CD45dimlin and CD34+lin cells; the latter used as reference control) before and after cytokine treatments by 14-day methylcellulose semisolid cultures in the presence of 25% serum substitute to avoid interference from serum factors (Bit 9500; StemCell Technologies) and SCF, IL-3, GM-CSF, G-CSF, Flt3-L, and erythropoietin, as reported (18, 20). Replicates of cloning assays were established in the different culture conditions but in the presence of SCF, TPO, and IL-6 to identify megakaryocytic aggregates (Meg-aggregates), as previously described (18). Colony formation was expressed as cumulative colony efficiency (CFU-GM plus BFU-E plus Meg-aggregates) both in terms of absolute number of colonies per number of seeded cells and in terms of percentage of seeded cells. Extended (12-wk) long-term culture-initiating cell (ELTC-IC) activity was assessed by limiting-dilution analysis (LDA) and 12-wk long-term culture on a murine stromal cell line engineered to produce human G-CSF and IL-3 (M2-10B4 cell line; a gift from Dr. C. Eaves (Terry Fox Laboratory, Vancouver, BC, Canada)) (18, 21). At wk 6, cultures were trypsinized, and cells were seeded on a new irradiated layer of M2-10B4 cells to circumvent cell senescence. freshly isolated CD34+CD133+CD7−CD45dimlin cells were tested in NK culture assays, which consisted of 21-day stroma-based culture on MS-5 murine cells in α-MEM supplemented with 10% horse serum, in the presence of IL-15 at 50 ng/ml. The starting population of CD34+CD133+CD7−CD45dimlin cells was seeded at 1 × 105 per well in 24-well plates, where a confluent stromal layer of MS-5 cells had been grown previously.
with 1% BSA, and further incubated with 20 μg/ml 7-amino actinomycin-
D (7-AAD; Molecular Probes) for an additional 30 min, before flow
cytometry analysis.

Measurement of lymphocyte divisions by CFSE

For cell-doubling experiments, cells were resuspended in PBS containing
2.5 μM CFSE (Molecular Probes) for 10 min at room temperature (22). To
quench the labeling process, an equal volume of FCS was added; after
washing with PBS supplemented with 3% FCS, cells were incubated over-
night with medium alone and then exposed to the various cytokine com-
binations, as detailed in the

Semi-quantitative RT-PCR

mRNA expression levels were evaluated by RT-PCR, normalizing the lev-
els of test RNA to that of the internal control, aldolase A. Total RNA
extractions were conducted with the RNeasy mini-kit (Qiagen, Hilden,
Germany), and RNA obtained from 2 × 10^6 cells was reverse transcribed
with 25 U of Moloney murine leukemia virus reverse transcriptase (PE
Applied Biosystems, Foster City, CA) at 42°C for 30 min in the presence
of random hexamers. Two microliters of these cDNA products were am-
plified with 1 U of AmpliTaq Gold (PE Applied Biosystems) in the pre-

cence of the specific primers for the mRNA of interest, together with the
aldolase-A primer (23). Reactions were conducted in the GeneAmp PCR
System 9600 (PE Applied Biosystems). Conditions were chosen so that
none of the amplification products obtained from the RNAs of interest
reached a plateau, and that the two primer sets did not compete with each
other. Amplification of GATA-3 mRNA was achieved by 30 cycles of 45 s
at 95°C, 45 s at 55°C, and 1 min at 72°C in the presence of 3 μM MgCl2.
Amplification conditions and primer sequences for the other markers stud-
ed in this report were described elsewhere (24–28). The PCR prod-
ucts were loaded onto 1.5% agarose gels and stained with ethidium bro-
mide. Each set of reactions included a no-sample negative control. The
ratio between the sample RNA to be determined and aldolase-A was cal-
culated to normalize for initial variations in sample concentration and as a
control for reaction efficiency. The following primers were used for ampl-
ification of GATA-3 mRNA: 5'-GTCCTGTGCGAACTGTCAGA-3' and
5'-GATCATCACCTGCACGCAC-3' for the primary PCR, and the 'T-CSR
11-primer and 5'-GGATCCACTGCCAAAGTAGGGTTGTTGGAATCAGG-3'
for the nested PCR. For both amplifications, PCR consisted of 30 cycles at 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min and resulted in a fragment ranging between 150 and 180 bp following the

Flow cytometry and immunofluorescence analysis

Cells were run through a FACScan flow cytometer (BD Biosciences),
equipped with an argon laser emitting at 488 nm. Details on instrument
requirements and settings were published elsewhere (30). FITC, PE, and
PerCP, or PE-Cy5 (Tricolor) signals were collected at 530, 575, and 670
nm, respectively; spectral overlap was minimized by electronic compen-
sation with Calibrite beads (BD Biosciences) before each determination
series. A minimum of 10,000 events was collected and acquired in list
mode using the CellQuest software (BD Biosciences).

Statistical methods

The approximation of population distribution to normality was tested pre-
liminarily using statistics for kurtosis and symmetry. Data were presented
as mean ± SD. Consequently, all comparisons were performed with Stu-
dent's t test for paired or unpaired data or with the ANOVA, as appropriate.
The criterion for statistical significance was defined as p ≤ 0.05.

Results

Purification of CD34^+ CD133^+ CD7^- lin^- cells

UCB mononuclear cells were enriched in CD34^- cells and lin^- cells by magnetic depletion of cells labeled with a mixture of mAb
directed against lymphoid, myeloid, and erythroid Ags, and by flow-
cytometric removal of cells reactive with anti-CD34 mAb.
The purity of sorted CD34^- lin^- cells always exceeded 98%, as assessed by FACS analysis (data not shown). CD133 and CD7 were found on 1.6–22.3% and on 8.5–39% of CD34^- lin^- cells, respectively, in the different UCB samples tested. Furthermore, freshly isolated CD34^- lin^- cells were reactive, albeit at low fre-

Phenotypic and molecular features of highly purified CD34^- CD133^+ CD7^- lin^- cells

The expression of informative differentiation Ags on highly purified
CD34^- CD133^+ CD7^- lin^- UCB cells was investigated by

![FIGURE 1](http://www.jimmunol.org/DownloadedFrom/326x155to536x392)

1 2 3 4 5 6 7 MW

A B C

CD133 PE

CD/TTC

CD7 FITC

10^4 cells was reverse transcribed

C...
multiparameter flow cytometry. The T cell and B cell lineage-associated Ags CD3 and CD22 as well as CD235a and CD11b, assigned to erythroid and granulocytic cells, respectively, were undetectable on CD34⁺CD133⁺CD7⁻lin⁻ UCB cells (Fig. 2). Accordingly, TCRαβ and TCRγδ as well as CD4 and CD8 Ags were absent. A readily discernible subset of CD34⁺CD133⁺CD7⁻lin⁻ UCB cells was reactive with anti-CD105 (10 ± 3%) and anti-CD90 mAb (12 ± 4%); similarly, expression of HLA-DR and Sca-1 was shown on a small subset of cells (<10%), in accordance with previous reports on putative stem/progenitor cells capable of regenerating human myocardium (31). Of interest, CD34⁺CD133⁺CD7⁻lin⁻ UCB cells uniformly expressed low levels of CD45 (mean fluorescence intensity equal to 27 ± 6 compared with 130 ± 15 in peripheral blood CD3⁻ T cells used as a control) and were virtually negative for Ags assigned to the granulocytic/monocytic differentiation pathway, e.g., CD33, CD13, CD64, CD65, as well as for the NK cell-associated Ags CD16 and CD56 (Fig. 2).

Next, we performed molecular studies to measure mRNA levels for the survival gene Bcl-2, cell cycle modulators (p27kip1, p21cip1, and TGF-β1), the lymphoid-associated transcription factor GATA-3, and differentiation proteins (CD34 and myeloperoxidase). Relative to the TF-1 cell line and to CD34⁺lin⁺ cells, CD34⁺CD133⁺CD7⁻lin⁻ cells displayed low Bcl-2 expression, high p21cip1 and TGF-β1 and intermediate p27kip1 expression, absence of CD34 and intermediate levels of myeloperoxidase (Fig. 3). The differential expression of GATA-3 in CD34⁺CD133⁺CD7⁻CD45lin⁻ compared with CD34⁺lin⁻ cells was of interest, given the undisputed role of GATA-3 in the promotion of T cell differentiation (32). Collectively, the observed molecular profile consisted of high expression levels of prototypic negative modulators of the cell cycle and low expression levels of survival-promoting proteins.

**FIGURE 2.** Phenotypic features of freshly isolated CD34⁺CD133⁺CD7⁻lin⁻ cells. CD34⁺CD133⁺CD7⁻lin⁻ cells were further analyzed for the expression levels of an array of differentiation/maturity Ags. Cells were gated in R1 based on forward-scatter (FSC)/side-scatter (SSC) characteristics. One representative experiment of six with similar results is shown. Markers were set according to the proper isotopic control. The percentage of cells staining positively for a given Ag is indicated in each bidimensional cytogram.

**Growth factor requirements and functional activity of highly purified CD34⁺CD133⁺CD7⁻CD45lin⁻ cells**

Next, we tested numerous growth factors for the ability to support survival and/or differentiation of human CD34⁺CD133⁺CD7⁻CD45lin⁻ cells in liquid cultures performed in the presence of horse/bovine serum and 10⁻⁷ M hydrocortisone (19). Based on data from preliminary experiments (not shown), corticosteroids and animal serum were included in all growth experiments, because their concomitant presence during the 48-h culture period reduced spontaneous cell death by 20% on average in any experimental condition tested as compared with medium supplementation with serum substitute alone. When used alone or in combinations, IL-3, IL-6, TPO, Flt3-L, IL-7, GM-CSF, IL-2, platelet-derived growth factor, and epidermal growth factor exerted no appreciable effects on growth, viability, and differentiation of CD34⁺CD133⁺CD7⁻CD45lin⁻ cells following 72 h of culture, as measured by viable cell counts and CD133 and CD7 expression. Of interest, SCF preserved cell viability and promoted the appearance of a population of cells that averaged 12% and expressed low levels of CD133. At variance with the TCRγ chain-signaling cytokines IL-2 and IL-7, IL-15 was capable of promoting CD34⁺CD133⁺CD7⁻CD45lin⁻ cell growth, with an average 2.4-fold increase in cell number, maintained cell viability, and induced expression of the T cell-associated Ag CD7 on an average of 15% of cultured cells on day 3.

Eight individual samples of purified CD34⁺CD133⁺CD7⁻CD45lin⁻ cells isolated from eight different UCB donors were assayed for direct cloning efficiency in semisolid methylcellulose culture in the presence of a combination of cytokines, as already detailed. Following 14 days of serum-free culture, no colonies of any lineage were scored when CD34⁺CD133⁺CD7⁻CD45lin⁻ cells were seeded at the concentration of either 1,000 or 5,000 cells
per plate (Table I). Conversely, after increasing cells to 10,000 per plate, five small nonhemoglobinized colonies on average were identified in replicate plates containing SCF, erythropoietin plus G-CSF, GM-CSF, IL-3, and Flt3-L (cloning efficiency, 0.05%). On the contrary, the CD34<sup>+</sup>/H11001</sup>/H11002</sup> counterpart isolated from the same UCB samples and used as a control cell population produced confluent colonies when seeded at 5,000 and 10,000 cells per plate, and hemoglobinized and nonhemoglobinized colonies averaged 100 at a starting concentration of 1,000 cells per plate (cloning efficiency, 10%; Table I). To assay for long-term functional activity of CD34<sup>+</sup>/H11002</sup>/H11002</sup>/CD133<sup>+</sup>/H11002</sup>/CD7<sup>+</sup>/H11002</sup>/CD45<sup>dim</sup>/lin<sup>+</sup> cells, we performed ELTC-IC assays through LDA and 12-wk culture on the murine stromal cell line M2-10B4, engineered to produce human G-CSF and IL-3. From these assays, we learned that human CD34<sup>+</sup>/H11002</sup>/H11002</sup>/CD133<sup>+</sup>/H11002</sup>/CD7<sup>+</sup>/H11002</sup>/CD45<sup>dim</sup>/lin<sup>+</sup> cells were endowed with low but measurable long-term activity (average ELTC-IC frequency of 1:12,000 in five independent experiments). For purposes of comparison, the CD34<sup>+</sup>/H11001</sup>/H11002</sup> counterpart from the same UCB samples showed an average frequency of 1:600 ELTC-IC, in accordance with previously published reports (18, 24).

CD34<sup>+</sup>/CD133<sup>+</sup>/CD7<sup>+</sup>/CD45<sup>dim</sup>/lin<sup>+</sup> cells cultured with SCF differentiate into CD34<sup>+</sup>/CD133<sup>+</sup> progenitors

Based on the results obtained with SCF and IL-15 after 72-h liquid culture, we first performed a more in-depth analysis of the effects produced by SCF on survival and expansion of CD34<sup>+</sup>/H11002</sup>/H11002</sup>/CD133<sup>+</sup>/H11002</sup>/CD7<sup>+</sup>/H11002</sup>/CD45<sup>dim</sup>/lin<sup>+</sup> cells maintained in culture for up to 7 days. This experimental condition was established in three replicate experiments with UCB cells from three different donors. In the absence of exogenous cytokines, cell count declined by 40% on average as compared with the starting cell population, and cell viability averaged 40% (Table II). Conversely, cultures maintained in the presence of SCF showed significant increases of cell counts, associated with cell viability scores that were consistently >80%. The onset of cell proliferation after exposure to SCF was also confirmed by cell-doubling experiments with the fluorescent dye

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### Table I. Comparison of cloning activities of freshly isolated CD34<sup>+</sup>/CD133<sup>+</sup>/CD7<sup>+</sup>/CD45<sup>dim</sup>/lin<sup>+</sup> and CD34<sup>+</sup>/lin<sup>+</sup> cells<sup>a</sup>

<table>
<thead>
<tr>
<th>Cell Subset</th>
<th>Cells per Plate</th>
<th>CFU-GM</th>
<th>BFU-E</th>
<th>Meg-Aggregates</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34&lt;sup&gt;+&lt;/sup&gt;/CD133&lt;sup&gt;+&lt;/sup&gt;/CD7&lt;sup&gt;+&lt;/sup&gt;/CD45&lt;sup&gt;dim&lt;/sup&gt;/lin&lt;sup&gt;+&lt;/sup&gt;</td>
<td>1,000</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD34&lt;sup&gt;+&lt;/sup&gt;/lin&lt;sup&gt;+&lt;/sup&gt;</td>
<td>5,000</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD34&lt;sup&gt;+&lt;/sup&gt;/lin&lt;sup&gt;+&lt;/sup&gt;</td>
<td>10,000</td>
<td>5 ± 1</td>
<td>58 ± 26</td>
<td>19 ± 10</td>
</tr>
<tr>
<td>CD34&lt;sup&gt;+&lt;/sup&gt;/lin&lt;sup&gt;+&lt;/sup&gt;</td>
<td>1,000</td>
<td>35 ± 19</td>
<td>Confluent</td>
<td>110 ± 30</td>
</tr>
<tr>
<td>CD34&lt;sup&gt;+&lt;/sup&gt;/lin&lt;sup&gt;+&lt;/sup&gt;</td>
<td>5,000</td>
<td>Confluent</td>
<td>Confluent</td>
<td>200 ± 50</td>
</tr>
<tr>
<td>CD34&lt;sup&gt;+&lt;/sup&gt;/lin&lt;sup&gt;+&lt;/sup&gt;</td>
<td>10,000</td>
<td>Confluent</td>
<td>Confluent</td>
<td>200 ± 50</td>
</tr>
</tbody>
</table>

<sup>a</sup> CFU-GM, BFU-E, and Meg-aggregates were generated from freshly isolated CD34<sup>+</sup>/CD133<sup>+</sup>/CD7<sup>+</sup>/CD45<sup>dim</sup>/lin<sup>+</sup> cells, as detailed in Materials and Methods. ND, Not detected. Results are expressed as the mean ± SD observed in eight independent experiments.
were performed in the presence or absence of SCF and/or 5000 CD34 cells were cultured in the presence of SCF, whereas 55 colonies on average were scored after culturing CD34 cells. Not detected. Results are expressed as the mean ± SD observed in four independent experiments.

a Expressed as the absolute number of colonies per 5000 seeded cells.

**Table II. Culture of CD34⁺CD133⁺CD7⁻CD45dimlin⁻ cells for 7 days in the presence or absence of SCF**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SCF</th>
<th>No Cytokine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell count (fold increase)</td>
<td>1.01 ± 0.3</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Viability (%)</td>
<td>88 ± 6</td>
<td>40 ± 10</td>
</tr>
<tr>
<td>CD133⁺ (%)</td>
<td>98 ± 5</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>CD34⁺ (%)</td>
<td>78 ± 3</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>CFU-GM</td>
<td>35 ± 7</td>
<td>ND</td>
</tr>
<tr>
<td>BFU-E</td>
<td>16 ± 5</td>
<td>ND</td>
</tr>
<tr>
<td>Meg-Aggregates</td>
<td>6 ± 2</td>
<td>ND</td>
</tr>
<tr>
<td>CFU-GMb</td>
<td>55</td>
<td>0.3</td>
</tr>
<tr>
<td>BFU-El</td>
<td>64</td>
<td>0.6</td>
</tr>
<tr>
<td>Meg-Aggregatesb</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

**FIGURE 4.** Phenotypic features of SCF-treated CD34⁺CD133⁺CD7⁻CD45dimlin⁻ cells. The expression of the stem cell-related Ag CD34 and CD133 was evaluated by flow cytometry after culturing for 7 days in the presence (left panels) or in the absence (right panels) of SCF at 10 ng/ml. All cultures were performed in α-MEM culture medium supplemented with 10% horse serum, 10% bovine serum, and 10⁻⁶ M hydrocortisone. The percentage of cells reactive with the anti-CD34 and/or the anti-CD133 mAb at the different time points is indicated in each bidimensional cytogram. Markers for quadrant analysis were set according to the proper isotypic controls. FSC, Forward scatter; SSC, side scatter. One representative experiment of four with similar results is shown.

**CD34⁺CD133⁺CD7⁻CD45dimlin⁻ cells cultured with IL-15 differentiate into lymphoid progenitors**

Following the observation that IL-15 promoted cell survival and induced CD7 expression in liquid culture, CD34⁺CD133⁺CD7⁻CD45dimlin⁻ cells were maintained with SCF acquired the CD133 marker by day 4 in the presence of exogenous IL-15 at 50 ng/ml. Under these experimental conditions, a population of putative lymphoid progenitors emerged that homogeneously expressed the T cell-associated Ag CD45, together with markers conventionally assigned to naive T cells, e.g., CD45RA (Table III). Accordingly, minute fractions of cells expressed the memory T cell-associated Ag CD45RO. Interestingly enough, high percentages of cells differentiated with IL-15 stained positively with both the anti-CD25 and the anti-CD44 mAb. These features were reminiscent of the early stages of normal T cell thymic development (12). In line with the exquisite sensitivity to IL-15, CD34⁺CD133⁺CD7⁻CD45dimlin⁻ cells expressed high levels of the IL-2/IL-15Rα chain (Table III). However, cells completely lacked expression of CD3, as well as of CD4 and CD8 Ag; consistently, either surface αβ or γδ chains of the TCR complex were undetectable. CD45 expression levels were significantly increased after culture, and IL-15-primed cells homogeneously acquired a
CD45 bright staining pattern. Conversely, most CD34−CD133−CD7−CD45 dimlin− cells cultured without IL-15 retained a CD45 dim phenotype (Table III). As shown in Table III, spontaneous cell differentiation occurred and cells maintained in the absence of exogenous IL-15 up-regulated the expression of the lymphoid-cell associated Ag CD7. Furthermore, 24 ± 5% of CD34−CD133−CD7−CD45 dimlin− cells cultured with medium alone displayed a CD44+CD25− phenotype as compared with 5 ± 2% of freshly isolated CD34−CD133−CD7−CD45 dimlin− cells. Data on phenotype and viability of IL-15-primed CD34−CD133−CD7−CD45 dimlin− cells are summarized in Table III.

When TCR gene rearrangement was investigated, the genes encoding the TCRγ chain were nonclonally rearranged, at variance with freshly isolated CD34−CD133−CD7−CD45 dimlin− cells, which failed to rearrange the TCRγ locus, as determined by a PCR analysis of amplified genomic DNA (Fig. 6). As expected, TCRγ chain gene rearrangements could be detected in CD34−CD133−CD7−CD45 dimlin− cells as well as in CD34−CD133−CD7+CD45 dimlin− cells, used as control cell fractions (Fig. 6).

Notably, CD34−CD133−CD7−CD45 dimlin− cells primed with IL-15 up-regulated mRNA transcripts for GATA-3 (Fig. 7); an anticipated finding of the molecular studies was the enhanced Bcl-2 and TGF-β1 expression levels after cytokine exposure, in line with previous studies on the survival-promoting effects of IL-15 (33). Myeloperoxidase mRNA was virtually undetectable, consistent with absence of myeloid commitment after IL-15-priming (Fig. 7). The molecular profile identified in this study indicates that CD34−CD133−CD7−CD45 dimlin− cells exposed to IL-15 differentiate into T cell precursors and selectively up-regulate survival genes as well as transcription factors associated with T cell development. The specificity of our findings was confirmed by the use of a blocking Ab directed against the IL-2/IL-15Rγ chain during culture in the presence of IL-15; under these conditions, the generation of lymphoid precursors from CD34−CD133−CD7−CD45 dimlin− cells was significantly and specifically inhibited (data not shown).

Priming of CD34−CD133−CD7−CD45 dimlin− cells with IL-15 was also associated with preserved survival and with significant longevity. Mean ± SD observed in four independent experiments performed in triplicate. One representative experiment is depicted in A. Data represent mean ± SD from four independent experiments performed in duplicate. One representative experiment is depicted in B. Lane 1, CD34−CD133−CD7−CD45 dimlin− cells (no cytokine); lane 2, SCF-treated CD34−CD133−CD7−CD45 dimlin− cells; and lane 3, TF-1 cell line.

**Table III. Culture of CD34−CD133−CD7−CD45 dimlin− cells for 7 days in the presence or absence of IL-15**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IL-15</th>
<th>No Cytokine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell count (fold increase)</td>
<td>2.5 ± 0.4 a</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>Viability (%)</td>
<td>98 ± 5†</td>
<td>38 ± 9</td>
</tr>
<tr>
<td>CD133+ (%)</td>
<td>2 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>CD34− (%)</td>
<td>4 ± 1</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>CD7− (%)</td>
<td>80 ± 2*</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>CD25+CD44+ (%)</td>
<td>81 ± 3*</td>
<td>24 ± 5</td>
</tr>
<tr>
<td>CD45 bright (%)</td>
<td>65 ± 7*</td>
<td>3 ± 0.2</td>
</tr>
<tr>
<td>CD45RO− (%)</td>
<td>9 ± 4</td>
<td>6 ± 3</td>
</tr>
<tr>
<td>CD45RA+ (%)</td>
<td>75 ± 7*</td>
<td>22 ± 4</td>
</tr>
<tr>
<td>CD132− (%)</td>
<td>95 ± 3*</td>
<td>2 ± 1</td>
</tr>
</tbody>
</table>

*CD34−CD133−CD7−CD45 dimlin− cells were cultured for 7 days with 10% horse serum, 10% bovine serum, and 10−6 M hydrocortisone in the presence or absence of 50 ng/ml IL-15. ND, Not detected. Results are expressed as the mean ± SD observed in four independent experiments performed in triplicate.
†Defined as cells showing a CD45-related fluorescence beyond the second decade of a four-decade logarithmic scale.
* p < 0.05 compared with cultures performed in the absence of exogenously added IL-15.
proliferation, compared with cultures maintained in the absence of IL-15 (Table III). Experiments with the fluorescent probe CFSE allowed the identification and quantification of up to six generations of progeny in cultures conditioned with IL-15; conversely, cells cultured in the absence of IL-15 were incapable of cell cycle progression beyond the second generation. Collectively, this kinetic analysis suggested that IL-15’s effects on the development of T cell precursors from CD34+/CD133+/CD7−CD45dimlin− cells were partly accounted for by induction of proliferation.

LDAs performed with CD34+/CD133+/CD7−CD45dimlin− cells plated at the concentration of one cell per well in the presence of IL-15 supplementation indicated that the average frequency of IL-15-responsive CD34+/CD133+/CD7−CD45dimlin− cells was 1/8 (range, 1/6–1/11) in three independent experiments performed in triplicate with three individual UCB samples. Finally, we wanted to clarify whether CD105+ mesenchymal stem cells contained within the starting population of CD34+/CD133+/CD7−CD45dimlin− cells (Fig. 2) possessed the ability to sustain the lymphoid differentiation process of CD34+/CD133+/CD7−CD45dimlin− cells by providing soluble factors or accessory signals. To accomplish this goal, we depleted CD105+ cells from CD34+/CD133+/CD7−CD45dimlin− cells before culturing in the presence of IL-15 (Fig. 8A). These experiments indicated that CD105+CD34+/CD133+/CD7−CD45dimlin− cells maintained the ability to differentiate along the lymphoid lineage, as shown by immunophenotypic (Fig. 8B) and molecular studies (not shown).

CD34+/CD133+/CD7−CD45dimlin− cells cultured on stroma supplemented with IL-15 differentiate into functional NK cells

To detect whether CD34+/CD133+/CD7−CD45dimlin− cells could give origin to terminally differentiated NK cells, a stroma-based 21-day culture system was established in the presence or absence of IL-15. On day 21, the expression of informative NK cell-associated Ags was investigated on cells recovered from the stromal

FIGURE 7. Molecular profile of IL-15-treated CD34+/CD133+/CD7−CD45dimlin− cells. mRNA levels for survival genes, cell cycle modulators, transcription factors, and differentiation proteins were measured with semiquantitative RT-PCR. The TF-1 cell line was used as reference control for gene expression levels. Data on cells cultured without IL-15 were presented for purposes of comparison. Absorbance relative to the housekeeping gene (aldolase A) was plotted in A. Data represent mean ± SD from four independent experiments performed in duplicate. One representative experiment is shown in B. Lane 1, CD34+/CD133+/CD7−CD45dimlin− cells (no cytokine); lane 2, IL-15-treated CD34+/CD133+/CD7−CD45dimlin− cells; and lane 3, TF-1 cell line.
IL-7 (40 ng/ml) developed no phenotypic features indicative of stroma supplemented with a combination of IL-2 (1000 IU/ml) and large granular lymphocyte morphology (data not shown). Collectively, these phenotypic features along with the expression of CD158a and CD158b were expressed on 10% of cells (data not shown). In addition, the NK cell-associated killer Ig-like receptors CD94 were expressed at high levels by the majority of cells expressing a given Ag is indicated in each bidimensional cytogram. One representative experiment of four with similar results is shown.

NK cell differentiation, further suggesting the specificity of IL-15 in the determination of the reported phenomena (Fig. 9D).

Finally, we attempted to test the NK cell activity of IL-15/stroma-primed CD34^−CD133^−CD7^−CD45dimlin^− cells. To this purpose, the K562 tumor cells were loaded with the fluorescent probe CFSE and then incubated with cytokine-primed CD34^CD133^−CD7^−CD45dimlin^− cells at graded E:T ratios. Results clearly documented that cells differentiated with IL-15 in the presence of a stromal support acquired functional features of NK cells, including the ability to lyse tumor cells in an MHC-unrestricted manner (Fig. 10).

**Discussion**

The human HSC compartment comprises primitive cells with different multipotentiality, proliferative capacity, and ability to generate committed progenitor cells through an orderly sequence of changes in gene expression and cell phenotype (1, 32–34). Although CD34 Ag expression has been conventionally assigned to human hemopoietic cells, recent studies have suggested that CD34^− cells might reside at earlier differentiation stages than CD34^+ cells (1, 35–37). In particular, CD34^−lin^− HSCs expressing the CD7 Ag possess limited differentiation potential within the myeloid lineage, but retain the ability to expand into NK cells if cultured on stroma supplemented with cytokines (11, 38). Studies by Gallacher et al. (10) identified complementary HSCs in human UCB, which coexist with CD34^CD133^+ and CD34^CD7^−lin^− cells, and possess a CD34^−CD133^+CD7^−lin^− phenotype and myeloid potential, giving rise to CD34^+CD133^+ cells after cytokine priming. However, whether there exist other HSC subsets that are endowed with lymphoid or myeloid/lymphoid differentiation potential under the instructive action of individual cytokines or cytokine combinations remains to be thoroughly investigated.

The objectives of the present investigation were the following: 1) to identify a relationship between the novel CD34^−CD133^−CD7^−CD45dimlin^− HSC subset and the previously described CD34^+CD133^−CD7^−lin^− and CD34^−CD7^−lin^− cells and 2) to...
evaluate the instructive action of individual cytokines on phenotype, function, and molecular profile of human CD34⁺CD133⁻CD7⁻CD45dimlin⁻ HSCs. To this purpose, suitable methodological approaches to investigate T cell differentiation potential were cytokine-sustained, serum-containing liquid cultures and the cultivation on established stromal cell layers in adjunct to cytokine supplementation, as previously reported by Storms et al. (11) for the assessment of the differentiation repertoire of CD34⁺CD7⁻lin⁻ UCB cells.

In this study, we isolated and characterized a previously undescribed subset of CD34⁺CD133⁻CD7⁻CD45dimlin⁻ HSCs from human UCB. These cells significantly differed from those studied by other investigators (10, 11), because they were selected based on the lack of CD133 and CD7 expression. At variance with the CD34⁺CD7⁺ lymphoid progenitors described elsewhere (11), CD34⁺CD133⁻CD7⁻CD45dimlin⁻ HSCs did not express measurable amounts of CD11b, but rather they displayed a CD105lowCD90lowCD117low phenotype.

Preliminarily, CD34⁺CD133⁻CD7⁻CD45dimlin⁻ HSCs were plated in suspension cultures with the objective to determine growth factor requirements. Among the various cytokines that we used, SCF and IL-15 were the only growth factors capable of sustaining cell viability. In line with these findings, both IL-2/IL-15Rγ chain (CD132) and SCFR (c-kit or CD117) were found on freshly isolated CD34⁺CD133⁺CD7⁺CD45dimlin⁻ cells. These data prompted us to evaluate the effects of SCF and IL-15 on the developmental fate of CD34⁺CD133⁺CD7⁺CD45dimlin⁻ HSCs, as prototypical myeloid-supporting and lymphoid-supporting cytokines, respectively. When cultured in the presence of SCF, CD34⁺CD133⁺CD7⁺CD45dimlin⁻ cells acquired a CD34⁺CD133⁺ phenotype, generated CFU-GM, BFU-E, and Meg-aggregates, manifested ELTC-IC activity, and up-regulated mRNA levels for myeloperoxidase. Furthermore, SCF-primed HSCs down-regulated mRNA signals for cell-cycle inhibitors, up-regulated Bcl-2 levels, and proliferated, as evaluated through cell-doubling experiments with CFSE. Particularly, differentiation of CD34⁺CD133⁺CD7⁺CD45dimlin⁻ cells toward the myeloid lineage occurred with sequential and coordinated appearance of CD133 and CD34 Ags. This interpretation of the data implies that CD34⁺CD133⁺CD7⁺CD45dimlin⁻ cells represent a more primitive HSC population than the previously described CD34⁺CD133⁺CD7⁻lin⁻ HSCs.

Interestingly, IL-15 promoted growth and differentiation of CD34⁺CD133⁺CD7⁺CD45dimlin⁻ HSCs into lymphoid progenitors with TCR rearrangement and expression of CD45RA and the T cell-associated Ag CD7. Of interest, these cells lacked markers typically found on mature lymphoid cells, e.g., surface membrane/intracellular TCR αβγδ chains, CD3, CD4, or CD8 Ags, suggesting absence of terminal T cell differentiation. mRNA signals for

FIGURE 10. Cytotoxicity of NK progenitors against K562 target cells. K562 cells were loaded with CFSE, as detailed in Materials and Methods. CD34⁺CD133⁻CD7⁻CD45dimlin⁻ cells primed with IL-15 (putative NK progenitor cells) and stromal cells were cocultured with graded numbers of NK-sensitive K562 cells or with NK-resistant (NK-res) Raji cells for 24 h, as indicated. A, The percentage of specific lysis was calculated by subtracting the fraction of 7-AAD⁻CFSE⁺ cells in control cultures not containing NK progenitors from the fraction of 7-AAD⁻CFSE⁺ cells in the cocultures containing NK progenitors (mean ± SD recorded in four independent experiments performed in duplicate). *, p < 0.05 compared with cultures performed in the absence of IL-15-primed progenitor cells and with cultures containing IL-15 primed HSCs and K562 cells at a 1:1000 E:T ratio. B, One representative experiment of four with similar results is shown. The percentage of lysed K562 cells (7-AAD⁻CFSE⁺ cells) is indicated in the upper right quadrant of each bidimensional cytogram.
the T cell-associated transcription factor GATA-3, one of the crucial molecules implicated in the early phases of T lymphopoiesis (38, 39), as well as gene activity for the survival/antiapoptotic factors TGF-β1 and Bcl-2 were strongly up-regulated after priming with IL-15. Similar to our findings, high GATA-3 mRNA levels were recently detected in common lymphoid progenitors differentiated from UCB CD34+/CD38−/CD7+ HSCs (40). CD34−/CD133+CD7−CD45dimlin− cells primed with IL-15 acquired a CD44+/CD25+ phenotype that closely resembled triple-negative thymic progenitors (41). Also, CD34+/CD133+CD7−CD45dimlin− HSCs displayed a modest ability to differentiate into cells with a CD44+/CD25−CD7+ phenotype in cultures maintained in the absence of exogenous IL-15, although cell viability was reproducibly poor. These observations imply that the effects of IL-15 on lymphoid differentiation of CD34+/CD133+CD7−CD45dimlin− HSCs might be, at least in part, accounted for by the promotion of cell survival.

Experiments documenting lack of terminal T cell maturation after priming with IL-15 alone prompted us to culture CD34+/CD133+CD7−CD45dimlin− cells on a stromal support in the presence or the absence of IL-15 supplementation. Consistent with the known actions of IL-15 on NK cell development and function (42), CD34+/CD133+CD7−CD45dimlin− cells cultured with stromal cells and IL-15 differentiated into a homogeneous population of CD3−CD56−CD16+CD10−CD94−/− cells, reminiscent of immature NK cells (43, 44). Killer Ig-like receptor activation was polyclonal, because cells coexpressed different receptors, e.g., CD158a and CD158b (data not shown). In our system model, stromal support was not dispensable for IL-15 to promote NK cell differentiation, because no phenotypic features of NK cells were detected after priming of CD34+/CD133+CD7−CD45dimlin− cells with IL-15 in the absence of stromal cells. Other cytokines belonging to the family of IL-2/IL-15Rγ chain-binding cytokines, e.g., IL-2 and IL-7, were ineffective at promoting T/NK cell differentiation, when used either alone or in combination, as also found by others (45). In contrast, stromal cells were incapable of sustaining NK cell development in the absence of IL-15 supplementation. Notably, the CD34−lin− counterpart exhibited minimal NK cell differentiation potential in the presence of a stromal layer and IL-15, when compared with CD34+/CD133+CD7−CD45dimlin− cells. When tested for their ability to lyse sensitive leukemic targets, cells differentiated with IL-15 and stromal cells exhibited measurable lytic responses, even at low E:T ratio, indicating the acquisition of the functional features of NK cells.

It must be emphasized that the failure to detect CD7+ lymphoid progenitors by flow cytometry within the starting population of CD34+/CD133+CD7−CD45dimlin− HSCs as well as the absence of mRNA signals for the TCR and the high frequency of IL-15-responsive progenitor cells measured with LDA argue against the possibility that the functional properties here assigned to CD34+/CD133+CD7−CD45dimlin− HSCs reflect the cytokine-driven expansion of contaminating T cells.

Collectively, CD34+/CD133−CD7−CD45dimlin− UCB cells were superimposable in terms of differentiation potential to the putative T cell/NK cell common progenitor that has been recently cloned from fetal liver and from mouse fetal thymus (46, 47). Conceivably, UCB CD34+/CD133+CD7−CD45dimlin− cells represent a primitive HSC population with an inherent NK/T cell differentiation capability. Coupled with previous descriptions of the coexistence of lymphoid-oriented CD34+/CD7−lin− cells and myeloid-oriented CD34+/CD133+CD7+lin− HSCs in UCB, our findings support a hierarchical view of the stem cell compartment, in which the very primitive CD34+/CD133+CD7−CD45dimlin− cells that we characterized in this study for the first time might reside in dynamic equilibrium with lymphoid-restricted (CD34+/CD7−lin−), transitional myeloid (CD34+/CD133+CD7−lin−), and myeloid-oriented (CD34+/CD133+) stem cells. Studies aimed at evaluating whether CD34+/CD133+CD7−CD45dimlin− cells possess multilineage in vivo reconstitution potential are underway in animal transplantation models.

A final implication of our findings pertains to the transplantation of highly purified CD34+ HSCs, which has enormously expanded in recent years for the treatment of malignant and nonmalignant disease (48). Based on the data presented in this study, it can be assumed that depletion of CD34+ HSC subsets with lymphoid differentiation potential contained within the unmanipulated HSC graft is expected to contribute to the delayed immunological reconstitution that is routinely observed after CD34-selected HSC transplantation (48). It remains to be evaluated whether ex vivo cytokine-driven expansion of CD34+ HSC subsets endowed with lymphoid developmental potential might be exploited to accelerate lymphoid recovery and boost antitumor responses after HSC transplantation.

References
cultures maintained on genetically engineered murine stromal cells. Blood 78:666.


