



Characterization of CD34⁺ subsets derived from bone marrow, umbilical cord blood and mobilized peripheral blood after stem cell factor and interleukin 3 stimulation

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Summary:

We characterized CD34⁺ cells purified from bone marrow (BM), mobilized peripheral blood (PB) and cord blood (CB) and we tried to establish correlations between the cell cycle kinetics of the CD34⁺CD38⁻ and CD34⁺CD38⁺ subpopulations, their sensitivity to SCF and IL-3 and their expression of receptors for these two CSFs. At day 0, significantly fewer immature CD34⁺CD38⁻ cells from CB and mobilized PB are in S + G2M phases of the cell cycle (respectively 2.0 ± 0.4 and $0.9 \pm 0.3\%$) than their BM counterpart ($5.6 \pm 1.2\%$). A 48-h incubation with SCF + IL-3 allows a significant increase in the percentage of cycling CD34⁺CD38⁻ cells in CB ($19.2 \pm 2.2\%$, $P < 0.0002$) and PB ($14.1 \pm 5.5\%$, $P < 0.05$) while the proliferative potential of BM CD34⁺CD38⁻ progenitors remains constant ($8.6 \pm 1.0\%$, NS). CD123 (IL-3 receptor) expression is similar in the three sources of hematopoietic cells at day 0 and after 48-h culture. CD117 (SCF receptor) expression, although very heterogeneous according to the subpopulations and the sources of progenitors evaluated, seems not to correlate with the difference of progenitor cell sensitivity to SCF nor with their proliferative capacity. Considering the importance of the c-kit/SCF complex in the adhesion of stem cells to the microenvironment, several observations are relevant. The density of CD117 antigen expression (expressed in terms of mean equivalent soluble fluorescence, MESF) is significantly lower on fresh PB cells than on their BM ($P < 0.017$) and CB ($P < 0.004$) counterparts, particularly in the immature CD34⁺CD38⁻ population (560 ± 131 , 2121 ± 416 and 1192 ± 129 MESF respectively); moreover, when PB and BM CD34⁺CD38⁻ cells are stimulated for 48 h with SCF + IL-3, the CD117 expression decreases by 1.5- and 1.66-fold, respectively. This reduction could modify the functional capacities of *ex vivo* PB and BM manipulated immature progenitor cells. *Bone Marrow Transplantation* (2000) 25, 377–383.

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Umbilical cord blood (CB), mobilized peripheral blood (PB) and bone marrow (BM) are currently used as sources of hematopoietic stem cells.^{1,2} However, several phenotypic and functional differences between hematopoietic stem cells from these three tissues have been described during the last few years.^{3–6}

The CD34⁺CD38⁻ phenotype corresponds to a primitive subpopulation of progenitor cells in BM as well as in CB and in mobilized PB, that can be distinguished functionally from the CD34⁺CD38⁺ population by several *in vitro* assays.⁷ Although for the three sources, the CD34⁺CD38⁻ phenotype is consistent in defining the most primitive progenitors,^{8,9} functional differences between CD34⁺CD38⁻ cells from the three tissues have been described. CD34⁺CD38⁻ cord blood cells have a higher cloning efficiency, proliferate more rapidly in response to cytokine stimulation and generate more progenitors than their counterpart in bone marrow.¹⁰

In this study, we characterized CD34⁺ cells purified from the three sources by comparing their cell cycle status and their capacity to form blast colonies in contact with BM stroma. Moreover, we evaluated the expression of receptors to SCF (CD117) and IL-3 (CD123) on the cell surface of each subpopulation at day 0 and 48 h after stimulation by SCF + IL-3. The rationale for utilizing this cytokine combination is its ability to promote *in vitro* expansion of CB CD34⁺ and CD34⁺CD38⁻ cells.¹¹ The aims of our study were to establish relations between the cell cycle kinetic of CD34⁺ subpopulations, their sensitivity to colony-stimulating factors and their expression of SCF and IL-3 receptors.

Materials and methods

Cells

Umbilical cord blood cells were collected after full-term deliveries. Immediately after delivery, the cord was clamped, the umbilical vein was catheterized aseptically and the sample collected by gravity into a 400 ml blood unit pack containing 35 ml citrate-phosphate-dextrose adenine-1 solution (CPD-A) (Baxter Fenwal, La Châtre, France).

Bone marrow cells were obtained by aspiration from the sternum of normal donors. The samples were anticoagu-

lated with 10 IU/ml heparin (Rhône-Poulenc Rorer, Brussels, Belgium).

Peripheral blood cells were obtained after cytopheresis of normal donors mobilized by 10 µg/kg of G-CSF (Neupogen, Amgen, Thousand Oaks, CA, USA).

Informed consent was obtained from normal donors before any sample collection.

CD34 positive cell selection on Midimacs immunomagnetic selection systems (Miltenyi Biotec, Bergisch Gladbach, Germany)

According to the manufacturer's instructions, 50 to 200 × 10⁶ mononuclear cells suspended in buffer containing PBS and 0.5% bovine serum albumin (BSA), were incubated for 15 min at 4°C with a blocking reagent (human IgG) and simultaneously with anti-human CD34 antibody (QBEND10). After washing, the cells were incubated for 15 min at 4°C with superparamagnetic MACS microbeads recognizing QBEND10. The cells were then washed and loaded on to a high gradient magnetic separation column (LS⁺ column) washed with 3 ml buffer. The unlabelled cells were washed five times through the column using 3 ml buffer. After removing the column from the magnetic separation unit, the labelled cells are collected by washing LS⁺ column with 5 ml buffer. Selected cells are washed once in PBS before use.

Suspension culture

CD34⁺ cells are plated in 24 well plates at 10⁴ to 10⁵ cells/ml in serum-free medium (CellGRO, BioWhittaker, Walkersville, MD, USA) with SCF (10 ng/ml) and IL-3 (20 ng/ml). Cells were maintained at 37°C under 5% CO₂ in 100% humidity for 2 days. Viable cell number, phenotype and cell-cycle status analysis were performed at day 0 and after 2 days of culture.

Phenotype analysis

Two-fluorescence analysis: Cell suspensions were incubated for 30 min at room temperature in the dark with fluorescein isothiocyanate (FITC) conjugated anti-CD38 (Immunotech, Marseille, France) associated with phycoerythrin (PE) conjugated anti-CD34 (HPCA-2, Becton Dickinson, San Jose, CA, USA), PE conjugated anti-CD117 (Immunotech) or PE conjugated anti-CD123 (Pharmingen, San Diego, CA, USA). After washing, the cells were analyzed using an EPICS-XL flow cytometer (Coulter, Hialeah, FL, USA). The percentage of positive cells was determined by reference to nonspecific staining with antibodies of the same isotype (IgG1/IgG1, Becton Dickinson). The density of CD117 and CD123 was also expressed as mean equivalent soluble fluorescence (MESF) evaluated by reference to calibration beads (Dako Fluospheres; Dako, Glostrup, Denmark).

Triple-fluorescence analysis: In addition, for several samples, cells were triple-stained using FITC-conjugated anti-CD38 and phycoerythrin-cyanin 5.1 (PE-Cy5) conjugated anti-CD34 (Immunotech) associated with either PE-

conjugated anti-CD117 or PE-conjugated anti-CD123. The percentages and the MESF of CD117 positive cells among CD38⁺ and CD38⁻ subpopulations were evaluated after gating on the CD34⁺ population. Non-specific staining was performed using the Opticlone (IgG1-FITC/IgG1-PE/IgG1-PE-Cy5) from Immunotech.

Double labelling analysis of DNA content and of CD38 expression

10⁵ cells were incubated for 30 min at room temperature with 20 µl FITC conjugated anti-CD38 (Immunotech). After incubation, cells were washed and resuspended in 100 µl of PBS. 20 µl of permeabilizing reagent (Coulter DNA prep reagent) were added and the suspension was mixed vigorously for at least 45 s. After mixing, 380 µl of propidium iodide (PI) solution (Coulter) were added and the cells were incubated for 24 h at 4°C in the dark. The cell cycle status of the cell suspension was analyzed by flow cytometry. Doublets were excluded by appropriate gating in the width vs area dot plot. Data were analyzed using Elite 4.0 Software (Coulter).

Blast-colony forming cell assay (Bl-CFC)

This assay is dependent on the ability of the stem cell population to recognize and attach to a preformed marrow derived stromal layer and to grow in this environment without addition of exogenous growth factors. The feeder layer is obtained by culturing during 4 to 6 weeks bone marrow mononucleated cells in the presence of methylprednisolone and is composed of fibroblasts, macrophages and fat cells.

Stromal layers were prepared by plating 5 × 10⁵ bone marrow mononuclear cells in 1 ml α-MEM (Gibco) containing 20% FCS supplemented with 2 × 10⁻⁵ methylprednisolone (Solumedrol) (Upjohn, Kalamazoo, MI, USA) in 35-mm Petri dishes. The stromal cultures were fed weekly by complete replacement of the culture medium. After 4 to 6 weeks, stromal layers are confluent.

At this time, 1 × 10⁴ cells from the starting CD34⁺ cell suspension and from cells recovered after 48-h culture were incubated for 2 h at 37°C and 5% CO₂ on confluent marrow-derived stromal layers to allow adherence to the stroma. The stromal layers were then washed three times with alpha-MEM to remove all cells which had not adhered. The cultures were then overlaid with 1 ml of 0.3% agar in alpha-MEM supplemented with 20% FCS and were incubated for 7 days at 37°C and 5% CO₂. All colonies closely associated to the stromal layer and containing more than 20 cells were counted.¹²

Statistical analysis

The comparisons between either phenotype or cell cycle status at day 0 and after 48-h culture or between phenotypes of CD38⁻ and CD38⁺ populations, in the same type of sample, are performed using the Student's paired *t*-test. Comparisons between phenotype from different types of sample are performed using the Student's *t*-test.

Table 1 Cell phenotype at day 0 and after 48 h stimulation using SCF + IL-3

	day 0		48 h	
	% CD34 ⁺	% CD34 ⁺ CD38 ⁻	% CD34 ⁺	% CD34 ⁺ CD38 ⁻
CB (n = 9)	85.9 ± 2.9	54.3 ± 3.3	89.5 ± 1.6	61.9 ± 5.3
BM (n = 5)	96.6 ± 0.8	29.7 ± 8.3	93.9 ± 2.7	32.5 ± 3.3
PB (n = 6)	87.9 ± 5.9	43.1 ± 7.9	93.5 ± 3.0	38.6 ± 14.4

Results

CD34CD38 expression of selected cells at day 0 and after 2 day stimulation by SCF + IL-3

As shown in Table 1, the purity of CD34⁺ cells, obtained using the Midimacs system, was greater than 85%. However, to exclude contamination by CD34⁻ cells, the phenotypes of the progenitor cells were studied by dual color fluorescence, and also by triple color fluorescence, analysis after gating on the CD34 positive cell fraction. After 2 days of culture, the number of cells remains constant for the three sources of hematopoietic stem cells evaluated, the expression of CD34 and CD38 antigens is not radically modified.

Cell cycle status of CD34⁺ cells from CB, BM and mobilized PB at day 0 and after 48-h stimulation by SCF + IL-3

In the first set of experiments, the cell cycle status of CD34⁺CD38⁻ and CD34⁺CD38⁺ cells is evaluated by flow cytometry. As shown in Table 2, cycle analysis of the two subpopulations indicates that at day 0, the large majority of the whole CD34⁺ cell population from CB as from BM and mobilized PB were in G0/G1 phases of the cell cycle. Significantly more CD34⁺CD38⁺ cells were in proliferative phases of the cell cycle (S+G2M) as compared with CD34⁺CD38⁻ cells, in CB, BM and PBSC. On the other hand, significantly more BM CD34⁺CD38⁻ cells were in S+G2M as compared with PB CD34⁺CD38⁻ cells (P = 0.028). No significant differences between cell cycle status of CD34⁺CD38⁻ cells are observed between CB and BM, nor between PB and CB.

After 48 h incubation of CD34⁺ cells in the presence of

SCF and IL-3, the percentage of BM CD34⁺CD38⁻ cells in S+G2M phase of the cell cycle does not significantly increase but in contrast, it increases by 7.7- and 10.2-fold for CB and PB, respectively.

Our data suggest that the CD34⁺CD38⁻ cells from BM analyzed at day 0 are more actively cycling than their PB and CB counterpart, however after 48-h incubation with SCF + IL-3 this percentage does not increase.

Clonogenic capacities of cells before and 48 h after stimulation with SCF + IL-3

To support our observations that CB and PB CD34⁺CD38⁻ cells are more proliferative after stimulation using SCF + IL-3 than at day 0, we have plated these cells in a culture system allowing the growth of stroma-dependent progenitors (BI-CFC). These cells are mainly present in the CD34⁺CD38⁻ fraction¹³ and we can thus expect that this assay reflects primarily the clonogenic capacities of the CD34⁺CD38⁻ fraction.

For CB and PBSC, we have obtained respectively 5.2 ± 1 (P < 0.0009) (n = 5) and 6.1 ± 1.2 (P < 0.022) (n = 4) fold more BI-CFC with CD34⁺ cells stimulated during 48 h by SCF + IL-3 than using fresh CD34⁺ selected cells. For BM, the number of BI-CFC was not significantly different before and after incubation with CSFs (n = 5). These results confirm the increased proliferative capacities of immature PB and CB CD34⁺CD38⁻ cells after incubation with SCF + IL-3 while BM immature progenitors maintain similar capacities.

CD117 expression on CD34⁺ subpopulations from CB, BM and mobilized PB at day 0 and after 48-h culture

The fact that CB and mobilized PB CD34⁺CD38⁻ cells seem to be more responsive to SCF and IL-3 than their BM counterpart, suggesting different sensitivity of progenitors to these growth factors, may result from different receptor expression on the cell surface. Therefore, we examined the expression of SCF and IL-3 receptors on CD34⁺CD38⁺ and CD34⁺CD38⁻ cells purified from CB, BM and PB. This expression is evaluated in terms of receptor density (MESF) (Figure 1) and of percentage of positive cells (Table 3). Due to the relatively high purity of CD34⁺ cells obtained using the Midimacs separation system, the results observed after two or triple fluorescence analysis were not different and were thus pooled.

Table 2 Cell cycle analysis of CD34⁺ cells and of the two subpopulations CD34⁺CD38⁻ and CD34⁺CD38⁺ at day 0 and after 48 h stimulation by SCF + IL-3

	CD34 ⁺			CD34 ⁺ CD38 ⁺			CD34 ⁺ CD38 ⁻		
	day 0	48 h	P	day 0	48 h	P	day 0	48 h	P
CB (n = 8)	5.9 ± 1.4	24.7 ± 3.5	0.003	22.7 ± 8.9	46.8 ± 5.5	NS	2.0 ± 0.4	19.2 ± 2.2	0.0002
BM (n = 8)	11.8 ± 1.4	23.4 ± 3.0	0.007	20.5 ± 2.2	35.9 ± 3.0	0.007	5.6 ± 1.2	8.6 ± 1.0	NS
PB (n = 6)	2.0 ± 0.6	28.7 ± 5.8	0.005	17.8 ± 7.9	50.2 ± 9.9	NS	0.9 ± 0.3	14.1 ± 5.6	0.05

Percentage of CD34⁺, CD34⁺CD38⁺ and CD34⁺CD38⁻ cells in S + G2M phases of the cell cycle, expressed as mean ± s.e.m. P represents significant difference between percentages observed at day 0 and after 48 h culture. It was calculated using the Student's paired t-test.

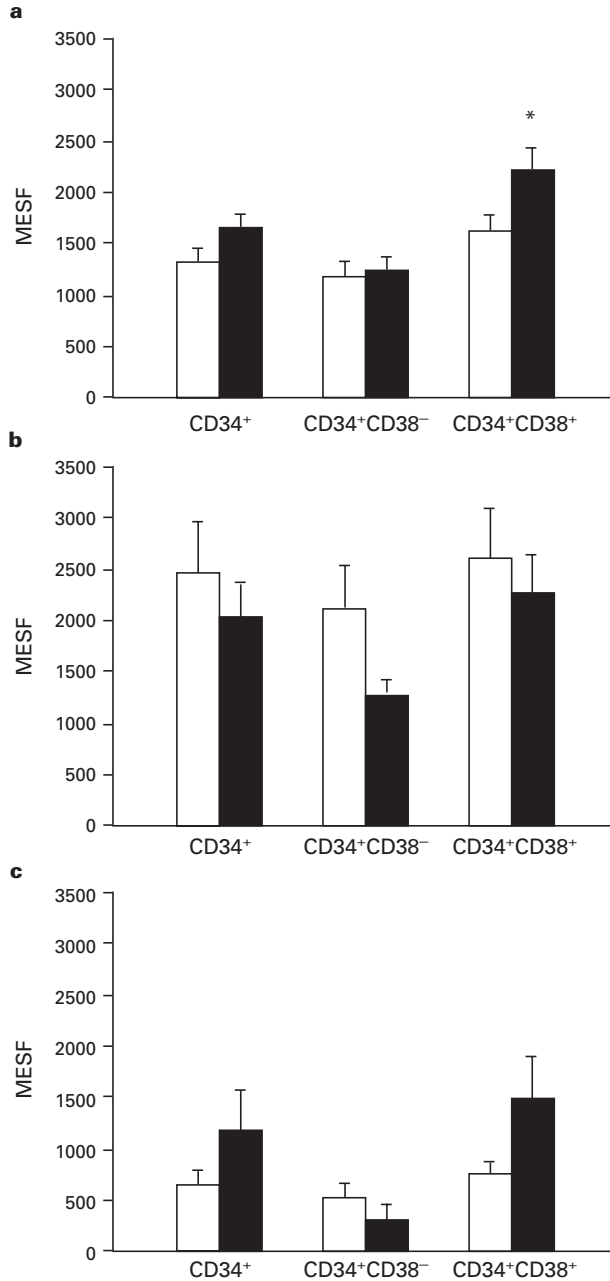


Figure 1 CD117 expression on (a) CB ($n = 9$), (b) BM ($n = 5$) and (c) PB ($n = 6$) CD34⁺ subpopulations at day 0, □; and after 48 h culture with SCF + IL-3, ■. Results are expressed as mean \pm s.e.m. of mean equivalent soluble fluorescence (MESF). *Represents significant difference between CD117 expression at day 0 and after 48 h culture.

When we compared the density of receptors on the cell surface (Figure 1), CD117 expression is significantly lower on CD34⁺CD38⁻ cells than on CD34⁺CD38⁺ cells, in CB ($P < 0.0004$) as well as in PB ($P < 0.011$) and in BM ($P < 0.02$). This difference between the two subsets remains significant after 48-h culture (respectively $P < 0.0001$, $P < 0.05$ and $P < 0.01$ for CB, PB and BM). On the other hand, it is clear that at day 0, on the immature CD34⁺CD38⁻ population, CD117 expression is significantly lower on mobilized PB cells than on their CB ($P < 0.001$) and BM ($P < 0.001$) counterparts. When the

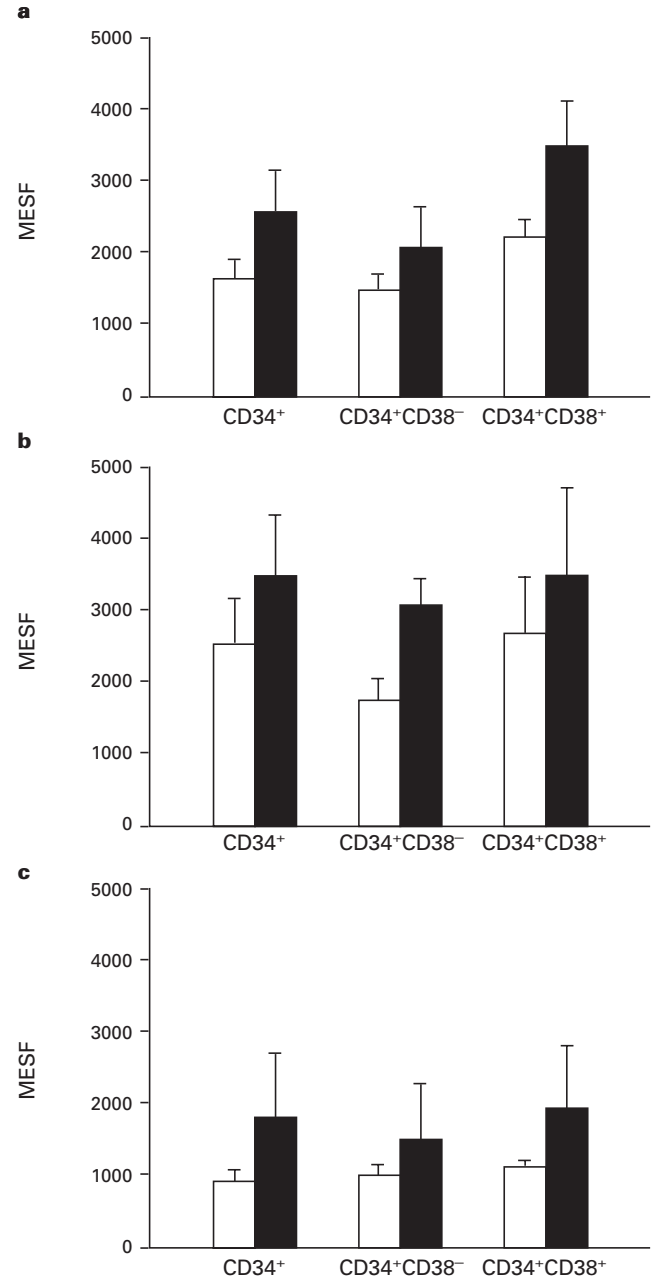


Figure 2 CD123 expression on (a) CB ($n = 9$), (b) BM ($n = 5$) and (c) PB ($n = 6$) CD34⁺ subpopulations at day 0, □; and after 48 h culture with SCF + IL-3, ■. Results are expressed as mean \pm s.e.m. of mean equivalent soluble fluorescence (MESF).

CD34⁺ cells are incubated for 48 h with SCF + IL-3, the CD117 expression on total CD34⁺ cells increases in CB and PB while it decreases in BM. This effect is probably due to the increase of CD117 expression on CD34⁺CD38⁺ cells from CB and PB and the decrease on BM cells. Surprisingly, CD117 expression decreases on PB and BM CD34⁺CD38⁻ cells while it remains stable in CB. The regulation of CD117 expression after short-term incubation with SCF + IL-3 seems very heterogeneous according to the type of samples and the subpopulation evaluated. Nevertheless, it could be noted that, after 48 h culture, CD117 expression

Table 3 CD117 expression on CD34⁺, CD34⁺CD38⁺ and CD34⁺CD38⁻ cells at day 0 and after 48 h culture with SCF + IL-3

	Day 0				48 h							
	CD34 ⁺	CD34 ⁺ CD38 ⁺	CD34 ⁺ CD38 ⁻	P ^a	CD34 ⁺	P ^b	CD34 ⁺ CD38 ⁺	P ^b	CD34 ⁺ CD38 ⁻	P ^b	P ^a	
CB (n = 9)	57.5 ± 7.1	64.5 ± 6.0	46.9 ± 7.8	0.0008	52.7 ± 2.6	NS	64.2 ± 5.8	NS	31.4 ± 7.6	NS	0.0008	
BM (n = 5)	67.2 ± 2.9	69.3 ± 2.3	45.8 ± 9.0	NS	48.2 ± 2.8	0.02	56.8 ± 4.6	NS	25.7 ± 6.2	0.04	0.019	
PB (n = 6)	43.1 ± 7.9	48.0 ± 8.5	20.3 ± 6.4	0.001	38.4 ± 8.1	NS	47.3 ± 8.9	NS	14.1 ± 6.3	NS	0.002	

Results are expressed as the mean percentage ± s.e.m. of CD117 positive cells in each subpopulation.

^aP represents significant difference between CD117 expression on CD34⁺CD38⁺ and CD34⁺CD38⁻ cells and are calculated using the Student's paired *t*-test.

^bP represents significant difference between CD117 expression at day 0 and after 48 h culture in the three subpopulations and are calculated using the Student's paired *t*-test.

on PB progenitor cells is significantly lower than on their CB and BM counterparts (respectively $P < 0.006$, < 0.0002 , < 0.004 and $P < 0.01$, < 0.01 , < 0.01 for the CD34⁺, CD34⁺CD38⁺ and CD34⁺CD38⁻ cells). When we compared the percentage of CD117 positive cells, the same differences between PB vs CB and BM progenitor cells, although less spectacular, are also observed. At day 0 as after 48 h, we did not observe a significant difference between CD117 expression on CB and BM cells.

CD123 expression on CD34⁺ subpopulations from CB, BM and mobilized PB at day 0 and after 48 h culture (Figure 2) (Table 4)

CD123 expression is not significantly different in the three sources of hematopoietic cells. After 48 h of culture the density of IL-3 receptors increases in all subpopulations while the percentages of positive cells remain constant. We did not observe significant differences between the three tissues.

Discussion

Mobilized peripheral blood (PB) and umbilical cord blood cells (CB) are two alternative sources of allogeneic stem cells to bone marrow (BM) for transplantation.¹ There are increasing reports demonstrating differences in phenotype and functional properties between hematopoietic progenitors from PB, CB and BM.^{5,10,14-16} The most striking difference between BM, PB and CB is in the hematopoietic

recovery following transplantation which is faster after PB and delayed after CB transplantation when compared to BM.^{1,2} The difference of engraftment kinetics between the three sources of progenitor cells are not yet fully understood. The impact of cell dose is probably important but remains controversial.¹⁷ On the other hand, it seems reasonable to hypothesize that qualitative difference between CD34⁺ populations of the three sources could also play a role in engraftment kinetics. Comparative studies of the CD34⁺ subpopulations in the three sources of stem cells may contribute to understanding the mechanisms involved in hematologic recovery after transplantation.

In this study, we have first identified the cell cycle status of CD34⁺CD38⁻ and CD34⁺CD38⁺ subpopulations in steady state BM, PB and CB samples. In accordance with previous studies,¹⁸⁻²² it appears that the great majority of CD34⁺ cells from CB and particularly PB, are in G0/G1 phases of the cell cycle whereas more than 10% of the BM CD34⁺ cells are in S + G2M phases. We have also observed this difference between on the one hand, CB and PB and on the other hand, BM, for the immature CD34⁺CD38⁻ fraction (<2% in S+G2M for PB and CB and >5% for BM). Short-term incubation of these cells with SCF + IL-3 allows a significant increase in the percentage of cycling CD34⁺CD38⁻ cells in CB and PB while the proliferative potential of BM CD34⁺CD38⁻ cells is not significantly modified. It seems thus possible to trigger during a short-term culture, the cycling status of PB and CB progenitor cells without radically modifying their number or their phenotype. Taking into account this particular aspect, it appears that the CB CD34⁺ progenitors seem to have more

Table 4 CD123 expression on CD34⁺, CD34⁺CD38⁺ and CD34⁺CD38⁻ cells at day 0 and after 48 h culture with SCF + IL-3

	Day 0				48 h							
	CD34 ⁺	CD34 ⁺ CD38 ⁺	CD34 ⁺ CD38 ⁻	P ^a	CD34 ⁺	P ^b	CD34 ⁺ CD38 ⁺	P ^b	CD34 ⁺ CD38 ⁻	P ^b	P ^a	
CB (n = 9)	60.5 ± 5.5	74.8 ± 4.2	60.5 ± 5.5	0.0004	57.1 ± 7.8	NS	67.9 ± 8.5	NS	40.5 ± 9.4	NS	0.004	
BM (n = 5)	65.9 ± 3.4	67.4 ± 4.1	45.8 ± 8.3	0.02	52.1 ± 3.2	NS	55.6 ± 5.8	NS	36.1 ± 7.3	NS	NS	
PB (n = 6)	68.1 ± 7.5	68.0 ± 8.5	49.3 ± 12.1	NS	64.8 ± 6.1	NS	69.1 ± 6.3	NS	48.0 ± 6.7	NS	0.0009	

Results are expressed as the mean percentage ± s.e.m. of CD123 positive cells in each subpopulation.

^aP represents significant difference between CD123 expression on CD34⁺CD38⁺ and CD34⁺CD38⁻ cells and are calculated using the Student's paired *t*-test.

^bP represents significant difference between CD123 expression at day 0 and after 48 h culture in the three subpopulations and are calculated using the Student's paired *t*-test.

similarities with PB than with BM CD34⁺ cells. These surprising results cannot explain the different behavior between CB and PB cells after transplant. Therefore, cell cycle differences are unlikely to explain the marked difference in post-transplant recovery between PB and CB.

Our results, demonstrating that PB CD34⁺ cells and particularly CD34⁺CD38⁻ cells respond rapidly to SCF + IL-3, seem to confirm the observation of Lemoli *et al*²³ which have shown that the majority of mobilized peripheral blood CD34⁺ cells, including LTC-IC, are not quiescent (G0 phase), but are in G1 phase of the cell cycle and are thus ready to progress into S-phase under CSF stimulation. The ability of CB and PB immature progenitors to exit G0/G1 phases more rapidly than their BM counterpart could derive from a difference in receptor expression for the two cytokines used (SCF and IL-3). We have thus investigated the expression of SCF and IL-3 receptors on the different CD34⁺ subpopulations and the short term *in vitro* modulation of this expression by cytokines.

It is well documented that the SCF/c-kit interaction plays a crucial role in the early stage of hematopoiesis. However, conflicting reports have described c-kit expression²⁴⁻²⁶ and little is known concerning the modulation of this expression on the immature and more committed progenitor cells. Therefore, it will be important to elucidate c-kit expression on different subpopulations of progenitors. Our results, in accord with those from several others groups,^{7,27,28} show that the c-kit expression on PB CD34⁺ cells is significantly lower than that of BM and CB CD34⁺ cells which express a high level of c-kit receptors. This difference, also observed in CD34⁺CD38⁺ cells, is particularly marked on immature CD34⁺CD38⁻ cells. Moreover, in PB, when we stimulated the CD34⁺ cells by SCF + IL-3, CD117 expression decreases on CD34⁺CD38⁻ cells while it increases on CD34⁺CD38⁺ cells. After 48 h culture, CD117 expression on PB CD34⁺CD38⁻ cells is thus very low. These results seem to be in contradiction with the apparent greater sensitivity of immature PB progenitors to SCF. In cord blood, CD117 expression remains constant in the CD34⁺CD38⁻ subpopulation and increases in CD34⁺CD38⁺ cells. This is contrary to BM progenitors in which CD117 expression decreases on all subpopulations studied. Our results indicate that CD117 expression although different between the three tissues, seems not to correlate with the difference in sensitivity of progenitor cells to SCF nor with the proliferative capacity of progenitors induced by SCF stimulation.

The low expression of c-kit on PB cells that we have observed in this study was previously related to the mobilization of CD34⁺ cells into the circulation after G-CSF administration.^{29,30} This observation gives a new importance to c-kit expression in mobilization and in adhesion of hematopoietic stem cells to bone marrow microenvironment. In fact, it is well known that c-kit is not only a cytokine receptor but also an adhesion molecule.^{31,32} The SCF/c-kit complex was previously shown to play an important role in the homing of hematopoietic stem cells to the bone marrow microenvironment.³³ As we postulated that the hematopoietic recovery after transplantation is dependent *in vivo* to homing of cells to bone marrow stromal cells and to proliferation of hematopoietic stem cells

in this microenvironment, we can suppose that *in vitro* down-regulation of the CD117 expression, as observed for the PB immature progenitors cells, may impair engraftment. This hypothesis is confirmed by data from Gothot *et al*³⁴ who showed that 36 h stimulation by cytokines activates cell cycle and decreased the ability of human PB cells to repopulate SCID/NOD mice and from Szilvassy *et al*³⁵ who recently observed the same decline in the homing capacity of murine progenitors after *ex vivo* culture.

In the same way, several authors have described that transplantations with *ex vivo* expanded CD34⁺ cells do not permit reconstitution of stable long-term hematopoiesis,^{36,37} indicating loss of repopulating capacities of immature progenitor cells during *ex vivo* culture. These observations, although controversial,³⁸ suggest that functional modifications could occur during the *ex vivo* manipulation and have an impact on the engraftment capacities of stem cells.

In conclusion, these experiments provide evidence that short term *ex vivo* culture can modify stem cells at different levels (their cell cycle status and the expression of adhesion molecules) and can influence the engraftment capacity of these cells. Moreover these modifications are very heterogeneous depending on the source of stem cells and the subpopulations studied. On the other hand, the *in vitro* proliferative, as well as adhesive, capacities of progenitor cells, although very important, do not seem to be the only limiting factors for the *in vivo* cell engraftment. Further studies will be needed to elucidate the relationship between cell behavior, cell cycle changes, expression of adhesion molecules, cytokine receptor expression and the capacity of cells to adhere to the bone marrow microenvironment.

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