HEMATOPOIESIS

Molecular characterization of early human T/NK and B lymphoid progenitor cells in umbilical cord blood

Rima Haddad\textsuperscript{1}, Philippe Guardiola\textsuperscript{2}, Brigitte Izac\textsuperscript{3}, Christelle Thibault\textsuperscript{4}, Jerry Radich\textsuperscript{2}, Anne-Lise Delezoide\textsuperscript{5}, Claude Baillou\textsuperscript{6}, François M. Lemoine\textsuperscript{6}, Jean Claude Gluckman\textsuperscript{1}, Françoise Pflumio\textsuperscript{3}, and Bruno Canque\textsuperscript{1}

From EMI-0013 Institut National de la Santé et de la Recherche Médicale - Université Paris 7, and Laboratoire d'Immunologie Cellulaire et Immunopathologie de l'Ecole Pratique des Hautes Etudes, Institut Universitaire d'Hématologie, hôpital Saint-Louis, Paris; Experimental Pathology, Fred Hutchinson Cancer Research Center, Seattle WA, USA; Département d'Hématologie, Institut Cochin, INSERM U567, Paris; Institut de Génétique et Biologie Moléculaire et Cellulaire, Strasbourg, France; Service de Biologie du Développement, hôpital Robert Debré AP-HP, Paris; CNRS UMR 7087, hôpital Pitié-Salpêtrière AP-HP, Paris, France.

Short title: lymphoid progenitors in human umbilical cord blood

Correspondence: Bruno Canque, Laboratoire d'Immunologie Cellulaire et Immunopathologie de l'EPHE, EMI-0013, Centre Hayem, hôpital Saint-Louis, 1 avenue Claude Vellefaux, 75475 Paris CEDEX 10, FRANCE.

Phone: +33 1 53 72 24 60 Fax: +33 1 53 72 22 37 E-mail: bruno.canque@chu-stlouis.fr

This work was supported by the Institut National de la Santé et de la Recherche Médicale, the Ministère de la Recherche Scientifique, the Association pour la Recherche sur le Cancer, the Comité de Paris de la Ligue Nationale Contre le Cancer, the Comité Leucémie de la Fondation de France, Eurocord III (QLRT-2001-01918), and the Société Française d'Hématologie.

Total number of words. Abstract: 183 Manuscript: 4835
Abstract

The early stages of human lymphopoiesis are poorly characterized. Here, we compared the lymphoid potential of a novel umbilical cord blood CD34⁺CD45RA⁺CD7⁺ hematopoietic progenitor cell (HPC) population with that of CD34⁺CD45RA⁺Lin'CD10⁺ HPCs, previously proposed as candidate common lymphoid progenitors. Limiting-dilution and clonal analysis, fetal thymic organ cultures and culture onto Notch ligand Delta-like-1-expressing OP9 cells, showed that although CD34⁺CD45RA⁺CD7⁺ HPCs could generate cells of the three lymphoid lineages, their potential was skewed toward the T/NK lineages. In contrast, CD34⁺CD45RA⁺Lin'CD10⁺ HPCs predominantly exhibited a B cell potential. Gene expression profiling with DNA micro-arrays confirmed that CD34⁺CD45RA⁺CD7⁺ HPCs selectively expressed T-lymphoid and NK lineage-committed genes while retaining expression of genes affiliated to the granulo-monocytic lineage, whereas CD34⁺CD45RA⁺Lin'CD10⁺ HPCs displayed a typical pro-B cell transcription profile and essentially lacked genes unrelated to the B lineage. In addition, both populations could be generated in vitro from CD34⁺CD45RA⁺CD7⁻ and CD34⁺CD45RA⁺Lin' HPCs with mixed lympho-myeloid potential, from which they emerged independently with different growth/differentiation factor requirements. These findings indicate that CD34⁺CD45RA⁺CD7⁺ and CD34⁺CD45RA⁺Lin'CD10⁺ HPCs correspond to multipotent early lymphoid progenitors polarized toward either the T/NK or B lineage, respectively.
Introduction

The immediate progeny of pluripotent hematopoietic stem cells is thought to correspond to common myeloid progenitors (CMPs) and common lymphoid progenitors (CLPs). CMPs are assumed to give rise to granulocytes and macrophages, as well as to the erythroid and megakaryocytic lineages, whereas CLPs are committed to generate either B lymphocytes (BLs) or T lymphocytes (TLs) and NK cells. Evidence for a primary segregation between CLPs and CMPs stems from in vivo transfer experiments in adult mice, where two populations of c-Kit<sup>lo</sup>Sca<sup>lo</sup>IL-7R<sup>+</sup> and FcγR<sup>lo</sup>CD34<sup>+</sup> hematopoietic progenitor cells (HPCs) isolated from the post-natal bone marrow (BM) were shown to selectively reconstitute either the lymphoid or erythro-megakaryocytic and granulo-monocytic lineages. Such dichotomous model of hematopoiesis remains however debated since there is also evidence that multi-lineage precursors co-express lymphoid as well as myelo-erythroid genes and that populations of early lymphoid progenitors (ELPs) retain some degree of multipotency. For example, AA4.1<sup>+</sup>FcγR<sup>+</sup> fetal precursors with TL and BL potential retain significant macrophage potential but fail to generate erythroid or granulocytic cells. In line with these findings, early GFP<sup>lo</sup>c-kit<sup>lo</sup>Sca-1<sup>+</sup> BL precursors from RAG1/GFP knock-in mice still express TL and macrophage potential when cultured under appropriate conditions. Lineage relationships among lymphoid progenitors also remain poorly characterized, since it has been proposed that ELPs subsequently differentiate into bipotent precursors of TLs and BLs or further segregate into biased CLP-B or CLP-T populations. As to early T lineage progenitors, although the immediate B220<sup>+</sup>c-Kit<sup>+</sup> progeny of murine c-Kit<sup>lo</sup>Sca<sup>lo</sup>IL-7R<sup>+</sup> HPCs has been shown to be present among the CD44<sup>+</sup>CD25<sup>+</sup> thymic DN1 subset, their contribution to post-natal murine thymopoiesis remains controversial since ikaros-deficient mice, which lack this population, have normal numbers of early thymic progenitors. Finally, that TL/NK cell and BL progenitors emerge independently during mouse embryogenesis, in the absence of prototypic CLPs, strongly suggests that fetal and post-natal lymphopoiesis correspond to largely independent processes, adding further complexity to the picture.

Only few reports address the ontogenetic relationships of human early lymphoid progenitors which remain poorly characterized. To date, two CD34<sup>+</sup> HPC populations with apparently lymphoid-restricted potential have been proposed as candidate CLPs: CD45RA<sup>hi</sup>LinCD10<sup>+</sup> cells...
from the BM and CD45RA⁺CD38⁻CD7⁺ cells from the umbilical cord blood (UCB) 24,25. Both were identified on the basis of reduced capacity to generate granulo-monocytic and erythroid cells, and substantial T, B and NK cell differentiation potential. However, the lack of comparative analysis of their intrinsic capacity to generate either BLs or TLs/NK cells hampers definitive conclusion, and the hypothesis that these populations actually correspond to progenitors already polarized T/NK or B lineages has to be considered. We have identified in the UCB a novel population of CD34⁺CD45RA⁺CD7⁺ HPCs with lymphoid potential that comprises bipotent NK and dendritic cell precursors and shares with CD34⁺CD1a⁺ post-natal thymocytes the capacity to differentiate into Langerhans cells via a TGF-β₁-independent pathway 26. Here, UCB CD34⁺CD45RA⁺CD7⁺ and CD34⁺CD45RA⁺Lin⁻CD10⁺ HPCs were compared for their lymphoid potentials and gene expression profiles 25,26. We show that, although both populations retain some degree of multipotency, the CD34⁺CD45RA⁺CD7⁺ HPCs are biased toward the TL/NK cell lineage, whereas the CD34⁺CD45RA⁺Lin⁻CD10⁺ HPCs correspond to pro-B cells.

Material and Methods

Isolation and immunolabeling of UCB and post-natal BM CD34⁺ cells

Normal UCB (laboratoire Sender, hôpital Saint-Vincent de Paul; service de Gynécologie-Obstétrique, hôpital Saint-Antoine; maternité, hôpital Robert Debré; Paris, France) and BM cells obtained from healthy BM graft donors were both collected according to institutional guidelines and processed in the same manner. Cord blood samples were collected only after informed consent was provided according to the Declaration of Helsinki. After Pancoll (Dutscher, Brumath, France) centrifugation, CD34⁺ cells, enriched to ≥85% with the midiMACS® system (Miltenyi Biotech France, Paris, France), were incubated for 30 min at 4°C in phosphate-buffered saline (PBS), 2% fetal calf serum (FCS; Dutscher), with CD34-phycoerythrocyanin 5 (PECy5) or CD34-allophycocyanin (APC) (clone 581), CD7-fluorescein isothiocyanate (FITC) (clone 8H8.1) (all from Beckman Coulter, Villepinte, France), CD45RA-phycoerythrin (PE) (clone HI100; BD Pharmingen, Le Pont de Claix, France), or CD10-PECy5 (clone A1B1; Beckman Coulter) monoclonal antibodies (mAbs) diluted 1:50 final. Cells were sorted based on CD7, CD45RA and CD10 expression (purity
>93%), using a FACS Vantage™ (Becton Dickinson, Mountain View, CA). To compare CD45RA<sup>+</sup>CD7<sup>+</sup> and CD45RA<sup>+</sup>Lin<sup>-</sup>CD10<sup>+</sup> HPC differentiation potential, pre-B cells were depleted to <10% from CD34<sup>+</sup> HPCs with CD19 mAb-coated magnetic Dynabeads (Dynal, Oslo, Norway) prior to sorting.

For phenotypic analysis, CD34<sup>+</sup> cells were incubated for 30 min at 4°C with mAbs (1:100 final) in PBS, 2% FCS, washed, and analyzed with a FACscalibur® (Becton Dickinson). Cells were labeled with PE-, PECy5-, or APC-conjugated CD34 mAb clone 581, and the following mAbs: CD10-PECy5, CD19-FITC (clone HIB19) or CD38-APC (clone HIT2, BD Pharmingen), CD127-PE (clone R34.34, Beckman Coulter). Isotype-matched FITC-, PE-, PECy5- and APC-conjugated irrelevant mAbs were from BD Pharmingen and Beckman Coulter.

**Assessment of NK cell and BL differentiation potential in bulk cultures**

NK cell differentiation was assessed by growing sorted CD34<sup>+</sup> HPC subsets at 37°C in a humidified 5% CO<sub>2</sub> atmosphere, in RPMI 1640, 10% FCS, 1% glutamine, 1% antibiotics ( Gibco BRL, Life Technologies, Cergy-Pontoise, France), with human recombinant cytokines: 50 ng/mL stem cell factor, 50 ng/mL Flt-3 ligand (FL; both from R&D Systems, Abingdon, UK), 100 U/mL interleukin (IL)-2, 20 ng/mL IL-7 and IL-15 (all from Tebu, Le Perray en Yvelines, France). Cultures were conducted for 3 weeks in 24- or 48-well plates (Dutscher) with or without murine MS5 stromal cells<sup>27</sup>, with half changes and cytokine addition every 6 - 7 days. Before culture initiation, MS5 cells were seeded in gelatin-coated plates and grown for 24 to 48 hrs in complete alpha-MEM medium ( Gibco BRL), 10% FCS, 1% antibiotics. BL differentiation was assessed by growing cells for 2 weeks in the MS5 cell-coated plates, in RPMI 1640, 3% FCS, 1% glutamine, 1% antibiotics, 50 µM β-mercaptoethanol, plus 50 ng/mL SCF, 100 ng/mL thrombopoietin (TPO) and 20 ng/mL IL-7 (both from Tebu). Cells were collected and labeled with CD56-PE (clone B159) and CD19-FITC (clone HIB19) (both from BD Pharmingen) or CD19-PE (clone J4.119; Coulter Immunotech) mAbs to detect NK cells and BLs, respectively.
Clonogenic assays

Erythroid and granulo-macrophagic potential of each CD34+ HPC subset was assessed by seeding 500 cells in 35 mm duplicate dishes (Dutscher), in 1.1 mL complete Methocult™ GF+ H4435 medium (Stem Cell Technologies, Meylan, France), supplemented with IL-3, IL-6, G-CSF, SCF, GM-CSF and EPO. Dishes were incubated at 37°C in humidified 5% CO2. Erythroid burst-forming colonies (BFU-E), granulocyte (CFU-G/GM) and macrophage (CFU-M) colony-forming units were counted under an inverted microscope on culture day 14, as reported 28.

Limiting-dilution assays and single-cell cultures

To assess NK cell progenitor frequency, cells were seeded at 300, 100, 30, 10, 3 and 1 cell/well into MS5 cell-coated 96-well plates (ATGC, Marne la Vallée, France) and cultured for 3 weeks in RPMI 1640, 10% human AB serum (Etablissement Français du Sang, Paris, France), 5% FCS, 50 µM β-mercaptoethanol, supplemented with SCF, FL, IL-2, IL-7, IL-15 as described above. BL progenitors were assessed by growing the cells, as above, with SCF, TPO and IL-7, in RPMI 1640, 3% FCS, 50 µM β-mercaptoethanol. Cultures lasted 2 to 3 weeks with half medium changes and fresh cytokines added every 6 - 7 days. Plates were examined weekly and cell-containing wells were scored under the microscope. At the end of cultures, BLs and NK cells were identified by CD19 and CD56 expression. The maximum likelihood estimate of NK cell or BL precursors was calculated according to the single-hit Poisson model. For single-cell suspension cultures, cells were individually plated by FACS onto MS5-coated 96-well plates and cultured in RPMI 1640, 10% human AB serum, 5% FCS, 50 µM β-mercaptoethanol, supplemented with SCF, TPO, IL-2 and IL-7. Cell-containing wells were scored on days 19 - 21, and NK cells, BLs and macrophagic cells were detected by labeling with CD56, CD19 and CD14 (clone M5E2, BD Pharmingen) mAbs.

Assessment of TL differentiation potential

TL differentiation potential of sorted CD34+ HPC subsets was first assessed in fetal thymic organ cultures (FTOC) essentially performed as reported 29,30. Briefly, thymic lobes were collected from day 14 - 15 NOD/SCID mice embryos. Hanging drops were prepared in Terasaki plates (Polylabo, Strasbourg, France) by adding 25 µL RPMI 1640, 10% human AB serum, 5% FCS, 50 ng/mL SCF, 5 ng/mL IL-2, and 20 ng/mL IL-7, with 0.2 to 2.5 x 10^4 cells/lobe (2 to 5 lobes/condition). Plates
were immediately inverted and incubated for 48 hrs in a humidified incubator before lobes were
removed, washed, transferred onto floating nucleopore filters (Millipore SA, France) in 6-well plates,
and cultured for 2 to 4 weeks. Lobes were subsequently removed, human cells were recovered by
mechanical disruption, pooled, and labeled with CD45-FITC, CD4-PECy5, CD8-APC or -PE-Cy5,
and TCRαβ-PE (all from Beckman Coulter) and/or with CD4-APC (BD Pharmingen), before FACS
analysis. Alternatively, cells were co-cultured onto OP9 murine stromal cells expressing murine
Notch ligand Delta-like-1 (OP9-DL1), which were produced by retroviral-mediated gene transfer
with a MIGR vector containing mouse Delta-like-1 cDNA kindly provided by A. Cumano (Institut
Pasteur, Paris). OP9-DL1 cells were seeded in gelatin-coated 48-well plates, and cultured for 24 to
48 hrs before initiating co-cultures in DMEM (GIBCO BRL), 10% FCS, 1% antibiotics. Co-cultures
of sorted CD34⁺ HPCs and OP9-DL1 cells were conducted for 2 - 4 weeks in RPMI 1640, 10%
human AB serum, 5% FCS, 50 µM β-mercaptoethanol, supplemented with SCF, FL, IL-2, IL-7, IL-
15. Media and cytokines were renewed every 6 to 7 days.

In vitro generation of CD45RAhiCD7hi and CD45RAhiLin-CD10+ HPCs
Purified CD34⁺ HPCs were labeled with CD34-APC, CD45RA-PE, CD10-PECy5, FITC-conjugated
CD7 and CD19 mAbs as previously described. CD45RAintCD7⁻ and CD45RAhiLin⁻ populations were
sorted and cultured onto MS5 cells in 48-well plates (Dutscher) in RPMI 1640, 10% FCS, 1%
glutamine, 1% antibiotics, 50 µM β-mercaptoethanol. Cultures were conducted for 4 to 6 days
without media change in the presence of 50 ng/mL FL, with or without 50 ng/mL recombinant
human thymic stromal lymphopoietin (TSLP; R&D Systems).

Micro-array sample preparation
Total RNA from CD45RAintCD7⁻ (biological triplicates), CD45RAhiCD7⁺ (biological triplicates), and
CD45RAhiLin⁻CD10⁺ HPC populations were extracted using the RNeasy Mini Kit (Qiagen, Hildren,
Germany). Only biological duplicates were available for CD45RAhiLin⁻CD10⁺ HPCs; one sample
obtained from an individual sorting experiment, the other corresponding to a total RNA pool of three
independent experiments. RNA quality control and quantification were monitored by
spectrophotometry and capillary electrophoresis using the RNA 6000 Pico LabChip® Kit and the
Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). RNA samples (starting amounts:
15 to 25 ng) were subjected to double amplification using the GeneChip® Eukaryotic Small Sample Target Labeling Assay Version II (Affymetrix, Santa Clara, CA). The resulting cRNAs were controlled and quantified using the Agilent 2100 Bioanalyzer. Amplification efficiency was monitored by hybridization to probe sets designed to 3’, middle and 5’ regions of housekeeping genes (Test3 Array, Affymetrix). Ten µg cRNA were hybridized to Affymetrix HG-U133A GeneChip arrays according to the manufacturer’s protocol. Quantitative scanning was performed using an Agilent GeneArray Scanner 2500A (Agilent Technologies). Probe set annotations for Affymetrix HG-U133A GeneChip arrays were downloaded from Affymetrix website and updated on August 18, 2003. Affymetrix GeneChip probe level data and background correction were obtained using the log scale robust multi-array analysis based on a log scale linear additive model available in Affy package 1.3.6 under Bioconductor / R 1.7.1 32 (http://www.bioconductor.org; http://www.r-project.org). Quantile normalization, “perfect match-only” model, and calculation of expression measures using median polish were considered, corresponding to the rma function available in the Affy package 33.

Micro-array data analysis

HPC populations were first compared two by two: CD45RA^{int}CD7^{-} vs. CD45RA^{hi}CD7^{+}, CD45RA^{hi}Lin^{-}CD10^{+} vs. CD45RA^{int}CD7^{-}, and CD45RA^{hi}Lin^{-}CD10^{+} vs. CD45RA^{hi}Lin^{+}CD7^{+}. Because of the limited number of replicates, the local pooled error test (LPE) was used for this purpose 34. Criteria used to consider genes as differentially expressed were: a fold change (FC) > 1.3, a raw p value < 0.05 according to the LPE test, an adjusted p value < 0.10 (false discovery rate < 10%) according to Benjamini and Hochberg’s procedure for multiple comparison adjustment 35, and a minimal average signal intensity for each considered probe sets above 70 in at least one of the two compared groups. To identify genes discriminating the three HPC populations, the classification analysis module of Array Miner 4.1 (Optimal Design, Brussels, Belgium), ClassMarker, was used. For this purpose, the complete dataset of the 22283 probe sets obtained with the rma function of Affy package was introduced in ClassMarker, and filtered according to the following criteria: maximal threshold, 16000; minimal fold change, 2.0; minimal absolute change, 100. Filtered data (n = 3640
probe sets) were subsequently introduced in a cross-validation analysis, and only probe sets with a signal to noise ratio > 2.00 were considered significantly associated with a cell population.

Results

Identification of UCB lymphoid progenitors according to CD45RA and CD7 expression levels

In order to compare the lymphoid potential of UCB CD34+CD45RAhiCD7+ HPCs with that of four other CD34+ cell populations, CD34+ cells were sorted according to CD45RA and CD7 expression levels, and tested for NK cell and BL potentials (Figure 1). Only the CD45RAhiCD7+ HPCs exhibited then a strong capacity to generate NK cells, with 70 ± 18% (mean ± SD, n = 3) CD56+ cells after 2-week culture with SCF, FL, IL-2, IL-7 and IL-15 (referred to as NK condition w/o MS5 thereafter), albeit with limited expansion (6- to 30-fold). These data were confirmed by culturing the CD45RAhiCD7+ cells with the same cytokine combination onto MS5 cells (referred to as NK condition thereafter) 36. Adding the stromal layer increased growth rates to 112 to 130 folds, with up to 95% CD56+ NK cells (data not shown). In parallel, the five cell populations were cultured for 2 weeks on MS5 cells with SCF, TPO and IL-7 to assess their differentiation capacity into BLs (referred to as B condition thereafter) 37. Again, the CD45RAhiCD7+ HPCs displayed the greatest potential, newly generated CD19+ BLs representing then 27 ± 17% (n = 6) of cells relative to ≤5% yields for the other populations. These data indicate that, of all the populations tested, only CD45RAhiCD7+ HPCs display mixed NK cell and BL differentiation potential.

CD45RA-CD7-, CD45RAintCD7-, CD45RAhiCD7- and CD45RAhiCD7+ HPCs were then tested for the capacity to form erythroid and granulo-monocytic colonies (Table 1). After 2-week culture in methylcellulose with IL-3, IL-6, G-CSF, SCF, GM-CSF and EPO, only CD45RA-CD7- HPCs displayed significant erythroid potential, which was consistent with their capacity to generate megakaryocytes in liquid cultures with SCF and TPO (data not shown). Conversely, only the CD34+CD45RA+ HPC populations comprised macrophage precursors. Of note, the CD45RAintCD7- HPCs were highly enriched in CFU-G/GM. These data confirm a report that erythroid potential segregates with the CD45RA- fraction of UCB CD34+ HPCs 38, and they indicate that acquisition of
lymphoid potential by CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs correlates with a decreased capacity to form both erythroid and granulocytic colonies.

**CD45RA<sup>hi</sup>CD7<sup>+</sup> and CD45RA<sup>hi</sup>Lin<sup>-</sup>CD10<sup>+</sup> HPCs differ in the capacity to generate NK cells and BLs**

Inasmuch as the above data suggested that CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs may correspond to a CLP population, we examined their ontogenetic relationship with previously reported CD45RA<sup>hi</sup>Lin<sup>-</sup>CD10<sup>+</sup> and CD38<sup>-</sup>CD45RA<sup>-</sup>CD7<sup>+</sup> candidate CLPs<sup>24,39</sup>. FACS analysis of UCB CD34<sup>+</sup> HPCs showed that the CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs were homogeneously CD38<sup>-</sup> (Figure 2A). In our hands, no CD7-expressing cell was detected among UCB CD34<sup>+</sup>CD38<sup>-</sup> HPCs, precluding further analysis of the CD38<sup>-</sup>CD45RA<sup>-</sup>CD7<sup>+</sup> population<sup>24</sup>. Inasmuch as CD38<sup>-</sup>CD45RA<sup>-</sup>CD7<sup>+</sup> HPCs has been shown to lack granulo-monocytic potential, it can be assumed that they do not overlap with CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs. Quadruple CD34-PECy5, CD45RA-PE, CD7-FITC and CD10-PECy5 mAb labeling showed that only a minority of CD34<sup>+</sup>CD45RA<sup>hi</sup> cells co-expressed CD7 and CD10 (Figure 2B). That the CD45RA<sup>hi</sup>CD7<sup>+</sup> and CD45RA<sup>hi</sup>Lin<sup>-</sup>CD10<sup>+</sup> HPC populations were independent was confirmed by the fact that only the latter HPCs homogeneously expressed CD127/IL-7R-α, which was barely detectable on CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs (data not shown). Interestingly, based on CD7, CD19, CD38 and CD127 expression, UCB CD45RA<sup>hi</sup>Lin<sup>-</sup>CD10<sup>+</sup> HPCs were undistinguishable from their postnatal BM homologues<sup>25</sup>. Finally, CD45RA<sup>hi</sup>Lin<sup>-</sup>CD10<sup>+</sup> HPCs were found to display similar granulo-macrophagic and erythroid potentials than CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs in methylcellulose assays (data not shown).

UCB CD45RA<sup>hi</sup>CD7<sup>+</sup> and CD45RA<sup>hi</sup>Lin<sup>-</sup>CD10<sup>+</sup> HPCs were then compared for the capacity to generate NK cells and BLs in limiting-dilution assays (Figures 3A,B). Because these populations display low cloning efficiency in the absence of feeder cells (data not shown), assays were performed on MS5 cells. In two independent experiments, NK cell precursor frequency varied between 1:4 and 1:10 among CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs relative to 1:50 and 1:100 for CD45RA<sup>hi</sup>Lin<sup>-</sup>CD10<sup>+</sup> HPCs. The two populations also markedly differed regarding the intrinsic capacity to generate NK cells. As shown in Figure 3A, CD56<sup>+</sup> NK cell percentages were always ≥77% in wells seeded with 300 to 30 CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs relative to ≤35% in CD45RA<sup>hi</sup>Lin<sup>-</sup>CD10<sup>+</sup> HPC cultures. Due to greater
overall cloning efficiency under the B condition, 5- to 16-fold greater BL precursor frequency was also noted among CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs (1:8 and 1:10 vs. 1:50 and 1:60), but CD45RA<sup>hi</sup>Lin<sup>CD10</sup> HPCs displayed stronger BL potential, with ≥75% CD19<sup>+</sup> cells in wells seeded with 300 to 30 cells vs. <40% in those seeded with CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs (Figure 3B).

To confirm these data, we established a single-cell clonal assay allowing for simultaneous differentiation of NK cells and BLs (referred to as B/NK condition thereafter). After 3-week culture, 43% NK cell clones, 24% mixed BL/NK and only 7% BL clones were recovered from positive wells seeded with CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs (Table 2). Again, CD45RA<sup>hi</sup>Lin<sup>CD10</sup> HPCs gave an inversed pattern with 63% BL clones, 9% NK clones, and no mixed BL/NK cell clones. Double-negative CD19<sup>+</sup>CD56<sup>+</sup> clones, noted irrespective of the population used, comprised a majority of CD14<sup>+</sup> cells (data not shown).

These findings indicate that CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs are enriched in progenitors with strong NK potential, whereas CD45RA<sup>hi</sup>Lin<sup>CD10</sup> HPCs comprise a majority of BL progenitors.

**CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs display enhanced TL differentiation potential**

FTOC were used to assess whether the differences noted between CD45RA<sup>hi</sup>Lin<sup>CD10</sup> and CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs extended to the capacity to generate TLs (Figure 4A). Only CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs displayed strong TL potential, with CD4<sup>+</sup>CD8<sup>+/−</sup>TCR<sub>αβ</sub><sup>+</sup> TLs representing about 35% of human cells recovered from thymic lobes. Only few mature CD4<sup>+</sup>CD8<sup>+</sup>TCR<sub>αβ</sub><sup>bi</sup> TLs were recovered from cultures initiated with CD45RA<sup>hi</sup>Lin<sup>CD10</sup> or bulk CD34<sup>+</sup> HPCs (less than 5% of human cells). Of note, CD45RA<sup>int</sup>CD7<sup>−</sup> cells lacked detectable TL potential (data not shown). Accordingly, human cell yields were 6- to 7-fold greater in thymic lobes seeded with CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs than with CD45RA<sup>hi</sup>Lin<sup>CD10</sup> HPCs or bulk CD34<sup>+</sup> cells. The difference was even more pronounced when considering CD4<sup>+</sup>CD8<sup>+</sup>TCR<sub>αβ</sub><sup>hi</sup> thymocytes (9- to 15-fold increase) and CD4<sup>+</sup>CD8<sup>+</sup>TCR<sub>αβ</sub><sup>bi</sup> mature TLs (35- to 50-fold increase).

To examine whether the T lineage potential of CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs correlated with a functional Notch signaling pathway, the same populations were assayed in cultures onto OP9-DL1 cells<sup>31</sup>. As expected, only CD45RA<sup>hi</sup>CD7<sup>+</sup> cells displayed then significant TL potential, albeit with limited 3- to 5-fold expansion (Figure 4B). After 2-week culture, 3.5% CD4<sup>+</sup>CD8<sup>+</sup>TCR<sub>αβ</sub><sup>+</sup> thymocytes and 15%
TCRαβ hi TLs were detected, with up to 11% mature TCRαβ hi TLs on culture week 4. Of note, TCRαβ hi TLs were predominantly CD4+CD8+ 31. CD45RA intCD7+ and CD45RA hiLin CD10+ HPCs did not differentiate into TLs on OP9-DL1 cells, bulk CD34+ HPCs expressing only marginal TL potential. Thus, only the CD45RA hiCD7+ HPCs respond to Notch ligand Delta-like 1 ligation.

**Gene expression profiling of CD45RA hiCD7+ and CD45RA hiLin CD10+ progenitors**

DNA micro-arrays were used to determine CD45RA hiCD7+, CD45RA hiLin CD10+ and CD45RA intCD7+ HPC gene expression profiles. CD45RA intCD7+ and CD45RA hiCD7+ HPCs displayed very close transcriptional profiles. Only 166 probe sets, representing 101 genes (complete cds or mRNA sequences) and 62 consensus sequences, were differentially expressed in the two populations. The complete lists of these genes may be found in supporting information Tables S1 and S2. As expected from their biological phenotype, TL lineage-committed genes CD7, CCR9, TRG, TRGC2, TRGV9, DNTT/TDT as well as the NK cell lineage-committed gene TYROBP/DAP12 were expressed to higher levels in CD45RA hiCD7+ cells. Relative to CD45RA intCD7+ HPCs, CD45RA hiCD7+ cells also over-expressed BL lineage-committed genes BLNK, CD24, IGJ, CD10/MME, VPREB1. The co-regulated serprocidins (AZU1, CTSG, PRTN3) as well as MPO, CSF1R, LYZ, ELA2, HCK, S100A8, CCL3, and CEBPD, which are usually expressed in myeloid cells, were also differentially expressed in CD45RA hiCD7+ cells. Comparatively, CD45RA intCD7+ HPCs displayed a more immature gene transcription profile with selective expression of stem cell-associated transcription factors TAL1, GATA3, HLF and MLLT3 40,41.

CD45RA hiLin CD10+ HPCs were more distantly related to CD45RA hiCD7+ (1200 probe sets differentially expressed) and CD45RA intCD7+ (1368 probe sets differently expressed) HPCs, with 857 common probe sets being differentially expressed in both comparisons, i.e., CD45RA hiLin CD10+ vs. CD45RA intCD7+ HPCs and CD45RA hiLin CD10+ vs. CD45RA hiCD7+ HPCs (Table S3). They expressed higher levels of transcripts for B-cell receptor components (IGLL1, CD79A, CD79B), BL-specific surface markers (CD10, CD19, CD22) or signaling molecules (BLK, BLNK), as well as transcription factors involved in BL lineage commitment (E2A/TCF3, PAX5, POU2AF1), which confirms that they correspond to prototypic pro-B cells. Regarding genes expressed in the TL or NK cell lineages, CD45RA hiLin CD10+ HPCs displayed lower expression of TRB, TRG, TRGC2
and TRGV9. The myeloid “signature” of CD45RAhiLinCD10+ HPCs was less clear with only low expression of myeloid lineage-affiliated transcripts.

Class prediction analysis extended and validated CD45RAhiCD7+, CD45RAhiLin-CD10+ and CD45RAintCD7- HPC gene transcription profiles. Among the 381, 27, and 27 probe sets that discriminated the three populations, it confirmed that AZU1, CCL3, CD7, CTSG, ELA2, LYZ, MPO, PRTN3, TRG, TRGC2, TRGV9, NKG7 and TYROBP were CD45RAhiCD7+ specific, whereas HLF, TAL1, HOXB2 and MLLT3 were among CD45RAintCD7- specific genes, and CD10, CD19, CD79B, VPREB1, VRPEB3, RAG1, SMARCA4, GALNAC4S-6ST, IGLJ3, E2A/TCF3, PAX5, POUF2A1, TLE1, BCL7A, BCL11A, IL7R, SOCS2 and BLNK were among CD45RAhiLin-CD10+ specific genes (Figure 5 A-C).

The three populations were finally examined for expression of known TL or NK cell markers, cytokine receptors, lymphoid signaling pathways, or transcription factors (data not shown). This disclosed that, whereas CD45RAhiCD7+ HPCs expressed low TRB levels, they lacked CD3 polypeptides, TRA, TRD and PTCRA. Accordingly, RAG1 and RAG2 expression was restricted to CD45RAhi LinCD10+ pro-B cells that also expressed IL7R and protein-tyrosine kinase LCK to higher levels. In addition, CD45RAhiLinCD10+ cells selectively expressed transcription factor LEF1 as well as dominant negative helix-loop-helix protein ID3. As to Notch signaling pathway, the three populations expressed Notch 1, Notch 2, and RBPSUH to similar levels, while DLK1 and HRY/HES1 were up-regulated in CD45RAhiCD7+ and CD45RAintCD7 HPCs.

Altogether, these results confirm the respective polarization of CD45RAhiCD7+ and CD45RAhiLinCD10+ HPCs towards the T/NK or B lineages, respectively, and they indicate that active transcription of TRG locus coincides with acquisition of TL potential.

In vitro generation of CD45RAhiCD7+ HPCs

The proximity of CD45RAhiCD7+ and CD45RAintCD7- HPCs disclosed by micro-array analysis led us to investigate their ontogenetic relationship. Because CD45RAintCD7- HPCs lack NK potential when cultured in the absence of MS5 feeders (≤ 2% of CD56+ cells; Figure 1), we first examined whether co-culture onto MS5 cells affected their capacity to generate NK cells. Hence, these cells were grown with SCF, FL, IL-2, IL-7 and IL-15, with or without MS5 cells. After 3 weeks, CD56+
cells represented 20 to 30% of total cells with, vs. <5% without, MS5 cells, indicating that CD45RA int CD7− HPCs acquire substantial NK cell differentiation potential under these conditions. Whether CD45RA int CD7− cells represented precursors of CD45RA hi CD7+ HPCs was then examined. CD45RA int CD7− (R1) and CD45RA hi Lin− (R2) HPCs populations were thus sorted and cultured onto MS5 cells in the presence of FL (Figure 6A). After 4 - 6 days under these conditions, CD45RA int CD7− cells had become uniformly CD45RA hi and 16 ± 11% were CD7+ (n = 7), whereas CD45RA hi CD7− cells represented up to 40% of CD45RA hi Lin− CD7+ HPC-derived cells (27 ± 19%; n = 4). In vitro-generated CD45RA hi CD7+ cells were then sorted and assessed for their lymphoid potential in limiting-dilution (Figure 6B). As for UCB-isolated counterparts, in vitro-generated CD45RA hi CD7+ cells expressed higher NK cell than BL potential, and they displayed substantial TL potential in co-culture with OP9-DL1 cells (Figure 6C). Altogether these data indicate that CD45RA hi CD7+ HPCs differentiate from CD45RA int CD7− via a stepwise process.

Whether CD45RA hi Lin−CD10+ HPCs differentiated from CD45RA int CD7− and CD45RA hi Lin− HPCs was also examined, but neither population generated CD10+ cells when cultured under the conditions described above (data not shown). Both populations were then cultured with a combination of one to four cytokines (SCF, FL, TPO, IL-7) onto MS5 or S17 murine cells 37,43,44, αSM-56 or SV40-56 human stromal cells 45, with similar negative results. Finally, CD45RA hi Lin− HPCs isolated from the UCB or adult BM were cultured onto MS5 stromal layer cells in the presence of FL and TSLP 46 (Figure 7). After 4 - 6 days, CD45RA hi Lin−CD10+ HPCs represented 0.5 to 1% of CD34+ cells derived from UCB CD45RA hi Lin− HPCs, while they reached 6% in cultures of adult BM cells. Of note, secondary-sorting experiments confirmed that, like their primary counterparts, in vitro-generated CD45RA hi Lin−CD10+ HPCs displayed predominant BL differentiation potential (data not shown).

Altogether, these data show that CD45RA hi CD7+ and CD45RA hi Lin−CD10+ HPCs emerge independently from CD45RA hi Lin− precursors, and they indicate that BM and cord blood CD45RA hi Lin− HPCs differ in their capacity to generate CD45RA hi Lin−CD10+ HPCs.

Discussion
Here, we found that, among six UCB CD34+ HPC populations identified on the basis of CD45RA, CD7 and CD10 expression, only CD45RA^{hi}CD7^{+} and CD45RA^{hi}Lin^{-}CD10^{+} HPCs displayed substantial lymphoid potential. Using semi-solid assays, we show in addition that although both populations had a reduced capacity to generate erythroid colonies, they nonetheless retained substantial granulo-monocytic differentiation potential. The results presented in this study also confirm that acquisition of CD45RA marker correlates with loss of erythro-megakaryocytic potential as well as with the capacity to generate macrophage colonies \cite{38}, arguing thus for an early partition of erythro-megakaryocytic and granulo-monocytic progenitors. Though conflicting with the current model of hematopoiesis, these findings are consistent with previous report that murine fetal liver Lin^{-}c-kit^{+}Sca-1^{high} HPCs generate myeloid cells, TLs and BLs but not erythroid cells or megakaryocytes \cite{9}.

As to the early stages of lymphopoiesis, the current paradigm postulates a unique founder CLP population from which TLs, BLs and NK cells differentiate \cite{47}. To date, most studies of human ELPs are based on the identification of populations endowed with the capacity to generate NK cells, TLs and BLs, and reduced granulo-monocytic and erythroid potentials \cite{3,24,25}. However, this should not be considered an absolute criterion given that even early CD34^{+}CD1a^{-} post-natal thymocytes retain the capacity to generate macrophages and dendritic cells \cite{49,50}. Here, at variance with previous reports \cite{24,25}, we used a double comparative approach based on quantification of each population’s intrinsic capacity to generate NK cells and BLs in limiting dilution and in mixed B/NK clonal assays, and on direct comparison of their lymphoid potentials. This led to the observation that CD45RA^{hi}CD7^{+} HPCs are highly enriched in NK cell precursors and display strong T potential, whereas CD45RA^{hi}Lin^{-}CD10^{+} HPCs comprise a majority of pro-B cells. Gene profiling of CD45RA^{hi}CD7^{+} and CD45RA^{hi}Lin^{-}CD10^{+} HPC populations confirmed their biological phenotypes by showing that CD45RA^{hi}CD7^{+} HPCs selectively express T/NK cell lineage-affiliated genes while CD45RA^{hi}Lin^{-}CD10^{+} HPCs display a typical B signature. Surprisingly, gene expression profiles also revealed that both CD45RA^{hi}Lin^{-}CD10^{+} and CD45RA^{int}CD7^{+} populations retained expression of globin genes despite a drastically reduced erythroid potential (data not shown), suggesting that overlap between the diverse hematopoietic differentiation programs may have been underestimated \cite{51}. Taken as a whole, these findings indicate that, although they retain some degree of multipotency,
CD45RA<sup>hi</sup>CD7<sup>+</sup> and CD45RA<sup>hi</sup>Lin<sup>-</sup>CD10<sup>+</sup> HPCs are endowed with either T/NK or B lineage biased lymphoid differentiation potential. Thus, neither population fulfills the defining criteria of prototypic CLPs, i.e., the overall similar capacity to generate NK cells, TLs and BLs together with lack of myeloid potential. However, because MS5 stromal cells have been previously shown to antagonize BL differentiation from mouse ELPs<sup>11</sup>, the hypothesis that the clonal B/NK assay used in this study could lead to underestimation of the BL differentiation potential of CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs should also be considered. Although such inhibitory effect has not been reported for human hematopoietic progenitors, one cannot exclude that prototypic CLPs could nonetheless be present among CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs.

Regarding the ontogenetic relationship of the CD34<sup>+</sup>CD45RA<sup>+</sup> cell populations examined here, we provide direct evidence that CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs differentiate from CD45RA<sup>int</sup>CD7<sup>-</sup> precursors via a stepwise process. These results are consistent with the immature gene expression profile of CD45RA<sup>int</sup>CD7<sup>-</sup> HPCs characterized by selective expression of stem cell transcription factors<sup>40</sup>, and with their mixed granulo-monocytic and lymphoid differentiation potential. That CD45RA<sup>int</sup>CD7<sup>-</sup> cells actually correspond to lympho-myeloid precursors is further supported by the observation that BL/NK containing clones derived from single CD45RA<sup>int</sup>CD7<sup>-</sup> HPCs seeded under the B/NK condition always comprised a significant fraction of CD19<sup>-</sup>CD56<sup>-</sup> myeloid cells (R. Haddad, unpublished data). Although both differentiate from CD45RA<sup>hi</sup>Lin<sup>-</sup> HPCs, the limited efficiency of CD45RA<sup>hi</sup>Lin<sup>-</sup>CD10<sup>+</sup> HPC in vitro generation does not allow conclusions regarding their ontogenetic relationship with CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs. That CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs represented precursors of CD45RA<sup>hi</sup>Lin<sup>-</sup>CD10<sup>+</sup> HPCs was also tested, but culture onto murine or human stromal layers supplemented with diverse cytokine combinations never yielded CD45RA<sup>hi</sup>Lin<sup>-</sup>CD10<sup>+</sup> HPCs from CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs (R. Haddad, unpublished data). For this reason, our working hypothesis is that the two populations differentiate independently from CD45RA<sup>hi</sup>Lin<sup>-</sup> precursors in response to signals provided by soluble factors such as FL or TSLP, or by stromal cell surface counter-receptors.

In conclusion, the results presented in this study show that UCB CD45RA<sup>hi</sup>CD7<sup>+</sup> and CD45RA<sup>hi</sup>Lin<sup>-</sup>CD10<sup>+</sup> HPCs correspond to multipotent ELPs polarized toward either the T/NK or B lineage, arguing thus for an early partition of TL/NK and BL precursors<sup>48</sup>.
Acknowledgements

We gratefully acknowledge the help of Pr. J. Milliez (service de Gynécologie-Obstétrique, hôpital Saint-Antoine) and Pr. J. Oury (Maternité, hôpital Robert Debré, Paris, France) for the gift of cord blood samples, and of Pr. E. Gluckman and Dr. P. Richard (service de Greffe de Moelle Osseuse, hôpital Saint-Louis, Paris, France) for providing BM samples. We are grateful to Dr. A. Cumano for the gift of OP9-DL1 stromal cells, and to Dr D. Chalmers (IMSERM EPI-119, Besançon, France) for the gift of αSM-56 and SV40-56 human stromal cells. We thank M. Yagello, F. Jourquin, N. Brunel and B. Dumas for technical assistance, and Pr. F. Sigaux (INSERM U462, hôpital Saint-Louis, Paris, France) for critical discussions.
References


Figure legends

Figure 1. NK cell and BL differentiation potential of UCB CD34⁺ HPC populations. Purified UCB CD34⁺ cells were sorted into CD45RA⁺CD7⁻ (R1), CD45RA⁺⁺CD7⁻ (R2), CD45RA⁺⁺⁺CD7⁺ (R3), CD45RA⁺⁺⁺CD7⁺ (R4) and CD45RA⁺⁻CD7⁺ (R5) populations, and cultured for 21 days with SCF, FL, IL-2, IL-7 and IL-15 (NK condition w/o MS5). Alternatively, cells were seeded onto MS5 cells with SCF, TPO and IL-7, and cultured for 14 days (B condition). At the end of the cultures, cells were harvested and labeled with CD56-PE and CD19-FITC mAbs before FACS-analysis: histograms and the indicated percentages of specifically labeled cells are based on control mAb labeling. In vitro generated NK cells were homogeneously CD8⁺CD56⁺ whereas a minority of CD10⁻CD19⁺ BLs co-expressed CD20. At culture initiation none of the populations tested did contain CD56⁺ cells; CD34⁺CD19⁺ pre-B cells represented 6 ± 2% (n = 3) of sorted CD45RA⁺⁺CD7⁺ (R2) cells. Data are from one of two experiments.

Figure 2. Immunophenotype of UCB CD34⁺ HPCs according to CD45RA expression levels. (A) CD38 and CD7 expression by CD45RA⁻ (R1), CD45RA⁺⁺⁺ (R2) and CD45RA⁺⁺ HPCs (R3). (B) CD10 and CD7 expression by CD45RA⁻ (R1), CD45RA⁺⁺⁺ (R2) and CD45RA⁺⁺ HPCs (R3). Prior to labeling, the CD34⁺ HPCs were depleted to <10% CD19⁺ pre-B cells. In all cases cells were gated based on expression of CD34 marker (data not shown). Data are from one representative experiment out of four.

Figure 3. NK cell and BL differentiation potential of CD45RA⁺⁺CD7⁺ and CD45RA⁺⁺Lin⁻CD10⁺ HPCs. Comparative analysis of CD45RA⁺⁺Lin⁻CD10⁺ and CD45RA⁺⁺CD7⁺ HPCs in limiting-dilution assay cultures under (A) the NK condition or (B) the B condition. Positive wells were scored and FACS-analyzed after 2 (B condition) or 3 (NK condition) weeks of culture. Upper panels show (A) NK cell and (B) BL precursor frequencies; lower panels display mean percentages ± SD of CD56⁺ and CD19⁺ cells per well. Differences of CD56⁺ and CD19⁺ cell percentages in wells from CD45RA⁺⁺Lin⁻CD10⁺ vs. CD45RA⁺⁺CD7⁺ HPC cultures seeded with 300 to 10 and 300 to 30 cells per well under the NK and B condition, respectively, were statistically significant (p ≤ 0.01;
Student’s t test). Results are representative of one experiment out of two under each culture condition.

**Figure 4. TL differentiation potential of CD45RA<sup>hi</sup>CD7<sup>+</sup> and CD45RA<sup>hi</sup>Lin<sup>-</sup>CD10<sup>+</sup> HPCs.** (A) FTOC assay: sorted CD45RA<sup>hi</sup>CD7<sup>+</sup>, CD45RA<sup>hi</sup>Lin<sup>-</sup>CD10<sup>+</sup> and bulk CD34<sup>+</sup> cells were cultured for 4 weeks in NOD/SCID mouse fetal thymic lobes before cells were recovered and FACS-analyzed; only CD45<sup>+</sup> (R1) human cells were further analyzed; percentages of positive cells are indicated; data are from one out of five experiments. (B) Co-culture with OP9-DL1 cells: CD34<sup>+</sup> HPC populations were cultured for 2 or 4 weeks onto OP9-DL1 cells with SCF, FL, IL-2, IL-7 and IL-15, before FACS analysis; percentages of positive cells are indicated; data are from one out of four experiments.

**Figure 5. Supervised analysis of CD45RA<sup>hi</sup>CD7<sup>+</sup>, CD45RA<sup>int</sup>CD7<sup>-</sup> and CD45RA<sup>hi</sup>Lin<sup>-</sup>CD10<sup>+</sup> HPC gene expression profiles.** Genes discriminating between the three cell populations were selected using the classification analysis module of Array Miner 4.1 (see Materials and Methods), and they were plotted according to average signal intensity (ASI). Relative expression levels of genes differentially expressed in (A) CD45RA<sup>hi</sup>CD7<sup>+</sup>, (B) CD45RA<sup>int</sup>CD7<sup>-</sup> and (C) CD45RA<sup>hi</sup>Lin<sup>-</sup>CD10<sup>+</sup> HPCs. Among the 381 probe sets differentially expressed in CD45RA<sup>hi</sup>Lin<sup>-</sup>CD10<sup>+</sup> HPCs, only those corresponding to genes underlying polarization toward the B lineage are presented.

**Figure 6. Lymphoid differentiation potential of in vitro generated CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs.** (A) Generation of CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs from CD45RA<sup>int</sup>CD7<sup>-</sup> and CD45RA<sup>hi</sup>Lin<sup>-</sup> HPCs: based on sorting gates set on CD34<sup>+</sup>CD7<sup>-</sup>CD19<sup>-</sup> cells, CD45RA<sup>int</sup>CD10<sup>-</sup> (i.e., CD45RA<sup>int</sup>CD7<sup>-</sup>) and CD45RA<sup>hi</sup>CD10<sup>-</sup> (i.e., CD45RA<sup>hi</sup>Lin<sup>-</sup>) cells were sorted and cultured onto MS5 cells with FL for 4 - 6 days; cells were gated based on high CD34 expression and FACS analyzed for CD45RA and CD7; percentages of labeled cells are indicated; data are from one of three experiments. (B) Analysis of NK cell and BL differentiation potentials of vitro generated CD45RA<sup>hi</sup>CD7<sup>+</sup> HPCs: CD45RA<sup>int</sup>CD7<sup>-</sup> HPC-derived CD45RA<sup>hi</sup>CD7<sup>+</sup> cells were sorted on culture day 6 and seeded in limiting-dilution under the NK or B conditions; upper panels show NK cell and BL progenitor frequencies; lower panels display mean percentages + SD of CD56<sup>+</sup> and CD19<sup>+</sup> cells in the corresponding replicate cell-containing wells; data are from one experiment of two. (C) TL potential of CD45RA<sup>int</sup>CD7<sup>-</sup> HPC-
derived CD45RA<sup>hi</sup>CD7<sup>+</sup> cells: sorted CD45RA<sup>hi</sup>CD7<sup>+</sup> cells were co-cultured for 3 weeks with OP9-DL1 cells, as indicated in the legend of Figure 4B, before FACS analysis; percentages of labeled cells are indicated; data are from one of two experiments.

**Figure 7. Generation of CD45RA<sup>hi</sup>Lin<sup>-</sup>CD10<sup>+</sup> from UCB and BM CD34<sup>+</sup>CD45RA<sup>hi</sup>Lin<sup>-</sup> cells.**

CD34<sup>+</sup>CD45RA<sup>hi</sup>Lin<sup>-</sup> cells were FACS-sorted from UCB or BM CD34<sup>+</sup> HPCs and cultured for 4 - 5 days onto MS5 cells in the presence of FL and TSLP. The recovered cells, gated based on CD34 expression (not shown), were then FACS analyzed. Data are from one of three experiments.
Haddad et al., Figure 1
Haddad et al., Figure 3

A. NK precursors

B. B precursors

CD45RA^hi^CD7^+^

CD45RA^hi^Lin^−^CD10^+^
A

Haddad et al, Figure 4A

mononuclear cells

CD45RA\textsuperscript{hi}CD7\textsuperscript{+}

CD45RA\textsuperscript{hi}Lin\textsuperscript{-}CD10\textsuperscript{+}

bulk CD34\textsuperscript{+}

CD45\textsuperscript{*} human cells (R1)

CD8 - APC

TCR\textsubscript{\alpha\beta} - PE

CD4 - PECy5

35

8

12

37

16

35

13

20

15

8

4

5

20

9

4
Haddad et al., Figure 6

A

B

NK precursors

B precursors

C

CD45RA - PE

CD7 - FITC

CD45RAhiLin-

CD45Raint CD7-

cells/well

0,1

1

10

100

0 2 5 7 5 1 0 0

1/6

CD56

CD19

CD8 - PECy 5

TCR αβ - PE

8.4%

8.8

0 25 50 75 100

0 25 50 75 100

negative wells (%)

negative wells (%)

CD56

CD19

cells/well

300 100 30

300 100 30

NK precursors

B precursors

For personal use only. on August 16, 2017. For personal use only.
Table 1. Assessment of erythroid and granulo-morphagic potential of UCB CD34⁺CD45RA⁻⁺⁻CD7⁻⁻⁻⁻ HPC populations.

<table>
<thead>
<tr>
<th>CD34⁺ HPC populations</th>
<th>CD45RA⁺⁺CD7⁻⁻</th>
<th>CD45RA⁺⁺⁺CD7⁻⁻⁻⁻</th>
<th>CD45RA⁺⁺⁺⁺CD7⁻⁻⁻⁻</th>
<th>CD45RA⁺⁺⁺⁺⁺CD7⁻⁻⁻⁻⁻⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp#1</td>
<td>Exp#2</td>
<td>Exp#1</td>
<td>Exp#2</td>
</tr>
<tr>
<td>BFU-E</td>
<td>94</td>
<td>96</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>CFU-G/GM</td>
<td>69</td>
<td>74</td>
<td>113</td>
<td>104</td>
</tr>
<tr>
<td>CFU-M</td>
<td>5</td>
<td>5</td>
<td>21</td>
<td>19</td>
</tr>
</tbody>
</table>

* Results are from 2 independent experiments
Table 2. Clonal analysis of BL and NK cell differentiation potential of CD45RA$^{hi}$CD7$^+$ and CD45RA$^{hi}$Lin$^-$CD10$^+$ HPCs$^a$.

<table>
<thead>
<tr>
<th>CD34$^+$ HPC populations</th>
<th>CD45RA$^{hi}$CD7$^+$</th>
<th>CD45RA$^{hi}$Lin$^-$CD10$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. cells seeded</td>
<td>418</td>
<td>148</td>
</tr>
<tr>
<td>total numbers of clones</td>
<td>58</td>
<td>11</td>
</tr>
<tr>
<td>NK clones (%)</td>
<td>43</td>
<td>9</td>
</tr>
<tr>
<td>B clones (%)</td>
<td>7</td>
<td>63</td>
</tr>
<tr>
<td>mixed B/NK clones (%)</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>CD19$^-$CD56$^-$ clones (%)</td>
<td>26</td>
<td>28</td>
</tr>
</tbody>
</table>

$^a$ Cells were seeded individually in MS5 cell-pre-coated wells and cultured for 3 weeks with SCF, TPO, IL2 and IL7. Clones were then harvested, labeled with CD56-PE and CD19-FITC mAbs, and FACS-analyzed. Data are pooled from two independent experiments.
Molecular characterization of early human T/NK and B lymphoid progenitor cells in umbilical cord blood

Rima Haddad, Philippe Guardiola, Brigitte Izac, Christelle Thibault, Jerry Radich, Anne-Lise Delézoide, Claude Baillou, François M Lemoine, Jean Claude Gluckman, Françoise Pflumio and Bruno Canque