Separation of Myeloid and Erythroid Progenitors Based on Expression of CD34 and c-kit

By Marg O. De Jong, Gerard Wagemaker, and Albertus W. Wognum

In this report, a novel approach is described to physically separate erythroid progenitors from monocyte and granulocyte progenitors, based on the expression of CD34 and Kit. Using biotin-labeled human Kit ligand (KL) and flow cytometry, Kit was detectable on 2% to 3% of the nucleated cells in the mouse bone marrow. Combination of biotin-KL with CD34 monoclonal antibodies (MoAb) showed that Kit was expressed on subsets of CD34low and CD34high cells. Our data clearly demonstrate that CD34high cells are more heterogeneous with respect to Kit expression than observed in studies using Kit MoAb. A small cluster, approximately 7% of the CD34high cells, expressed CD34 at submaximal levels and stained brightly with biotinylated KL. This CD34high*/Kit1 fraction contained predominantly erythroid progenitors (burst-forming units-erythroid; BFU-E). The majority of the granulocytic and monocytic progenitors (colony-forming units-granulocyte/macrophage; CFU-GM) were CD34low.

Kitmed. Some BFU-E were also detected in the CD34high*/Kitmed and CD34low*/Kitlow fractions at low frequency. In the latter subset, most erythroid colony-forming units (CFU-E) were recovered. Using three-color flow cytometry, we analyzed expression of Kit in relation to that of CD34 and the class II major histocompatibility antigen, RhLA-DR. The most immature bone marrow cells that can be identified in vitro, ie, CD34+/RhLA-DRlow cells, were Kitmed. The CD34low*/Kitlow and CD34low*/Kitmed subsets predominantly contained the more mature RhLA-DRhigh cells. Our results demonstrate that erythroid precursors express c-kit at much higher levels than monomyeloid precursors and pluripotent progenitors. The differences in expression levels of CD34 and c-kit can be exploited to isolate BFU-E populations that are virtually devoid of nonerythroid cells.

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differences in techniques and reagents used to detect and isolate Kit-expressing cells. The ability to distinguish kit<sup>low</sup>, kit<sup>med</sup>, and kit<sup>hi</sup> cells is influenced by the binding affinity of the Kit MoAb. In addition, some Kit MoAbs inhibit outgrowth of KL-responsive cells, which may impede kit<sup>low</sup> cells from being isolated. Kit-expressing cells can be distinguished and physically separated from immature multipotent cells and committed monocyte and granulocyte progenitors on the basis of CD34 and kit expression.

**MATERIALS AND METHODS**

**Biotinylation.** Recombinant human kitL (a gift from Dr S. Gillis, Immunoex, Seattle, WA<sup>2</sup>) was biotinylated using biotin-N-hydroxy succinimide ester (NHS-Biotin; Pierce, Rockford, IL) as described previously for other growth factors. Briefly, NHS-Biotin dissolved in dimethyl sulfoxide (DMSO) was added to 10-μg aliquots of kitL in 0.1 mol/L carbonate-bicarbonate buffer, pH 8.4, containing 0.02% (vol/vol) Tween-20 at molar biotin:protein (BP) ratios of 10:1, 100:1, or 300:1. A control sample was incubated with DMSO without biotin. After 3 hours of incubation at room temperature in the dark, biotin= molecules were separated from the remaining free biotin molecules in the samples by size exclusion chromatography on a 1-mL Sephadex G-25 column (Pharmacia, Uppsala, Sweden), equilibrated in phosphate-buffered saline containing 0.02% (vol/vol) Tween-20. To test the efficiency of the biotinylation, biotin-KL was adsorbed onto streptavidin-agarose beads. This preparation was washed in HH with fetal calf serum and azide. Fluorescence signals were amplified by incubating the cells for 30 minutes on ice with streptavidin-phycocerythrin (streptavidin-PE, 1:150 vol/vol; Molecular Probes, Eugene, OR). After each incubation the samples were washed in HH with fetal calf serum and azide. Fluorescence signals were amplified by incubating the cells for 30 minutes on ice with biotinylated PE MoAb and streptavidin-PE as described earlier. During the last streptavidin-PE incubation, cells were double-stained with a CD34 MoAb (antibody 566; provided by Dr T. Egeland, The National Hospital, Oslo, Norway) that was labeled with fluorescein isothiocyanate (FITC; Sigma) using standard procedures. For three-color analysis, cells were also incubated with a peridinin chlorophyll protein (PerCP)-labeled antibody against the human class II histocompatibility antigen HL-A-DR (Becton Dickinson, Mountain View, CA) that crossreacts with rhesus monkey Rh-LA-DR antigens. To study the expression of the transferrin receptor, double-staining experiments with biotin-KL and FITC-conjugated CD71 MoAb (Becton Dickinson) were performed. Sorted cells with low CD34 expression were incubated with FITC-labeled CD71 MoAb as well.

Samples were analyzed using a FACSscan or sorted using a FACS Vantage (Becton Dickinson, San Jose, CA). Cells were illuminated with the 488-nm line of an argon ion laser. Green FITC fluorescence was measured through a 530-nm/30-nm bandpass filter. Orange PE fluorescence was measured through a 575-nm/25-nm or a 585-nm/40-nm bandpass filter. Red PerCP fluorescence was measured through a 650-nm longpass filter. Cells were analyzed in a light scatter window as indicated in Fig 2C to include cells with intermediate to high forward light scatter (FLS) and low to intermediate perpendicular light scatter (PLS) properties and to exclude granulocytes, dead cells, and cellular debris.

**In vitro culture in semisolid medium.** Sorted populations were assayed for their content of CFU-GM, CFU-E, and BFU-E by in vitro colony formation in semisolid methylcellulose culture medium. In 35-mm petri dishes (Becton Dickinson), unsorted cells were plated at a concentration of 50,000 per dish; sorted subsets were plated at 10,000 per dish (for cells from the light scatter window as shown in Fig 2C) or at 500 to 1,000 per dish (for subsets of CD34<sup>high</sup> cells) in 1 mL methylcellulose medium as described. Methylcellulose cultures included the following components: 0.8% (vol/vol) methylcellulose in αMEM (GIBCO, Gaithersburg, MD), supplemented with 10% (vol/vol) fetal calf serum (FCS), 0.05 mmol/L L-β-mercaptoethanol, 5 ng/mL human IL-3, and 10% (vol/vol) conditioned medium of the 5637 cell line. Cultures were maintained at 37°C in a humidified atmosphere of 16% CO2 in air. To determine the biologic activity of biotin-KL, 5 × 10<sup>5</sup> cells per well of 96-well microtiter plates (Falcon 3072; Becton Dickinson Labware, Lincoln Park, NJ) were cultured in 200 μL medium containing serial dilutions of growth factor. The cells were cultured for 40 to 48 hours, after which 0.25 μCi H-thymidine was added to each well. The cells were harvested after 16 to 18 hours of thymidine incorporation, and the radioactivity was measured in a liquid scintillation counter. At a BP ratio of 10.1, KL retained all of its biologic activity, while the biotinylation efficiency was greater than 99%, as determined by adsorption onto streptavidin-agarose beads.

**Low-density BM cell preparation.** BM aspirates from young adult rhesus monkeys (Macaca mulatta) from the TNO Primate Center, Rijswijk, The Netherlands, were collected in Banks’ HEPES buffered salt solution (HH) with heparin and DNase. The buffy coat fraction was collected after centrifuging the cells for 15 minutes at 2,500 rpm at room temperature. Low-density cells were obtained by centrifugation for 30 minutes at 2,000 rpm at room temperature over a discontinuous bovine serum albumin (BSA) density gradient consisting of 25%, 23%, 22%, 21%, and 17% (wt/vol) BSA in 0.2 mol/L Tris-buffer/phosphate buffer, pH 7.2. Fractions were collected and washed in HH. Erythrocytes were lysed using 10 mmol/L potassium bicarbonate, 155 mmol/L ammonium chloride, pH 7.4, containing 0.1 mmol/L EDTA.

**Immunocytochemical staining and flow cytometry.** Cells were stained overnight on ice with biotin-KL (1 mmol/L) in HH containing 2% (vol/vol) fetal calf serum, 2% (vol/vol) thymus monkey serum, 0.05% (wt/vol) sodium azide, and DNase (0.5 mg/mL). Similar results were obtained by incubation for 2 hours on ice. Specificity of binding of the biotin-KL samples was determined by incubating the cells with biotin-KL in the presence of either the blocking Kit antibody SK-1 (ascites 1:200 dilution; provided by Dr V. Broudy, University of Washington, Seattle, WA) or a 1:100-molar excess of unbiotinylated KL. The cells were incubated for 30 minutes on ice with streptavidin-phycocerythrin (streptavidin-PE, 1:150 vol/vol; Molecular Probes, Eugene, OR). After each incubation the samples were washed in HH with fetal calf serum and azide. Fluorescence signals were amplified by incubating the cells for 30 minutes on ice with biotinylated PE MoAb and streptavidin-PE as described earlier. During the last streptavidin-PE incubation, cells were double-stained with a CD34 MoAb (antibody 566; provided by Dr T. Egeland, The National Hospital, Oslo, Norway) that was labeled with fluorescein isothiocyanate (FITC; Sigma) using standard procedures. For three-color analysis, cells were also incubated with a peridinin chlorophyll protein (PerCP)-labeled antibody against the human class II histocompatibility antigen HL-A-DR (Becton Dickinson, Mountain View, CA) that crossreacts with rhesus monkey Rh-LA-DR antigens. To study the expression of the transferrin receptor, double-staining experiments with biotin-KL and FITC-conjugated CD71 MoAb (Becton Dickinson) were performed. Sorted cells with low CD34 expression were incubated with FITC-labeled CD71 MoAb as well.

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adding G-CSF. BFU-E cultures were grown in the presence of EPO, KL, and 0.2 mmol/L bovine hemin. CFU-E cultures were grown in the presence of EPO and 0.14 mmol/L hemin. Cultures were maintained in a humidified atmosphere of 10% CO₂ in air. Colonies were counted at day 4 (CFU-E) and day 11 or 12 (CFU-GM and BFU-E). Data of duplicate dishes were expressed as average number of colonies counted, assuming that crude colony counts are Poisson-distributed.

**RESULTS**

**Biotin-KL staining of MO7e cells and rhesus monkey BM cells.** Cytochemical staining properties of biotin-KL on MO7e cells and on rhesus monkey BM cells were studied using flow cytometry. All cells of the KL-responsive cell line MO7e and a fraction of the BM cells were stained brightly with biotin-KL in combination with PE-conjugated streptavidin (Fig 1A and B). Because the number of kit⁹⁹⁹ cells in unfractionated BM was very low (2% to 3% of the nucleated cells, corresponding to 7% of the cells inside the light scatter window, indicated in Fig 1C), Kit expression was also studied on cells inside a window based on high CD34 expression (Fig 1C). Within the CD34⁺ subset, 30% of the cells were kit⁺⁺. The fluorescence signal of MO7e as well as BM cells, incubated with biotin-KL in the presence of either unlabeled KL or the blocking anti-Kit antibody SR-1, was almost identical to that of control cells incubated without biotin-KL. This indicated that binding of biotin-KL to these cells was specific and due to binding to Kit.

**Distribution of c-kit on rhesus monkey BM cells.** Because of the low frequency of kit⁺⁺ cells in unfractionated BM (Fig 1), the relation between CD34 and Kit expression was studied on low-density BM cells (Fig 2). Expression of Kit was detectable on 15.2% ± 6.1% (three different experiments) of the low-density BM cells inside the light scatter window indicated in Fig 2. Combination of biotin-KL with CD34 MoAb showed that 30% to 50% of these Kit-positive cells were CD34⁺⁺. The CD34⁺⁺ cells were heterogeneous with respect to Kit expression. A small subset, containing 7% of the CD34⁺⁺ cells, expressed high levels of c-kit (region 1 in Fig 2A), CD34 expression on these CD34⁺⁺/kit⁺⁺ cells was lower than on another subset that expressed Kit at intermediate levels (CD34⁺⁺/kit⁺⁺, region 2), which indicated that the latter population included the more immature cells. This kit⁺⁺ fraction contained more than 60% of the CD34⁺⁺ cells. The remainder of CD34⁺⁺ cells had no detectable Kit expression (CD34⁺⁺/kit⁺⁻, region 3). Finally, a subset of cells with low CD34 expression ex-
pressed c-kit in a range from low to high. Most cells in this region, particularly those with high Kit expression, showed a small shift in fluorescence intensity after staining with the CD34 MoAb, as compared with cells stained with isotype-control MoAb. Therefore, this subset was designated CD34low (CD34neg/kitneg; region 4).

As shown in Fig 2C, CD34neg/kitneg and CD34pos/kitneg cells (regions 1 and 2) displayed light scatter properties characteristic of immature, blast-like cells, i.e., intermediate to high FLS and low PLS. CD34pos/kitneg and CD34pos/kitpos cells (regions 3 and 4) were more heterogeneous with respect to light scatter and also contained cells with relatively high PLS. Additionally, small cells with low FLS were found in the CD34pos/kitneg fraction (region 3).

Analysis of RhLA-DR expression on the different subsets (Fig 2D) indicated that most of the CD34pos/kitneg and CD34pos/kitpos cells (regions 1 and 3) were RhLA-DRbright, whereas the CD34pos/kitneg cluster (region 2) contained RhLA-DRdim and RhLA-DRbright cells at almost equal frequencies. The presence of RhLA-DRdim cells in region 2, in combination with the high CD34 expression of this subset, indicated that very immature cells express Kit at low to intermediate levels. RhLA-DR expression was also low on the CD34neg/kitneg cells, but these represent relatively mature cells, as CD34 levels on these cells were very low.

Colony-forming potential of different fractions of rhesus monkey BM. We assayed the functional abilities of the subsets, discussed in the previous section, in standard colony assays in semisolid culture media. The results of two such experiments are shown in Table 1. Most CFU-GM were found in the CD34pos/kitneg fraction, which was 20 to 30 times enriched in CFU-GM as compared with the low-density cells. A much lower proportion of CFU-GM was recovered in the CD34pos/kitneg subset, and less than 1% of the CFU-GM were found in the CD34pos/kitneg fraction.

The CD34pos/kitneg fraction contained at least 30-fold more BFU-E than CFU-GM. As shown in Table 1, 200 to 400 of every 1,000 CD34pos/kitneg cells developed into a BFU-E colony, compared with one to three of every 1,000 low-density cells, demonstrating an enrichment of 100- to 200-fold.
Some BFU-E were detected in the CD34⁺/kit⁺/CD71⁺ fractions and the CD34⁺/kit⁺/CD71⁻ fractions, but at 20- to 50-fold lower frequencies than in the CD34⁺/kit⁻ fraction. The erythroid origin of kit⁺ cells was confirmed by double-staining of cells with biotin-KL and CD71 MoAb, which showed that kit⁺ cells expressed high levels of the transferrin receptor (Fig 3).

The number of CFU-E colonies recovered after sorting was very low (Table 1). Most of the CFU-E were present in the CD34⁺/kit⁻/CD71⁺ fraction, suggesting that this fraction contained differentiating erythroid cells. In agreement with this, erythroblasts were the predominant cell type identified in cytocentrifuge preparations from this fraction (data not shown). Moreover, restaining of sorted CD34⁺/kit⁺ cells with CD71 MoAb showed high CD71 expression on these cells (Fig 4). However, because of the low CFU-E recovery, we cannot exclude the possibility that CFU-E were present in other subsets as well.

Growth factor responses in liquid culture. To study the effect of different growth factors on the short-term proliferation and differentiation of the various subsets, 1,000 cells per well were sorted into liquid medium containing different (combinations of) cytokines. The highest proliferation was found in the wells with cells from the CD34⁺/kit⁺/CD71⁺ and CD34⁺/kit⁻/CD71⁺ fractions when cultured in the presence of KL + IL-3 + GM-CSF. FACS analysis of the different fractions after 7 days of culture showed mainly erythroid (CD71⁺/CD11b⁺/RLA-DR⁻/CD10⁻/CD10d⁻) cells in the CD34⁺/kit⁺ fractions, and granulocytic (CD71⁻/CD11b⁺/RLA-DR⁻/CD10⁻/CD10d⁻) and monocytic (CD71⁻/CD11b⁺/RLA-DR⁻/CD10⁻/CD10d⁻) cells in the CD34⁺/kit⁻/CD71⁻ fractions. Examples of CD71 and RLA-DR expression on cultured erythroid cells and on cultured granulocyte and monocyte precursors are shown in Fig 5A and B, respectively. In agreement with these findings, cytospin preparations showed cells from the erythroid lineage in the CD34⁺/kit⁺ and CD34⁺/kit⁻ cell cultures, and monocyted cells in the CD34⁺/kit⁺/CD71⁻ and CD34⁺/kit⁻ cell cultures (data not shown).

To further characterize the long-term differentiation potential of the various fractions, individual cells were sorted directly into separate wells of 96-well microtiter plates and

Table 1. Colony Formation In Vitro of Different Sorted Fractions of Low-Density Rhesus Monkey BM Cells

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Region</th>
<th>Sorted Fraction</th>
<th>% Recovery</th>
<th>No. of Colonies/10³ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>V</td>
<td>CD34⁺/kit⁺</td>
<td>0.33</td>
<td>14.5 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>CD34⁺/kit⁻</td>
<td>3.89</td>
<td>181.5 ± 9.5</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>CD34⁺/kit⁻</td>
<td>2.06</td>
<td>16.0 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>CD34⁺/kit⁻</td>
<td>3.70</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>Low density</td>
<td>100</td>
<td>9.3 ± 0.2</td>
</tr>
<tr>
<td>B</td>
<td>V</td>
<td>CD34⁺/kit⁺</td>
<td>0.45</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>CD34⁺/kit⁻</td>
<td>3.30</td>
<td>101.5 ± 7.1</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>CD34⁺/kit⁻</td>
<td>2.65</td>
<td>13.5 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>CD34⁺/kit⁻</td>
<td>5.88</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>Scatter fraction</td>
<td>100</td>
<td>3.6 ± 0.4</td>
</tr>
</tbody>
</table>

Cells inside a light scatter window as shown in Fig 2C are sorted on the basis of CD34 and kit expression into regions as shown in Fig 2A. Results of two independent sorting experiments (A and B) are shown. Data from duplicate dishes are expressed as average number of colonies per 10³ cells plated ± SE (the square root of the absolute number of colonies counted; see Materials and Methods). Recovery was calculated relative to the unsorted low-density fraction (experiment A), or relative to the fraction sorted inside a light scatter window as shown in Fig 2C (experiment B).
cultured in the presence of IL-3, GM-CSF, and KL for a period of 4 weeks. No significant difference was seen between cultures with and without EPO (data not shown). Ninety percent of the wells with CD34pos/kithi cells and 81% with CD34pos/kitmed cells contained proliferating cells, whereas only 33% of the CD34pos/kitneg cells showed proliferation (Table 2). There was a large difference between the fractions with respect to the amount of cells produced in the wells and the nature of these clones. In general, the kithi clones proliferated faster and were exhausted sooner than the kitmed clones, which continued to grow up to 4 weeks after sorting. The number of cells in the kitneg clones remained very low. Microscopic inspection of the wells showed erythroid cells in the kithi clones and granulocytic and monocytic cells in the kitmed and kitneg clones. About 25% of the wells with kithi cells (Table 2) proliferated sufficiently to perform a FACs phenotyping experiment, resulting in 46 erythroid (CD71pos/CD11bneg/RhLA-DRneg); G, granulocytic (CD71neg/CD11bpos/RhLA-DRpos); and M, monocytic (CD71neg/CD11bneg/RhLA-DRpos).

DISCUSSION

Expression of Kit on murine65-68 and human69-72 BM cells has been studied extensively using Kit-specific MoAb. However, the exact expression pattern of Kit during the early stages of hematopoietic cell differentiation especially is still not clear. Differences between published results may partially be explained by the use of different MoAbs, because the ability to distinguish kithi, kitmed, and kitneg cells is influenced by the binding affinity of the Kit MoAb. Moreover, as Kit MoAb can inhibit outgrowth of kitneg cells,72,42,46,47 it

![Image](https://example.com/image1)

**Table 2. Proliferation of Different Subsets of CD34pos Low-Density Rhesus Monkey BM Cells**

<table>
<thead>
<tr>
<th>Region</th>
<th>Fraction</th>
<th>No. of Wells (of a total of 192)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CD34pos/kithi</td>
<td>173</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E: 130</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G: 46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M: 0</td>
</tr>
<tr>
<td>2</td>
<td>CD34pos/kitmed</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E: 51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G: 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M: 42</td>
</tr>
<tr>
<td>3</td>
<td>CD34pos/kitneg</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E: 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G: 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M: 0</td>
</tr>
</tbody>
</table>

Cells were incubated with biotin-KL and CD34 MoAb and sorted one cell per well into liquid medium containing IL-3, GM-CSF, and KL with or without EPO. Wells in which proliferation occurred were counted. Before reaching confluency, clones with extensive proliferation were then expanded into 1-mL cultures. Expanded clones that contained sufficient numbers of cells were stained with antibodies against CD71, CD11b, and HLA-DR.

Abbreviations: E, erythroid (CD71pos/CD11bneg/RhLA-DRneg); G, granulocytic (CD71neg/CD11bpos/RhLA-DRpos); and M, monocytic (CD71neg/CD11bneg/RhLA-DRpos).
is possible that the kit<sup>pos</sup> cells do not develop optimally in culture if high-affinity MoAbs are used for sorting. This would lead to a serious underestimation of the number of colony-forming cells in the kit<sup>pos</sup> subset. Cell staining methods based on the ligand itself cause no such inhibition. In addition, such methods by definition provide more reliable information about the capacity of the cells to bind and respond to a specific ligand than staining with MoAb against the receptor. In this study, we have used biotinylated KL to examine the expression of Kit on subsets of low-density rhesus monkey BM cells. We were able to detect Kit on 2% to 3% of the nucleated cells, similar to frequencies previously obtained for human BM using Kit MoAb. 46 Double-staining with biotin-KL and CD34 MoAb showed Kit expression on subsets of CD34<sup>pos</sup> as well as CD34<sup>low</sup> rhesus monkey BM cells.

A small fraction of CD34<sup>pos</sup> cells with a high Kit expression was detected, consisting almost exclusively of BFU-E. In line with this, CD34<sup>pos</sup>/kit<sup>th</sup> cells produced erythroblasts in liquid suspension cultures, and the kit<sup>th</sup> cells expressed high levels of the transferrin receptor. Some BFU-E were also found in the kit<sup>med</sup> population. This might reflect insufficient separation of this subset and the kit<sup>th</sup> cells, although the kit<sup>th</sup> cells are quite a distinct cluster. It is also possible that these kit<sup>med</sup> cells represent a separate population, eg, one that is more immature than the kit<sup>th</sup> BFU-E, because the kit<sup>th</sup> cells express CD34 at a lower level than the kit<sup>med</sup> population. Heterogeneity within the BFU-E population has also been observed by Simmons et al., 46 who detected a small CD34<sup>pos</sup>/kit<sup>th</sup> BFU-E fraction in sorting experiments using kit MoAb YB5.B8. Using another MoAb, NU-c-kit, Gunji et al. 46 separated CD34<sup>pos</sup> BM cells into kit<sup>th</sup>, kit<sup>med</sup>, and kit<sup>low</sup> subsets. In contrast with our results, these investigators found the highest BFU-E frequencies in the kit<sup>low</sup> subset, whereas BFU-E frequencies in the kit<sup>th</sup> subset were very low. 42 Unfortunately, no information was provided about the overall BFU-E recovery in the sorted fractions, so it cannot be ruled out that colony formation by kit<sup>th</sup> cells was underestimated. This might be in accordance with results reported by Broudy et al., 40 who showed that outgrowth of a small subset of human BFU-E is not inhibited by the Kit antibody SR-1. Such inhibition apparently does not occur with cells stained with biotinylated KL, as demonstrated by the high recovery of BFU-E and CFU-GM after sorting (Table 1).

Most CFU-E were present in the CD34<sup>low</sup>/kit<sup>pos</sup> fraction. This population was almost completely erythroid, as demonstrated by high CD71 expression. The results indicating that BFU-E as well as CFU-E display Kit correlate well with the insufficient erythropoiesis and the occurrence of macrocytic anemia in /W /W mutant mice, which do not have functional c-kii. Also in accordance with these results, Papayannopoulos et al. 48 have shown that BM cells that had been isolated via either immune adherence to the SR-1 antibody or immune rosetting using the YB5.B8 antibody were highly enriched for erythroid cells. Binding studies with 125I-KL on cultured BFU-E progeny showed that pro-erythroblasts labeled much more densely than erythroblasts. 40 Although Kit is present on both BFU-E and CFU-E, KL is necessary only for BFU-E outgrowth, but not for CFU-E and later erythroid cells. 40, 49 Collectively, the data suggest that Kit expression reaches its maximum at the BFU-E stage and gradually declines during terminal erythroid differentiation. This pattern of Kit expression appears similar to that of CD71 7 and the EPO receptor. 59, 71

The CD34<sup>pos</sup>/kit<sup>th</sup> fraction contained virtually no colony-forming cells. Although the presence of very immature cells in this fraction cannot be ruled out completely, most of the cells in this subset are more mature, based on the relatively low CD34 and high RhLA-DR expression, characteristic of activated and differentiating cells. These CD34<sup>pos</sup>/kit<sup>th</sup> cells appear to represent primarily monocyte and granulocyte precursors. In addition, part of the CD34<sup>pos</sup>/kit<sup>th</sup> fraction has low FLS and PLS properties and probably consists of B-lymphocyte precursor cells. In previous studies, Kit has been detected on only small subsets of CD34<sup>pos</sup>/CD10<sup>pos</sup> and CD34<sup>pos</sup>/CD19<sup>pos</sup> B-lymphocyte precursors. 44 This is consistent with an involvement of KL in early but not later stages of B-cell development. 14, 15, 29, 72, 73

Part of the CD34<sup>pos</sup>/kit<sup>med</sup> cells showed high CD34 and low RhLA-DR expression, a phenotype that has previously been associated with the most immature cells that can be identified in human BM. 45, 74, 75 Recently, we have shown that the CD34<sup>pos</sup>/DR<sup>atl</sup> rhesus monkey BM subset contains multipotential progenitors with high proliferative capacity. 45 Preliminary results from transplantation experiments indicate that the cells that can reconstitute lethally irradiated rhesus monkeys are also present in this subset. In accordance with the expression of Kit on immature rhesus monkey BM cells, murine BM cells expressing Kit were enriched for hematopoietic stem cells. 65, 66, 76 These results support the conclusion that Kit is already expressed at low to intermediate levels at a very early stage of hematopoiesis. Further studies focusing on subsets of CD34<sup>pos</sup>/kit<sup>med</sup>/RhLA-DR<sup>atl</sup> rhesus monkey BM cells will be useful to establish the importance of individual hematopoietic growth factors during stem cell proliferation and differentiation, and to provide candidate stem cell fractions for transplantation studies.

In summary, our data are consistent with a model in which immature, multipotent progenitors are CD34<sup>pos</sup>/kit<sup>med</sup>. Along the mononuclear lineages, these cells differentiate into CD34<sup>pos</sup>/kit<sup>med</sup> CFU-GM. Expression of Kit declines after the CFU-GM stage, and the cells lose CD34 expression. Along the erythroid lineage, CD34<sup>pos</sup>/kit<sup>med</sup> progenitors differentiate to kit<sup>th</sup> BFU-E, which gradually lose CD34 when they differentiate into CD34<sup>low</sup>/kit<sup>th</sup> CFU-E. This is followed by a gradual disappearance of Kit expression during terminal differentiation into mature red blood cells. The ability to distinguish the CD34<sup>low</sup>/kit<sup>th</sup> population provides a method to obtain highly enriched BFU-E populations that are devoid of nonerythroid cells.

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