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Characterization of CD34+, CD13+, CD33− cells, a rare subset of immature human hematopoietic cells

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Background and Objectives. Hematopoietic progenitor cells that express CD34 are heterogeneous in their lineage affiliation and degree of maturation. Expression of CD13 and CD33 antigens indicates myeloid lineage association, but the precise sequence of expression of these two markers during differentiation is unclear. We noted the presence of CD34+ cells expressing CD13 but lacking CD33, a subset of cells not yet well characterized. In this report we describe the prevalence and the immunophenotype of this cell subset.

Design and Methods. We studied the immunophenotype of immature myeloid cells in human bone marrow samples from 11 healthy transplantation donors and in 4 cord blood samples. We used four-color flow cytometry and a large panel of monoclonal antibodies directed against lineage and differentiation-associated antigens. Three additional bone marrow samples were analyzed after immunomagnetic sorting of CD34+ cells. We focused our analysis on the subset of cells defined by the expression of CD34 and CD13 and the lack of CD33.

Results. We found CD34+, CD13+, CD33− cells in all 11 bone marrow and 4 cord blood samples studied. These cells represented 0.5±0.5% (mean ± SD) and 0.8±1.2% of mononucleated cells, respectively. CD34+, CD13+, CD33− cells appeared to be more immature than those expressing CD33 because of their light scatter characteristics (smaller size and lower granularity), the expression of markers associated with early hematopoietic cells (CD90, CD133 and CD117), and the absence of lineage-associated markers.

Interpretation and Conclusions. These findings suggest that the expression of CD13 precedes that of CD33 during myeloid differentiation, and that CD34+, CD13+, CD33− cells are at an early stage of human myeloid cell differentiation.

Key words: CD34+ cells, flow cytometry, hematopoiesis, minimal residual disease, leukemic myeloblasts.

Expression of CD34 on the cell surface denotes hematopoietic cells that can form colonies of different hematopoietic lineages *in vitro*[1-8, 22–26] and reconstitute hematopoiesis *in vivo*.[9-12] In bone marrow, CD34 is expressed by a heterogeneous pool of hematopoietic progenitors of various lineages at different stages of differentiation.[13-21] CD34+ cells that are committed to differentiate into myeloid cells are commonly recognized by the surface expression of CD13 and CD33. However, the precise sequence of expression of these markers during hematopoietic differentiation is unclear. Moreover, the relative proportion of CD34+ cells expressing either marker is not well established. While some investigators found a predominance of CD13 expressing cells compared to those expressing CD33, others found comparable percentages of CD34+ cells expressing the two markers.[29-36]

In studies of normal bone marrow and cord blood we noted the presence of a small but distinct population of CD34+ cells expressing CD13 but lacking CD33. We, therefore, applied a large panel of monoclonal antibodies using three and four-color flow cytometry to describe the prevalence and the immunophenotype of this rare cell subset.

Design and Methods

Cells

Bone marrow samples were taken from 14 healthy transplantation donors, aged 1 to 51 years (median 22 years). Cord blood samples were taken from 4 healthy full term neonates immediately after vaginal delivery. The institutional review board approved this study and informed consent was obtained from patients and their guardians. Mononucleated cells (<1.077 g/mL) were obtained

after centrifugation on a density gradient using Ficoll-Paque (Pharmacia LKB, Uppsala, Sweden) and washed three times in phosphate-buffered saline (PBS) and resuspended at 0.5-1.0×10^6/mL in PBS containing 0.5% bovine serum albumin.

Immunomagnetic cell sorting

CD34$^+$ cells from 3 bone marrow samples were isolated by incubating 10^8 cells in 300 μL of PBS with 100 μL of Fc receptor blocking reagent and 100 μL of CD34 (QBEND10)-conjugated magnetic beads (CD34 direct isolation kit; Miltenyi Biotec, Bergisch, Gladbach, Germany) followed by incubation for 30 min at 4-8°C. After washing with PBS, cells were processed through a MS$^+$ separation column (Miltenyi Biotec) placed in a magnetic field, and labeled cells were retained. For higher purity, a second run with a new column was applied to the recovered CD34$^+$ fraction. Viable isolated CD34$^+$ cells were enumerated by trypan blue dye exclusion and purity was checked by flow cytometry after double staining with anti-CD34 PE and CD45 PerCP (Becton Dickinson, San Jose, CA, USA).

Immunostaining and flow cytometric analysis

One hundred microliters of cell suspension were incubated with antibodies conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinine chlorophyll protein (PerCP) and allophycocyanine (APC) for 10 min at 20°C. After two washes, cells were resuspended in 0.5 mL of 0.5% paraformaldehyde and analyzed with a FACScalibur flow cytometer equipped with two lasers (488 and ~635 nm) using CellQuest™ and PAINT-a-GATE plus™ software programs (Becton Dickinson). Four-color antibody combinations (Table 1) included anti-CD13 FITC (Dako, Glostrup, Denmark) or PE (Becton Dickinson), CD34 PerCP (Becton Dickinson), CD33 APC (Becton Dickinson), and one of the following FITC or PE monoclonal antibodies: anti-MPO, CD2, CD79a (Dako); anti-HLA-DR, CD3, CD7, CD19, CD11b, CD14, CD15, CD38, CD45, CD56 (Becton Dickinson), anti-CD133 (Miltenyi Biotec); anti-CD65 (Caltag Laboratories, San Francisco, CA, USA); anti-CD41a, CD117, anti CD90 (Coulter/Immunotech, Miami, FL, USA). Three-color antibody combinations (Table 1) included anti-CD13 FITC (Dako), CD34 PerCP (Becton Dickinson) and one of the following anti-CD33 PE antibodies: clone p-67-6 (IgG1, Becton Dickinson), and clone WM53 (IgG1, ImmunoQuality, Groningen, The Netherlands). For intracellular staining, surface labeled cells were treated with Fix and Perm™ (F/P, An der Grub, Vienna, Austria) according to the manufacturer's instructions. Isotype-matched non-reactive antibodies conjugated to FITC, PE, PerCP and APC (Becton Dickinson) were used as negative controls. All the antibodies used in this study were titrated, and used at optimal concentration to avoid non-specific staining.

Table 1. Monoclonal antibody combinations used to analyze the immunophenotype of CD34$^+$/CD13$^+$/CD33$^+$ and CD33$^-$ cell subsets.

IgG FITC/CD13 PE/CD34 PerCP/CD33 APC
CD2 FITC/CD13 PE/CD34 PerCP/CD33 APC
CD7 FITC/CD13 PE/CD34 PerCP/CD33 APC
CD19 FITC/CD13 PE/CD34 PerCP/CD33 APC
CD65 FITC/CD13 PE/CD34 PerCP/CD33 APC
CD15 FITC/CD13 PE/CD34 PerCP/CD33 APC
CD41 FITC/CD13 PE/CD34 PerCP/CD33 APC
CD45 FITC/CD13 PE/CD34 PerCP/CD33 APC
HLA-DR FITC/CD13 PE/CD34 PerCP/CD33 APC
cyMPO FITC/CD13 PE/CD34 PerCP/CD33 APC
CD90 FITC/CD13 PE/CD34 PerCP/CD33 APC
CD14 FITC/CD13 PE/CD34 PerCP/CD33 APC
CD13 FITC/IgG PE/CD34 PerCP/CD33 APC
CD13 FITC/CD11b PE/CD34 PerCP/CD33 APC
CD13 FITC/CD117 PE/CD34 PerCP/CD33 APC
CD13 FITC/CD56 PE/CD34 PerCP/CD33 APC
CD13 FITC/Glycophorin A PE/CD34 PerCP/CD33 APC
CD13 FITC/CD38 PE/CD34 PerCP/CD33 APC
CD13 FITC/CD133 PE/CD34 PerCP/CD33 APC
CD13 FITC/cyCD79a PE/CD34 PerCP/CD33 APC
CD13 FITC/cyCD3 PE/CD34 PerCP/CD33 APC
IgG FITC/IgG PE/IgG PerCP/IgG APC
CD13 FITC/CD33 PE/CD34 PerCP

To define the immunophenotype of CD34$^+$, CD13$^+$, CD33$^-$ cells we used the following protocol: first, 10,000 non-gated events were acquired. These were then gated as follows: gate R1 surrounded the light-scatter dot plot of mononucleated cells, excluding debris and mature granulocytes; gate R2 was drawn on the fluorescence dot plot to include all cells expressing either CD13 or CD33. Electronic events falling within both gates 1 and 2 were selectively recorded. At the time of the analysis, we drew a gate (R3) surrounding CD34$^+$ cells with low/intermediate side scatter (SSC); R3-gated cells expressing both CD13 and CD33, or expressing CD13 but lacking CD33 were enclosed by gates R4 and R5, respectively. The expression of leukocyte markers among these two selected populations was then analyzed (Figure 1). To calculate the prevalence of various cell subsets among the total fraction of CD34$^+$ cells, one additional cell aliquot, labeled with IgG1 FITC, CD13 PE, CD34 PerCP and CD33 APC, was acquired with a live gate consisting of R1 (viable mononucleated cells) and a second gate around all cells with CD34 expression and low to intermediate SSC characteristics.

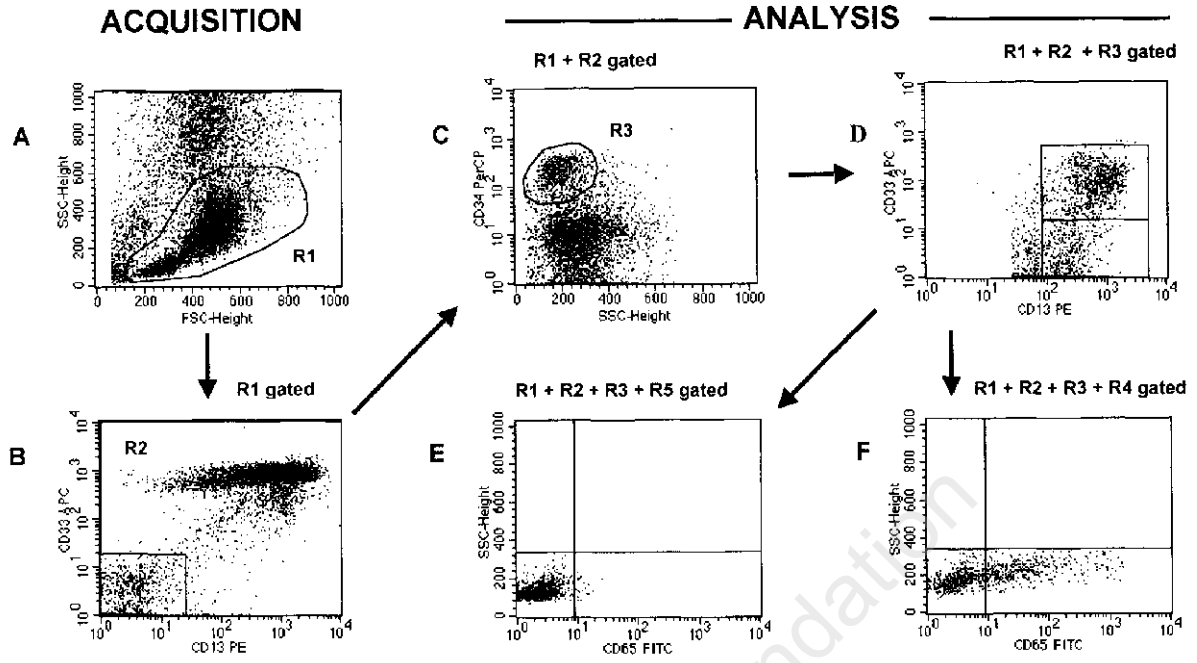


Figure 1. Four-color flow cytometric anlysis of CD34+, CD13+, CD33− and CD34+, CD13+, CD33+ cells in normal bone marrow. After analyzing 10,000 total cells, two gates were drawn: one included events with light scatter characteristics of mononucleated cells (R1, panel A) and the other included cells expressing CD13 and CD33 (R2, panel B). Events falling within both gates 1 and 2 were selectively recorded. The immunophenotype of cells expressing CD13 and CD33 was then analyzed; gate R3 (panel C) identified CD34+ cells with low to intermediate SSC characteristics; of these, gate R4 surrounded cells expressing both CD13 and CD33, while gate R5 defined cells expressing CD13 but lacking CD33 (panel D). Expression of a fourth antigen (e.g. CD65) was then measured in CD13+, CD33+ and CD13+, CD33− cells (panels E and F, respectively).

Results

Prevalence and light scatter characteristics of CD34+, CD13+, CD33− cells in normal bone marrow and cord blood

During the immunophenotypic characterization of normal myeloid cell differentiation, we identified a subset of cells that expressed CD34 and CD13 but lacked CD33. In bone marrow (n = 11), cells with this phenotype represented 0.5±0.5% (mean ± SD) of mononucleated cells and 22.0±10.3% of CD34+ cells. Interindividual variations in the distribution of this subset did not appear to be age-related (data not shown). In cord blood (n = 4), cells with the CD34+, CD13+, CD33− phenotype formed 0.8±1.2% of mononucleated cells and 11.8±11.5% of CD34+ cells (Table 2). CD13+, CD33− cells had light scattering properties that were reminiscent of those of early myeloid cells or small monocytes. When compared to the CD33+ counterpart, this population contained cells of smaller size and lower granularity as demonstrated by forward (FSC) light scatter and SSC measurements (Figure 2). The intensity of CD34 expression was indistinguishable from that measured in CD33+ cells: mean fluorescence intensity (MFI) was 194.7 in CD13+, CD33- cells versus 192.7 in CD13+, CD33+ cells. Expression of CD13 was lower in these cells than in their CD33+ counterpart (MFI: 122.1 versus 460.9).

Immunophenotype of CD34+, CD13+, CD33− cells

To characterize the immunophenotype of CD34+, CD13+, CD33− cells in normal bone marrow and cord blood, we used four-color flow cytometry and a variety of lineage and maturation-associated markers in combination with CD34, CD13 and CD33. The median number of CD34+ cells analyzed (gate R3 in Figure 1) was 1,500 (range 770–5,728). Both CD34+, CD13+, CD33− and CD33+ bone marrow subpopulations expressed either low levels or an absence of surface antigens associated with T (CD7 and CD2) or B (CD19) cell lineages (Table 3).

Table 2. Prevalence of CD34⁺/CD13⁺/CD33⁺ and CD34⁺/CD13⁺/CD33⁻ cell subsets in normal bone marrow and cord blood.

	CD13⁺/CD33⁺ subset mean±SD % (range)		*CD13⁺/CD33⁻ subset mean±SD % (range)*	
	% of mono-nucleated cells	*% of CD34⁺ cells*	*% of mono-nucleated cells*	*% of CD34⁺ cells*
Bone marrow (n = 11)	1.8±1.2 (0.5-4.0)	48.8±14.4 (23.0-67.1)	0.5±0.5 (0.1-1.2)	22.0±10.3 (2.0-44.4)
Cord blood (n = 4)	1.0±0.5 (0.5-1.7)	67.2±22.2 (38.0-91.1)	0.8±1.2 (0.1-2.5)	11.8±11.5 (1.8-27.0)

Abbreviations: n, number of donors tested.

Myeloid-associated antigens such as CD65, CD15 and CD14 were not expressed in CD34⁺, CD13⁺, CD33⁻ cells, while CD65 and CD15 were clearly detectable (mean 22.3±11.9% and 28.7±15.8%, respectively) among CD34⁺, CD13⁺, CD33⁺ cells. Expression of myeloperoxidase (MPO) was markedly higher in the CD13⁺, CD33⁺ population than in the CD33⁻ cells (46.2±13.3% versus 7.5±6%) suggesting a more advanced myeloid commitment within the former cell subset. Expression of CD11b was low in both CD33⁻ and CD33⁺ cell populations (14.4±16.6% and 6.3±3.9%, respectively). Natural killer (CD56), megakaryocytic (CD41a) and erythroid (glycophorin-A) antigens were not expressed in either CD34⁺ subsets. Maturation-associated antigens such as CD45, HLA-DR, CD38, CD133 and CD117 were expressed in the great majority of CD34⁺, CD13⁺, CD33⁻ and CD33⁺ cells (Table 3). A lower expression of HLA-DR was observed in the CD13⁺, CD33⁻ subset compared to that in the CD13⁺, CD33⁺ one (MFI 151±70 versus 398±180, respectively). No significant differences were observed in the levels of expression of CD45, CD38, CD133 and CD117 (MFI 84.5±93 versus 85±53.5; 50.3±13.4 versus 53.2±24; 67 versus 68 and 827±424 versus 399±181, respectively).

We also studied 4 cord blood samples and found that the immunophenotype of CD34⁺, CD13⁺, CD33⁻ cells was similar to that of their marrow equivalent. However, no CD34⁺, CD13⁺, CD33⁻ cells expressing lineage-associated antigens were found in cord blood (Table 3).

To ensure that our observations were not peculiar to the anti-CD33 antibody clone used (p-67-6), the fluorochrome conjugated to it (APC), or the gating strategy applied, we analyzed the immuno-

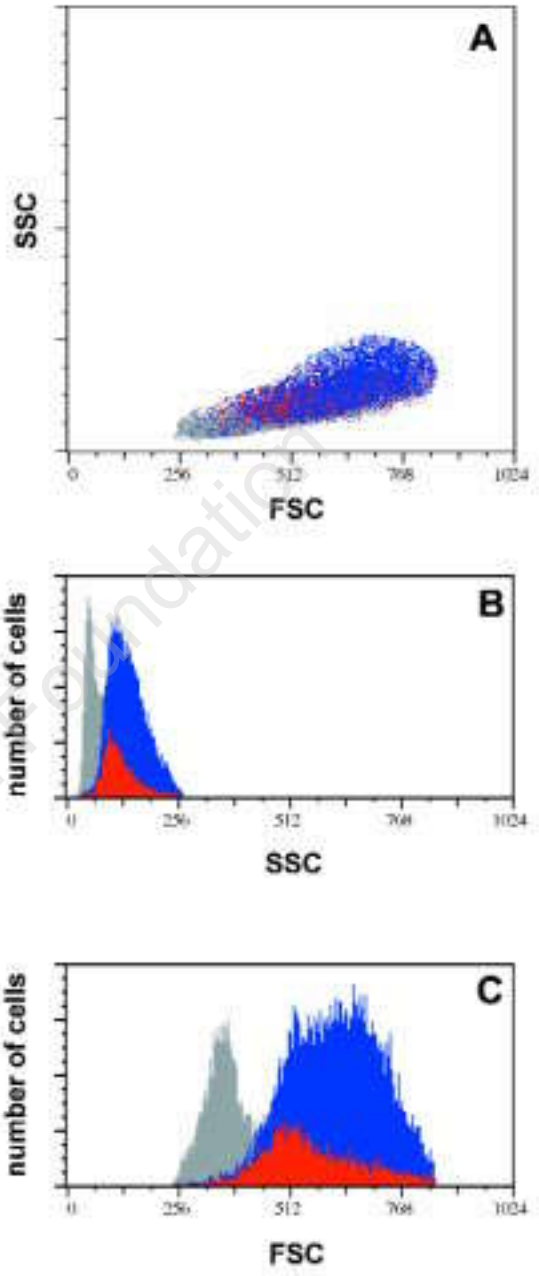
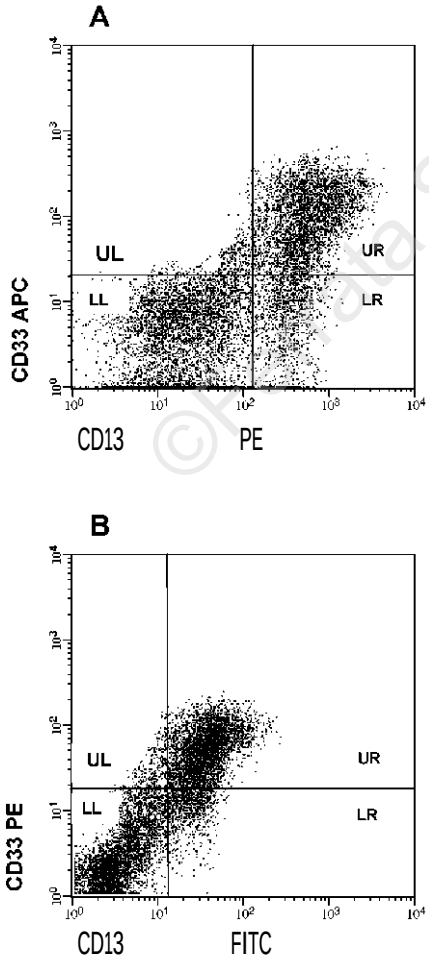


Figure 2. Light scatter characteristics of CD34⁺ cell subsets defined according to CD13 and CD33 reactivity. Four-color immunofluorescence staining was performed with IgG1 FITC, CD13PE, CD34 PerCP, CD33 APC. Dot plots depict forward light scatter (FSC = cell size; x axis) and side light scatter (SSC = granularity; y axis). CD34⁺, CD13⁺, CD33⁻; CD34⁺, CD13⁺ CD33⁺; CD34⁺, CD13-, CD33⁻ cells are depicted in red, blue and grey, respectively (panel A). Histograms show SSC (panel B) and FSC (panel C) characteristics. Red, blue and grey areas represent fluorescence distribution of CD34⁺, CD13⁺, CD33⁻; CD34⁺, CD13⁺, CD33⁺; CD34⁺, CD13⁻, CD33⁻ cells, respectively.

Table 3. Phenotype of CD34+/CD13+/CD33− and CD34+/CD13+/CD33+ cell subsets in normal bone marrow and cord blood.

	Bone marrow			Cord blood		
Antigen	*n*	*CD13+/CD33+ cells mean % ± SD (range)*	*CD13+/CD33- cells mean % ± SD (range)*	*n*	*CD13+/CD33+ cells mean %±SD (range)*	*CD13+/CD33− cells mean %±SD (range)*
CD2	4	neg	neg	nd	nd	
CD7	6	<10.0	<10.0	3	neg	neg
CD3[a]	3[b]	<10.0	<10.0	nd	nd	
CD19	7	neg	<10.0	3	neg	neg
CD79a[a]	3[b]	<10.0	<10.0	nd	nd	
CD65	6	22.3± 11.9 (11.9-45.3)	neg	3	28.3±2.8(25.0-30.0)	<10.0
CD11b	6	<10.0	14.4±16.6 (0.0-47.8)	4	neg	neg
CD15	4	28.7±15.8 (20.0-52.5)	neg	3	neg	neg
CD117	6	91.6±4.9 (82.0-94.9)	94.7±3.0 (91.4-98.6)	4	90.9±9.4 (80.0-96.2)	82.4±14.8 (61.0-99.6)
CD14	3	neg	neg	4	<10.0	neg
MPO[a]	8[b]	46.2±13.3 (33.0-73.6)	<10.0	3	10.6±1.7 (8.7-12.0)	neg
CD56	7	neg	neg	nd	nd	
CD41a	4	<10.0	<10.0	3	neg	neg
Glycophorin A	4	neg	neg	nd	nd	
CD90	3[b]	3.1±1.5 (1.8-5.2)	13.3±6.3 (8.2-22.2)			
CD38	4	98.6±0.7 (97.6-99.4)	96.7±3.0 (92.4- 98.7)	4	98.8±0.9 (97.5-99.7)	98.4±1.8 (96.0-100)
CD45	5	98.7±0.9 (97.6-99.7)	99.4±1.0 (97.9-100)	3	99.5±0.5 (99.0-100)	99.6±0.6 (99.0-100)
CD133	3	79.3±1.3 (78.0-80.7)	60.5±2.6 (57.9-64.2)	3	93.0±1.7 (91.3-94.8)	96.3±1.5 (95.1-98.0)
HLA-DR	4	95.8± 1.7 (93.2-96.9)	96.8± 3.1 (92.5-100)	3	94.0±2.0 (92.0-96.0)	98.5±0.5 (98.0-99.0)

Abbreviations: n, number of donor tested; neg, negative (expression of the antigen was considered negative when less than 10 positive events were detected by flow cytometry); nd, not determined. [a]means cytoplasmic antigen expression; [b]means that in three patients, of those tested, the antigen expression was determined after immunomagnetic purification of CD34+ cells.



phenotype of CD34+ cells purified (≥95% pure) from three bone marrow samples by immunomagnetic sorting and by labeling them with two triple antibody combinations: 1) anti-CD13 FITC, CD34 PerCP, and either CD33 PE p-67-6 or WM53 clones; 2) anti-CD13 PE, CD34 PerCP and CD33 APC (Figure 3). We detected the CD13+, CD33− cell subset in all three cases, although the proportion of these cells among CD34+ cells was higher in samples labeled with the APC-conjugated p-67-6 clone (mean 14.3±6.2%) than in those labeled with the PE-conjugated p-67-6 antibody or with the WM53 antibody (mean 8.2±3.3% and 5.7±2.2%, respectively).

Figure 3. Flow cytometric analysis of normal bone marrow CD34+ cell subsets defined according to their CD13 (x axis) and CD33 (y axis) expression. CD34+ cell selection was carried out as described in the Methods. Four-color immunofluorescence staining was performed with IgG1 FITC, CD13PE, CD34 PerCP, CD33 APC (clone p-67-6, showed in panel A) and with CD13 FITC, CD33 PE (clone WM53), CD34 PerCP (panel B). Dot plots, subdivided into four quadrants (Low-Left, LL; Low-Right, LR; Upper-Right, UR; Upper-Left, UL), represent the fluorescence distribution of the antibodies tested. CD13−/CD33− (LL), CD13+/CD33− (LR), CD13+/CD33+ (UL) and CD13−/CD33+ (UL) events are shown. Isotype-matched control antibodies were used to set up the background level.

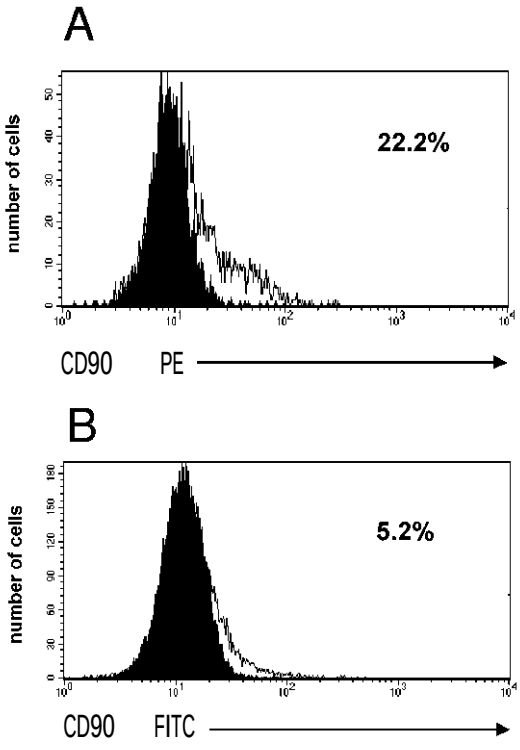


Figure 4. Flow cytometric analysis of CD90 expression in bone marrow CD34+, CD13+, CD33− cells (panel A) and CD34+, CD13+, CD33+ cells (panel B). Four-color immunofluorescence staining of purified CD34+ cells was performed with CD90 FITC, CD13 PE, CD34 PerCP and CD33 APC as described in the Methods. The histograms shown are from one representative case of three studied. Open areas represent fluorescence distribution of CD90 antibody and solid areas represent that of the isotype-matched control antibody. Numbers within the histograms are percentages of CD90 positive cells.

To investigate the degree of maturation of CD34+, CD13+, CD33− cells further, we tested the expression of CD90, a marker that has been reported to be expressed by the most immature fraction of hematopoietic precursors.[39-41] The mean percentage of CD34+ cells expressing CD90 was 2.3±0.2%; a higher proportion of CD90+ cells was found among CD13+, CD33− cells than among CD13+, CD33+ cells (13.3±6.3 versus 3.1±1.5%, Table 3 and Figure 4).

Cytoplasmic expression of the lineage specific-associated markers MPO, CD79a and CD3 was analyzed in three different CD34+ cell subsets defined according to their CD13 and CD33 reactivity (Figure 5), using four-color staining as indicated in Table 1. CD34+ cells lacking CD13 and CD33 expression had high levels of the B-cell marker CD79a (mean 60.3% ± 29.2%), but did not express MPO (less than 1% positivity), whereas CD34+, CD13+, CD33+ cells expressed high levels of MPO (mean 46.2±13.3 %), but did not contain B-cells (less than 1% of CD79a expression, Table 3). Some CD34+, CD13+, CD33− cells expressed, although in a low percentage, both CD79a and MPO (mean 6.0±3.2% and 7.5±6.0%, respectively), suggesting that this cell subset includes some B-cell progenitors in addition to myeloid progenitors. Cytoplasmic CD3, a marker of T-cell differentiation, was negative on all CD34+ cells, irrespective of their CD13 and CD33 expression. The distribution of B-cell progenitors among these three cell subsets was further assessed by CD19, CD13, CD34 and CD33 surface staining of sorted CD34+ cells from one bone marrow sample; as shown in Figure 6, most of the CD13−, CD33− cells expressed the CD19 antigen (71%). The expression of this marker was clearly detectable in a minority of CD13+, CD33− cells (13%), while it was absent in CD13+, CD33+ cells.

In all samples studied, CD34+ cells with the phenotype CD13−, CD33+ were extremely rare (Figure 3), had no homogeneous light scatter distribution, and were negative for CD90 (data not shown).

Discussion

We characterized the prevalence and the immunophenotype of a subset of immature hematopoietic cells which express CD34 and CD13 but lack CD33. These cells were reported to contain primitive hematopoietic progenitors with pluripotent and vigorous, albeit delayed, *in vitro* cell growth.[28] We took advantage of four-color flow cytometry to characterize the immunophenotype of CD34+, CD13+, CD33− cells further. The majority of CD34+, CD13+, CD33− cells lacked markers associated with the myeloid lineage, such as CD65 and CD15, but a minor proportion (approximately 7-8%) were reactive for MPO. They also lacked reactivity for the T-lineage (CD7 and cytoplasmic CD3), megakaryocytic lineage (CD41a) and erythroid lineage (glycophorin A). Expression of CD79a and CD19, indicative of B-cell commitment, on these cells was generally low (less than 10%). Cord blood CD34+, CD13+, CD33− cells were even more phenotypically immature than those found in bone marrow, and did not express any lineage-associated marker. Expression of CD133 and CD117 was compatible with the relative immaturity of this cell subset,[41-43] although no significant differences were observed in the level of expression of these two markers between CD34+, CD13+, CD33− and CD34+, CD13+,

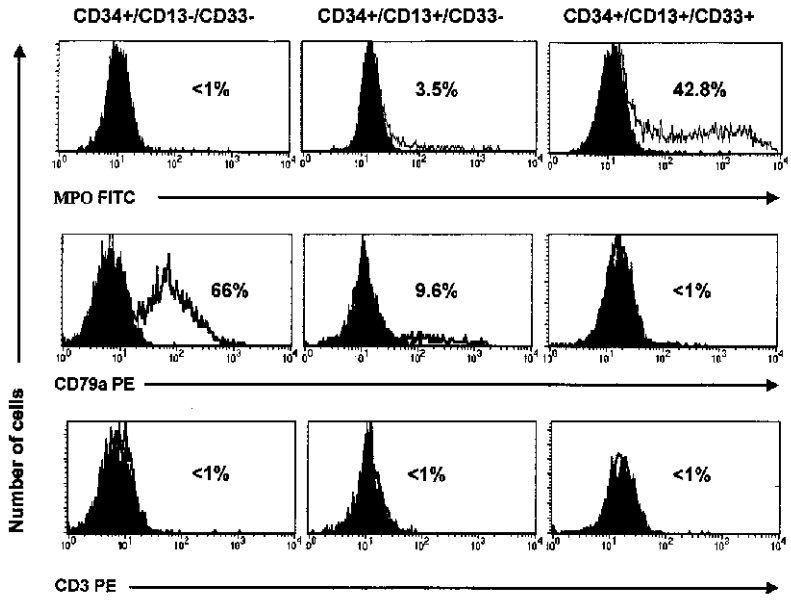
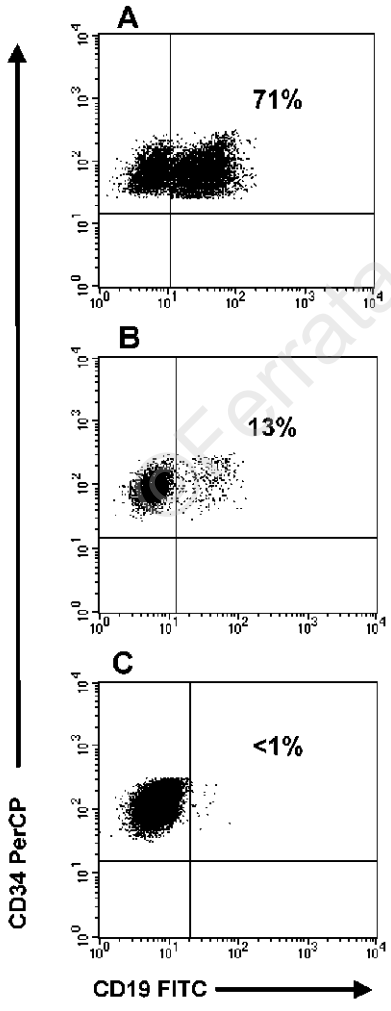


Figure 5. Flow cytometric analysis of MPO (top panels), CD79a (middle panels) and CD3 (bottom panels) cytoplasmic expression in bone marrow CD34+ cells. Four-color immunofluorescence staining of purified CD34+ cells was performed as described in the Methods and Table 1. The histograms shown are from one representative case of three studied. Reactivity of each antibody was analyzed in CD13−, CD33− (left panels), CD13+, CD33− (central panels), and CD13+, CD33+ (right panels) cell subsets. Open areas represent fluorescence distribution of the antibodies tested and solid areas represent that of the isotype-matched control antibody. Numbers within the histograms are percentages of positive cells. The cell number is shown on the y axis.



CD33+ cells. Likewise, CD38 expression was indistinguishable between these two cell populations. However, the CD33− subset had increased levels of CD90, a feature of immaturity.[39-41] In addition, CD34+, CD13+, CD33− cells had lower levels of HLA-DR and CD13 compared to those measured in the CD13+, CD33+ compartment. It has been previously observed that there is a positive correlation between the degree of maturation of myeloid progenitors and the level of HLA-DR[16] and CD13.[20]

Expression of CD13 and CD33 antigens is commonly associated with cells committed to the myeloid lineage and occurring very early during myeloid cell maturation. Nevertheless, it has been a matter of debate whether expression of CD13 pre-

Figure 6 [left]. Flow cytometric analysis of CD19 surface expression in bone marrow CD34+ cells. Staining of purified CD34+ cells was performed with anti-CD19 FITC, CD13 PE, CD34 PerCP and CD33 APC antibodies. The reactivity of anti-CD19 was analyzed in CD13−,CD33− (panel A); CD13+, CD33− (panel B); and CD13+, CD33+ (panel C) cell subsets. Dot plots, subdivided into four quadrants, represent the fluorescence distribution of anti-CD34 (y axis) and anti-CD19 (x axis) antibodies. Percentages of CD19+ cells are indicated. Isotype-matched control antibodies were used to set up the background level.

cedes that of CD33 during this process. Several authors who studied the immunophenotype of CD34+ cells in bone marrow, in cord blood and in peripheral blood have reported an increased expression of CD13 compared to that of CD33,[15,29–33] implying the existence of CD34+ cells expressing CD13 but not CD33. By contrast, others did not find substantial differences in the percentage of CD34+ cells expressing these two markers.[19,34-36] The interpretation of these results is, however, complicated by the fact that none of the above studies simultaneously examined CD13 and CD33 expression on CD34+ cells with three-color staining; the simple comparison of percentages may be misleading especially when small cell populations are examined. Our demonstration of a CD34+, CD13+, CD33– cell subset was confirmed using purified CD34+ cells and was not dependent on a particular clone or fluorochrome used for CD33 detection. This result suggests that CD13 is expressed earlier than CD33 during myeloid differentiation. In line with this notion are the findings of Boezeman *et al.*[28] who reported that sorted CD13+, CD33– cells gave rise to large colonies of mixed, erythroid and myeloid morphology, while CD13+, CD33+ cells yielded smaller colonies predominantly of myeloid origin.

San Miguel *et al.*[37] and Terstappen *et al.*[38] found that the CD34+, CD13+, CD33– phenotype occurred in approximately 20% of cases of acute myeloid leukemia studied. The high proportion of leukemic cases with this phenotype is in sharp contrast with the rarity of equivalent normal cells, and these authors used this immunophenotype to monitor the presence of residual leukemic cells in bone marrow. Although in some cases with small numbers of normal cells with the CD34+, CD13+, CD33– phenotype this approach may be informative, our study indicates that investigators should be aware of the possibility of false-positive results caused by the mistaken identification of normal myeloid progenitors as leukemic myeloblasts.

Contributions and Acknowledgments

GG contributed to drafting the article, perform laboratory experiments and conception ; DC contributed to conception and design. GG and DC should be considered as the principal authors. The remaining authors have taken specific responsability for the the following parts: E C-S and ET for analysis and interpretation of data ; OM for laboratory experiments; AB for critically revising the manuscript. We thank Mrs. E. Paccagnini for secretarial support.

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Disclosures

Conflict of interest: none.

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Peer Review Outcomes

What is already known on this topic

Conflicting results about the existence of $CD34^+/CD13^+/CD33^-$ cells have been provided by previous studies.

What this study adds

This study confirms the presence of $CD34^+/CD13^+/CD33^-$ cells in adult bone marrow and in cord blood and describes their antigenic features of early myeloid progenitors.

Manuscript processing

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Potential implications for clinical practice

The presence of $CD34^+/CD13^+/CD33^-$ cells in normal bone marrow must be considered in studies aimed to identify residual AML cells during remission in patients with $CD13^+/33^-$ blasts.

Dario Ferrero, Associate Editor