Characterization and Functional Analysis of Adult Human Bone Marrow Cell Subsets in Relation to B-Lymphoid Development

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To study the frontiers between pluripotent stem cells and committed progenitors and to further define the B-cell pathway in adult bone marrow (BM), CD34+ subpopulations and CD34+ B-lineage cells were analyzed by multiparameter flow cytometry, studied by light and electron microscopy, and in short-term and long-term cultures (LTC). While the total CD34+ cells represent 4.9% ± 0.8 of BM mononuclear cells within the lymphoid-blast window, 73.8 ± 3.5%, 14.4 ± 1.8% and 8.8 ± 2.9% of them were CD34+ CD10– CD19–, CD34+ CD10+ CD19+, and CD34+ CD10+ CD19+, respectively. CD34+ CD10+ CD19+ cells represent a small homogeneous TdT+ cM– blast population. Although expressing CD34 and high level of HLA-DR antigens, like myeloid committed progenitors, they did not generate LTC, myeloid, and T lymphoid colonies suggesting that the CD34+ CD10+ CD19+ population represents exclusively B-lymphoid committed progenitors. By contrast, all myeloid progenitors and LTC-initiating cells were found in the CD34+ CD10– CD19– cell fraction. This fraction appeared more heterogeneous and contained CD38– HLA-DR low small cells, larger blasts, and promonocyte-like cells exhibiting small peroxidase-positive granules. Interestingly, CD10 was also present on CD34+ CD19– cells. This population mainly coexpressed CD33 and gave rise to macrophagic colonies.

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MATERIALS AND METHODS

Cells

Human heparinized bone marrow was obtained after informed consent either from fragments of femur bones removed during orthopedic surgery from patients free of hematologic diseases or by iliac crest aspiration from normal volunteer donors for allogenic transplantation. Bone marrow cells from orthopedic surgery were first centrifuged at 200 g for 10 minutes to remove the fat. Bone marrow mononuclear cells (BMMC) were then isolated by ficoll-hypaque gradient (density 1.077 g/mL). Cells were washed and then stored overnight at 107 cells/mL in phosphate buffered saline (PBS).
+ 20% fetal calf serum (FCS). Under these conditions, 87.6 ± 7.3% of viable cells were recovered the following day.

**Monoclonal Antibodies (MoAbs) and Fluorescent Reagents**

CD10 (J5), Phycoerythrin (PE) conjugated CD10 (J5-PE) and CD33 (My 9-PE), biotinylated CD19 (B4-BIOT) MoAbs, and isomatched mouse (Ms) Ig were purchased from Coulter (Coultronics, Margency, France). CD38-PE and HLA-DR-PE antibodies were purchased from Becton Dickinson (Sunnyvale, CA). Fluorescein conjugated (FITC) CD34 (8G-12 FITC) was a kind gift from Dr. P.M. Lansdorp (Terry Fox Laboratory, Vancouver, Canada). Anti-human FITC conjugated Ig M (μ chain specific) and anti-human FITC conjugated Ig A (α chain specific) were obtained from Tago (Burlingame, CA). Streptavidin allophycocyanin (APC) and goat anti-mouse APC were purchased from Molecular Probes (Eugene, OR). Normal Ms Ig were obtained from Biosys (Compiègne, France).

**Cell Surface Staining**

For triple staining with CD34, CD10, and CD19 MoAbs, BMNMC at 10^5 cells/mL were first stained in PBS 2% FCS with CD19-BIOT MoAb (5 μL/10^6 cells) for 20 minutes at 4°C, washed, and then stained with CD34-FITC (0.5 μg/10^6 cells), CD10-PE (5 μL/10^6 cells) MoAbs, and APC (1 μL/10^6 cells) for 20 minutes at 4°C. Labeled cells were kept on ice until analysis and sorting. In some cases, HLA-DR-PE, CD33-PE, or CD38-PE (20 μL/10^6 cells) MoAbs were used instead of CD10-PE. For triple staining with CD34, CD18, CD10 MoAbs, or with CD34, HLA-DR, CD10 MoAbs or with CD34, CD33, CD10 MoAbs, cells were first incubated with CD10 (5 μL/10^6 cells), then with GAM-APC (5 μL/10^6 cells). Before adding the other combinations of MoAbs, Ms Ig (17 μg/10^6 cells) was used to block any free binding sites on GAM-APC. As negative control, cells were stained with conjugated isotype matched nonspecific Ms Ig and APC or GAM-APC.

**Flow Cytometry**

Flow cytometry analysis and cell sorting were performed on a two air cooled laser cytometer EPICS Elite (Coultronics, Margency, France). Fluorescence attributable to FITC and PE labeled MoAbs was determined using excitation by an argon laser operating at 488 nm and adjusted at 15 mW. APC was excited with an helium neon laser operating at 633 nm and adjusted at 10 mW. Forward and side scatter measurements were made using linear amplification and all fluorescence measurements were made with logarithmic amplification. Cells labeled with individual antibodies were used to set the voltage, and gain setting and compensation levels on the flow cytometer. For analysis and sort, a gate was drawn according to a low to moderate forward scatter (FS) and a very low side scatter (SS) (see Results). Within this gate, the proportion of positive cells was estimated by determining first the channel number where the negative control and the test sample curves crossed each other. For analysis on triple stained cells, a minimum of 180,000 ungated events or 30,000 events gated on CD34+ cells were acquired in listmode using a software Elite, which permits the identification of multiple cell populations using multidimensional analysis. In some cases, simultaneous expression of four antigens was monitored using sequential combination with three MoAbs and then deduced by comparing the results for each triple labeling procedure with another (see results).

Cells were sorted at 2,000 cells per second and collected in PBS containing 50% FCS. The cell viability evaluated by trypan blue exclusion was always 95%. The purity, which attained 96%, was assessed by analyzing immediately after sorting an aliquot of the sorted cells.

**Intracellular Staining**

For intracellular staining, sorted cells were cytocoentrifuged 12 minutes at 750 rpm. For intracytoplasmic μ chain (μc) staining, slides were kept either at room temperature or at −20°C for several days before staining in order to decrease the background due to the remaining fluorescence present on the sorted cells. Both techniques gave similar results. Then, slides were fixed 10 minutes in cold methanol, washed three times in PBS, saturated 10 minutes in PBS 1% BSA, and incubated 40 minutes in a humid dark atmosphere with anti-human μ chain-FITC or α chain-FITC (as negative control) antibodies at a final dilution of 1/100. After three more washes, slides were mounted and 200 cells per slide were counted with a fluorescence microscope. The percent surface μ chain positive cells was determined by subtracting the percentage of α chain positive (nonspecific staining <3%) from the percentage of μ chain positive. As positive control, an EBV μ chain positive cell line generated in our laboratory was used.

Terminal deoxynucleotidyl transferase (TdT) was detected using an immunoperoxidase kit obtained from Supertechs Inc (Bethesda, MD). Briefly, unfixed slides were stored in a desiccator at room temperature before use, then slides were fixed 30 minutes in absolute methanol at 4°C, air dried, and stained according to the Supertechs’ procedure. Slides were mounted and counted by bright field microscopy. As positive controls, Supertechs slides and a B ALL TdT positive from our laboratory were used. As negative control, the rabbit anti-TdT antibody was replaced with PBS (0% of the cells were positive).

**Preparation for Light and Electron Microscopy**

For light microscopy, sorted cells (purity >96%) were cytocoentrifuged as described above, fixed, and stained with a May-Grünwald Giemsa coloration. At least 200 cells per slide were examined by two independent cytologists. For electron microscopy, a modified micromethod from Anderson was used. First, 5,000 to 10,000 cells per fraction were sorted into 3% of glutaraldehyde in 0.1 mol/L phosphate buffer, incubated 1 hour, centrifuged, washed, fixed in 1% osmic acid solution, washed again in PBS, and placed in Beem capsules. Capsules were centrifuged, and 5 to 10 mL of human plasma and 3% of glutaraldehyde in 0.1 mol/L phosphate buffer were gently added to the pelleted cells. After 1 hour incubation, cells were dehydrated and embedded in epon. This method allowed to manipulate a solid block in which an invisible pellet of cells was lodged. For the detection of peroxidase activities, cells fixed by glutaraldehyde were incubated for 1 hour in Graham Karnovsky medium and treated as above. The thin stained sections were examined using a Philips CM10 electron microscope.

**Methylcellulose Colony Assay for Myeloid Progenitors**

Cells were plated in 35 mm dishes (Greiner, FRG), in 1.1 mL of IMDM containing 0.8% methylcellulose (Fluka 4000, Buchs, Switzerland), 30% FCS, 1% BSA, 100 μmol/L 2-mercaptoethanol (Sigma), 2 mmol/L glutamine, 100 U/mL penicillin, 10 μg/mL streptomycin, 3 U/mL recombinant human erythropoietin (rh Epo) (Boehringer, Mannheim, FRG), 100 U/mL rh Interleukin 3 (IL3) (Genzyme, Boston, MA), 200 U/mL rh granulomacrophagic colony stimulating factor (GM-CSF) (Genetics Institute, Cambridge, MA).

Duplicate cultures were incubated in a humidified atmosphere containing 5% CO2 in air at 37°C. BMNMC were plated at 5 × 10^4 per dish, and sorted fractions were plated at various cell concentra-
tions (10^3 to 10^5 cells per dish) depending of their phenotype. Burst forming unit erythroid (BFU-E) and colony forming unit granulomacrophagic (CFU-GM) were counted between 16 and 18 days culture. BFU-E were subdivided into primitive and mature sub-
classes according to their number of clusters. CFU-GM colonies were subdivided into CFU-G/GM and CFU-M, but in some experiments, CFU-
GM colonies were subdivided into CFU-G/GM and CFU-M (see Results).

Long-Term Marrow Cultures

LTC were established and maintained as described elsewhere. Briefly, cells were suspended in 2.5 mL of long-term culture me-
dium (Terry Fox Laboratory media, Vancouver, Canada), supple-
mented with 10^{-8} mol/L hydrocortisone hemisuccinate (Sigma, St Louis, MO) in 35 mm tissue culture dishes (Falcon, Becton Dickin-
son, Plymouth, UK) on a preestablished irradiated (15 Gy) normal feeder layer obtained from allogeneic marrow as follows: LTCs
were initiated 3 to 6 weeks before the experiment, adherent layers were trypsinized, irradiated with 15 Gys, and replated in 35 mm tissue culture dishes at 3 

Results were presented as the mean ± standard error of the mean (SEM).

Statistical Analysis

Results were presented as the mean ± standard error of the mean (SEM).

RESULTS

Immunophenotyping Patterns of CD34+ Cells and B-Cell Precursors

Light scatter properties. The study of the light scatter properties of CD34+ cells and B-cell precursors indicate that they are contained in a window, so called lymphoid blast gate representing 50.9 ± 2.55% of the total BMMNC (Fig 1A) and already known to contain all the myeloid progeni-
tors, ie, clonogenic and LTC-IC (unpublished data). Within the CD34+ cells, CD34+ CD10- CD19- cells and CD34+ CD10+ CD19- cells have a low to intermediate FS and a very low SS (Fig 1B and C), while CD34+ CD10+ CD19+ cells (Fig 1D) are somewhat smaller (lower FS) and more homogeneous. Interestingly, CD34+ cells expressing B cell antigens, ie, CD10+ CD19+ or CD19+ alone have, like CD34+ CD10+ CD19+ cells, very low FS and SS (Fig 1E and F). Data from 21 separate experiments indicate that within the lymphoid blast gate, CD34+ CD10+ CD19+, CD34+ CD10+ CD19+, CD34+ CD10+ CD19+, and CD34+ CD10- CD19+ fractions represent 3.7% ± 0.7%, 0.7% ± 0.2%, 0.3% ± 0.04%, 5.1% ± 0.8%, and 8.5% ± 0.9% of the BMMNC, respectively.

Distribution of CD34, CD10, and CD19 antigens in the lymphoid blast gate. The fluorescence profile of BMMNC stained with CD34, CD10, and CD19 conjugated MoAbs is presented in Fig 2A through C. The mean percentage (n = 21) of CD34+ cells, CD10+ cells, and CD19+ cells within the lymphoid-blast gate is 4.9% ± 0.8%, 7.5% ± 1.1%, and 14.4% ± 1.2%, respectively.

The analyses of the distribution of CD10 and CD19 on CD34+ gated cells (Fig 2D and Fig 3A) indicate that CD34+ CD10+ CD19+ cells and CD34+ CD10+ CD19+ cells represent 14.4% and 8.8% of the total CD34+ cells, respectively, whereas no CD34+ CD10- CD19+ cells are detected. This strongly suggests that, in adult bone marrow, all CD34+ CD19+ cells coexpress CD10.

Analyses performed on CD10+ gated cells (Fig 3B) or on CD19+ gated cells (Fig 3C) indicate that CD34+ CD10+ CD19+ cells represent 10.7% and 4.4% of the total CD10 and CD19 cells, respectively, whereas CD34- CD10- CD19+ cells represent 67.2% and 35.3% of the total CD10 and CD19 cells, respectively. Interestingly, one can see (Fig 3B) that 22.1% of the CD10+ cells do not coexpress the CD19 B-cell-specific antigen.

Therefore, we studied whether CD34- CD10- CD19+ cells might express CD33 usually present on myeloid cells. For this purpose we used sequential triple combinations with CD34, CD10, CD19, and CD33 MoAbs. Analysis performed on 30,000 gated CD34+ cells shows that CD34+ CD10+ cells are CD33+ (Fig 2E), while 8.2% of CD34+ cells are CD10+ and CD33+ (Fig 2F). Thus, the comparison of the different triple stainings clearly indicates that CD34+ CD10+ CD19+ cells (8.8% of the CD34+ cells) mainly express the CD33 Ag. This suggests that CD10 might be present on myeloid progenitor cells.

Expression of CD38 and HLA-DR antigens. Because CD38 and HLA-DR antigens are useful to distinguish very primitive CD34+ cells from myeloid clonogenic progeni-
tors, we studied the expression of these two antigens on CD34+ and CD34- cells expressing B-lymphoid markers using triple staining procedure with combination of CD34, CD10, CD19, and HLA-DR MoAbs. Analyses within CD34+ cells indicate that CD34+ CD19+ are HLA-
DR+ (Fig 2G) and CD34+ CD19+ are CD38+ (Fig 2H). Assuming from Fig 2D that CD34+ CD19+ cells are always CD10+, these data indicate that like clonogenic myeloid CD34+ progenitors, CD34+ CD10+ CD19+ cells are also CD38 and HLA-DR positive. Additional experiments show that, in the CD34+ CD10+ (also CD19+) cell fraction, 15% and 7.5% are HLA-DR+ and CD38+, respectively (Table 1). These cells, ie, CD34+ HLA-DR+ or CD34+ CD38+ express high levels of CD34+ Ag and represent a small-sized and homogeneous population (not shown).

Similar cytometric analysis were also performed on CD10+ gated cells or on CD19+ gated cells. The data are summarized in Table 1 and show that CD34+ cells coexpressing CD10 and CD19 antigens are CD38+ and HLA-
DR+. Interestingly, CD38 antigen is only expressed in 20% of the CD34+ CD10+ CD19+ cells, which represent newly formed and mature B cells.

μ Chain and TdT expression. In order to determine the expression of μ chain and TdT on the different cell fractions, sorted cells were cytocentrifuged and stained as de-
scribed in the Materials and Methods section. As shown in Fig 4, TdT is strongly expressed on CD34+ CD10+ CD19+ cells and progressively decreases along the B lymphoid devel-
development, while intracytoplasmic \( \mu \) chain expression appears on cells that no longer display CD34. Interestingly, very few CD34+ CD10- CD19- cells are TdT positive. These data indicate that the majority of CD34+ CD10+ CD19+ cells are TdT+ and \( \mu^- \).

**Morphologic Aspect of CD34+ Subpopulations and CD34- B-Lineage Cells**

**Light microscopy.** As described in the Materials and Methods section, at least 200 cells per slide were studied. The results show that CD34+ CD10- CD19- cells are composed of blast cells but are not homogeneous by their size (Fig 5A). Most of them exhibit one or two large nucleoli and in some cases the nonbasophilic archoplasm may contain some azureophilic granules (Fig 5B). CD34+ CD10+ CD19- sorted cells also represent a heterogeneous population with some cells identical to CD34+ CD10- CD19- cells, while others have a very compact chromatin. Interestingly, as in the CD34+ CD10- CD19- subpopulation, mitotic cells are also observed (Fig 5C). The morphologic aspect of CD34+ CD10+ CD19+ cells is illustrated in Fig 5D; some of them are of smaller size with a high nuclear cytoplasmic ratio. Surprisingly, in the CD34+ CD10+ CD19+ cell fraction, nucleoli are rather frequent in the nucleus (Fig 5E). Because of the low number of CD34+ CD10+ CD19- cells, this cell fraction has not been examined.

**Electron microscopy.** The morphologic aspect confirmed the observations made by light microscopy. The different cells presented in the Figs 6, 7, and 8 are representative of seven separate cell sorts that gave identical results. Blasts from CD34+ CD10- CD19- cell fraction show a nucleus with predominant euchromatin. The structure of the nucleoli is either loose (Fig 6A) or compact (Fig 6B). Monoribosomes are abundant but the endoplasmic reticulum cisternae are rare or absent (Fig 6A, B). Some of the CD34+ CD10+ CD19+ blasts exhibit few clustered granules and contain long cisternae of endoplasmic reticulum and numerous mitochondria (Fig 6C). After incubation with diaminobenzidine medium, all the small granules are peroxidase-positive (inset Fig 6C). In contrast to the usual bone marrow promonocytes, the peroxidase activity is never detected in the nuclear envelope, endoplasmic reticulum, and Golgi apparatus. These results suggest that these blasts stop very early the production of peroxidase-positive granules and represent a subpopulation of CD34+ promonocytes. Interestingly these cells are never found in the CD34+ CD10+ CD19+ cell fraction. Although CD34+ CD10+ CD19+ cells contain several blasts exhibiting a morphology similar to that shown in Fig 6A and B, the majority of them have a
Fig 2. Expression of CD10, CD19, CD33, CD38, and HLA-DR antigens within the lymphoid-blast window. Single fluorescence profile for CD34-FITC (A), CD10-PE (B), and CD19-APC (C). Analysis was performed on 180,000 gated events in the lymphoid-blast window. Three color analysis showing the expression of various antigens was evaluated by gating on 30,000 CD34+ cells. (D) Coexpression of CD10-PE and CD19-APC; (E) expression of CD33-PE and CD19-APC; (F) coexpression of CD33-PE and CD10-APC; (G) coexpression of HLA-DR-PE and CD19-APC; (H) coexpression of CD38-PE and CD19-APC. All fluorescence profiles are presented in a logarithmic scale. A representative of 21 and 5 separate experiments for (A) through (D) and for (E) through (H) is shown, respectively.
nucleus with more heterochromatin and nucleoli (Fig 7A, B). Other cells have a very irregular and indented nucleus (Fig 7C). Cells in mitosis (Fig 7D) are also observed. In the CD34+ CD10+ CD19- or CD34+ CD10- CD19+ fractions, the development of endoplasmic reticulum is more evident (Fig 8) and the loose or compact nucleoli persist (Fig 8A and B). As seen by light microscopy, a nucleolus also remains in the nucleus of many CD19+ cells (Fig 8C). Interestingly by comparing Figure 8A and D taken at the same magnification, it turns out that the size of mitochondria clearly increases in mature B cells lacking CD10 (Fig 8D). These morphologic data indicate that CD34+ CD10+ CD19+ cells strongly resemble the other CD34+ subpopulations, but are a little bit more homogeneous.

**Culture Assays**

To compare the growth abilities of the different CD34+ subpopulations and CD34- B-lineage cells, we used various culture assays capable of detecting myeloid progenitors. When sorted cell fractions are plated in myeloid short-term assay, we observe that myeloid and erythroid colonies (Table 2) are only present in the CD34+ CD10- CD19- subset, while no colonies are detectable under our culture conditions in the CD34+ CD10+ CD19+ cell fraction. Interestingly, CD34+ CD10+ CD19+ cells are capable of giving rise to ~30% (40 of 130) of the macrophagic colonies within the total CD34+ population, indicating that CD10 is expressed by progenitors that still retain at least some myeloid potential.

To measure the number of LTC-IC contained in the different sorted cell fractions, cells were plated in LTC as described in the Materials and Methods section. The results show in Table 3 that myeloid clonogenic cells detectable after 5 weeks culture, are only present in the CD34+ CD10- CD19- cell fraction.

These data indicate that CD34+ CD10+ CD19- cells contain both clonogenic myeloid progenitors and LTC-IC, whereas CD34+ CD10+ CD19+ cells have lost their myeloid potential and may consequently represent B-lymphoid committed progenitors.

**DISCUSSION**

The hematopoietic system is composed in the bone marrow of a cellular hierarchy where the most primitive cells, which possess a pluripotent potential, proliferate and differentiate into committed progenitors giving rise through multiple steps of differentiation to circulating blood cells. This continuous orderly process is characterized by coordinated changes both at the gene level and in the expression of intracellular and cell surface antigens. In this report we focused our attention on early B-lymphoid progenitors in adult bone marrow. For this purpose, we sorted by multi-parameter flow cytometry the various CD34+ subpopula-
Table 1. Percentage CD38 and HLA-DR Positive Cells Within CD34+ Subpopulations and CD34- B- Lineage Cells

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<tr>
<th>CD38+ Cells (%)</th>
<th>HLA-DR+ Cells (%)</th>
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<tr>
<td>CD34+ CD10- CD19-</td>
<td>92.5</td>
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<tr>
<td>CD34+ CD10+ CD19+</td>
<td>100</td>
</tr>
<tr>
<td>CD34+ CD10- CD19+</td>
<td>100</td>
</tr>
<tr>
<td>CD34+ CD10+ CD19+</td>
<td>20</td>
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The number of single, double, and triple positive cells using various combinations of three MoAbs were calculated using a software Elite. Then, the percentage of CD38 or HLA-DR positive cells were deduced by comparing the combinations of three MoAbs to one another. Results for CD38 and HLA-DR were obtained from three and five separate experiments, respectively.

* In the CD34+ CD10- CD19+ cell fraction, 15% of the cells were HLA-DRlow, whereas 85% were HLA-DRhigh.

First, we analyzed the distribution of CD10 and CD19 antigens on CD34+ cells. While CD34+ CD10+ CD19+ cells and CD34+ CD10+ CD19+ cells represented 14.4% and 8.8% of the total CD34+ cells, respectively, no CD34+ CD10+ CD19- cells were detectable. These findings indicate that CD34+ CD19+ cells are always CD10+ and suggest that CD19 is not expressed before CD10 antigen in adult bone marrow. This is of particular interest because of the controversy concerning the sequential expression of CD10 and CD19 antigens. Indeed, based on the identification of CD10+ CD19+ leukemic cells, Nadler et al have proposed that CD19 expression precedes CD10 expression, whereas other studies carried out in fetal liver, where CD10+ CD19- cells are in larger number than CD10+ CD19+ cells, indicate that CD10 antigen expression precedes the acquisition of CD19 antigen. These apparent discrepancies might be due to the fact that most of the studies on human B lymphopoiesis have been carried out either on fetal tissues (ie, fetal liver or fetal bone marrow) or on leukemic B cells. Therefore, we suggest that the antigenic distribution might be different in the fetal and adult B lymphoid pathway.
Fig 6. Ultrastructural aspect of CD34⁺ CD10⁻ CD19⁻ blast cells. (A) Aspect of a blast at low magnification (X 5,000). The enlargement shows a large loose nucleolus (Nu) surrounded by heterochromatin. In the cytoplasm, there are numerous monoribosomes (X 25,000). (B) Low magnification (X 5,000) of another smaller blast with an indented nucleus. At higher magnification (X 25,000), the round nucleolus appears compact. (C) Blast with different characteristics. A large nucleolus is also present in the nucleus with an irregular contour. In contrast with the two other blasts from A and B, this blast exhibits in the cytoplasm long cisternae of endoplasmic reticulum (ER) and clustered small granules (Gr) (X 12,200). (C, inset) Identical blast that has been incubated in the diaminobenzidine medium. The very small granules are peroxidase positive. Compare their size with that of mitochondria (X 35,000).
Fig 7. Ultrastructural aspect of CD34+ CD10+ CD19+ blast cells. (A) This blast with a loose nucleolus has a nucleus with heterochromatin distributed mainly around the nuclear envelope and surrounding the nucleolus (× 8,900). (B) Another blast with two nucleoli. A centriole (Ce) is in the middle of a Golgi zone, which deforms the nucleus (× 8,900). (C) Larger blast with highly indented nucleus and small multiple nucleoli (arrows) (× 8,900). (D) Cell in mitosis (× 8,900).

is reinforced by the fact that we did not find any CD34+ CD10+ CD19+ cells in cord blood (our unpublished data) in line with others. Although our data did not determine whether some CD10+ CD19+ cells may be committed to the B lineage, the isolation of various CD34+ subpopulations should enable us to answer this question either by using the B-cell colony assay recently described by McGinnes et al or by developing a human counterpart of the murine lymphoid long-term culture. Recently, such a culture assay in the presence of stromal cells and IL7 has been described for fetal human B-cell precursors.

Further characterization of CD34+ CD10+ CD19+ cells indicate they were TdT+ and cμ+. Interestingly, we also found that 2% of CD34+ CD10+ CD19+ cells were TdT+ (Fig 4). These results are in agreement with other data suggesting that TdT is either expressed on B cell progenitors before the acquisition of CD19 and CD10 Ags or on CD34+ cells that likely include progenitors cells for both B and T lineage. The concept of a common lymphoid progenitor is still unclear albeit a subpopulation of lymphoid progenitors coexpressing CD19, CD7, and CD34 has been identified in human fetal BM. Interestingly, we also found CD34+ CD7+ CD19+ cells and CD34+ CD7+ CD19+ cells in adult bone marrow (not shown). The demonstration that such a putative common lymphoid progenitor can differentiate both into phenotypically mature T and B cells would be proven by using B lymphoid30 and prothymocyte assays. However, in preliminary experiments performed with CD34+ CD10+ CD19+ and CD34+ CD10+ CD19+ subpopulations we did not get any T cell clones (data not shown). This negative result might be due to the extremely low frequency of CD34+ CD7+ cells, the most immature prethymic T lineage progenitor, which only represent 5% of the total CD34+ cells and give rise in limiting dilution assay to T cell clones at a frequency of 1 in 35 to 53. In order to demonstrate whether CD34+ CD10+ CD19+ cells still retain some T lymphoid potential, further experiments on purified CD34+ CD7+ CD19+ cells that are also CD10+ are now in progress.

Morphologic and functional studies indicated that
CD34⁺ CD10⁻ CD19⁺ cells had a blast morphology like other CD34⁺ cells but were more homogeneous and of a smaller size, and were not capable of growing in short-term clonogenic myeloid culture nor in LTC. This loss of myeloid potential is in agreement with previous studies on human fetal hematopoietic tissues showing that purified CD10⁺ lymphoid progenitors were not capable of giving rise to CFU-GM colonies. However, the presence of mitosis

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<th>Table 2. Detection of the Number of Myeloid Clonogenic Progenitors in the Different Subpopulations</th>
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<tr>
<td>Mononuclear cells</td>
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<tr>
<td>3-8 Clusters</td>
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<td>---</td>
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<tr>
<td>Total CD34⁺</td>
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<td>Total CD34⁻</td>
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<td>CD34⁺ CD10⁻ CD19⁺</td>
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<td>CD34⁺ CD10⁺ CD19⁺</td>
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Absolute number of progenitors are given for 10,000 cells. Results represent the mean ± SEM of (n) separate experiments performed in duplicate.

* CFU-G/M included CFU-GM, CFU-G, and CFU-M.
Table 3. Recovery of Committed Progenitors From Sorted Cell Fractions After 5 Weeks in Long-Term Studies

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<tr>
<th>Fraction</th>
<th>BFU-E</th>
<th>CFU-GM</th>
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<tr>
<td>Total mononuclear cells</td>
<td>131 ± 17</td>
<td>279.5 ± 28.5</td>
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<tr>
<td>CD34+ CD10- CD19+</td>
<td>4,640 ± 740</td>
<td>9,300 ± 0</td>
</tr>
<tr>
<td>CD34+ CD10+ CD19-</td>
<td>0</td>
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<td>CD34- CD10+ CD19+</td>
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<tr>
<td>CD34- CD10- CD19+</td>
<td>0</td>
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One representative experiment of four separate experiments is shown. Sorted cell fractions were seeded in LTC. After 5 weeks, nonadherent and adherent cells were recovered and plated in duplicate in methylcellulose. Absolute number of progenitors are given for 10^6 plated cells in LTC.

(Figs 5C and 7D), indicates that this population is not quiescent and might grow under appropriate culture conditions.

Second, we studied CD34+ CD10- CD19- cells (ie, 73%-74% of total CD34+ cells), which appeared phenotypically more heterogeneous. Indeed, some of them contained clustered granules and were peroxidase-positive (Fig 6C). Several arguments indicate that these cells are indeed CD34+ promonocytes and do not represent a contaminant: they are numerous in all CD34+ CD10+ CD19- fractions and are absent from all CD34+ CD10+ CD19+ fractions studied; these cells have also been described in two other studies on purified CD34+ cells82 and express early (CD13, CD33) and more heterogeneous. Indeed, some of them contained clustered granules and were peroxidase-positive (Fig 6C). Several arguments indicate that these cells are indeed CD34+ promonocytes and do not represent a contaminant: they are numerous in all CD34+ CD10+ CD19- fractions and are absent from all CD34+ CD10+ CD19+ fractions studied; these cells have also been described in two other studies on purified CD34+ cells82 and express early (CD13, CD33) and late (CD14, CD15) myeloid markers.83 In addition, using immunogold labeling on a suspension of unseparated bone marrow cells, such promonocytes have also been identified with anti-CD34 antibody (IBG, personal data). These cells probably are maturing into macrophages in vitro. Other CD34+ CD10- CD19+ cells were smaller with compact nucleoli (Fig 6B). The latter cells, which present a minor component of CD34+ cells are CD38+ and HLA-DRlow and express higher levels of CD34 antigen. Such cells, capable of initiating long-term bone marrow culture in vitro,67 have an aspect similar to the murine WGA+ Rh123+ cell46 and might be identical to the CD34+ Thy1+ cells recently described as a human hematopoietic pluripotent stem cell population.2 Interestingly, when we examined the expression of the CD38 and HLA-DR Ags on CD34+ CD10+ CD19- cells, we found that these cells were positive for both Ags like myeloid committed progenitors within the CD34+ CD10+ CD19+ subset. These findings indicate that CD38 and HLA-DR antigens are expressed coordinately with lineage specific antigens on CD34+ cells and represent general markers of commitment in hematopoiesis.

Third, we observed that 8.8% of CD34+ cells were CD34+ CD10- CD19+. Further studies indicated that these cells were mainly CD33+ (see Fig 2F), but such a cell population has not been found by others.29 Interestingly, CD34+ CD10+ CD19- cells, when plated in culture, gave rise to macrophagic colonies. From these findings, one can speculate about the existence of a common progenitor to the macrophagic and lymphoid lineages in human bone marrow inasmuch as recent published data have shown the presence of bipotential precursors of B cells and macrophages in murine fetal liver.45 In view of our results, we propose that the hierarchical organization of the CD34+ subpopulations in adult bone marrow would be as follows: 1) CD34+ CD38+ HLA-DR low cells would represent the most primitive pluripotent stem cell; 2) then this small sized population (with a morphology similar to Fig 6B) begins to be committed and to express CD38 and a high level of HLA-DR Ags; 3) within this population of CD34+ CD38+ HLA-DR high cells, one can suppose that some of them commit to the myeloid lineage and others represent a common progenitor to the macrophagic and lymphoid lineages. The latter cells, which still retain some ability to generate macrophagic colonies, are CD34+ CD10+ and might coexpress either CD33 and/or T and B associated markers including CD7, CD19, and TdT; 4) finally, CD34+ CD10+ CD19+ cells, which have lost their myeloid potential, may represent the earliest B-lymphoid committed progenitor capable of differentiating into CD34+ CD10+ CD19+ CD44+ pre-B cells and newly formed CD34+ CD10- CD19+ B cells.

Therefore, the characterization of these different stages of the B lymphoid pathway from the most immature CD34+ cells to the mature B lymphocytes will allow us to study their response to growth factors such as interleukin-7 and kit-ligand, which have been shown to stimulate, in combination with stromal cells, the proliferation of pre-B cells in mice.46 Such a strategy, might provide the appropriate culture conditions to develop B-lymphoid specific assays in human adult bone marrow.

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Characterization and functional analysis of adult human bone marrow cell subsets in relation to B-lymphoid development

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