Further Phenotypic Characterization and Isolation of Human Hematopoietic Progenitor Cells Using a Monoclonal Antibody to the c-kit Receptor

By Robert A. Briddell, Virginia C. Broudy, Edward Bruno, John E. Brandt, Edward F. Sroul, and Ronald Hoffman

A mouse antihuman monoclonal IgG antibody, termed stem cell receptor-1 (SR-1), specific for a determinant of the c-kit ligand receptor (KR), was used as an immunologic probe to analyze KR expression by human bone marrow hematopoietic progenitor cells. Monoclonal antibodies to CD34 and HLA-DR were used in a multicolor staining protocol in conjunction with SR-1 to further define the phenotypes of various classes of hematopoietic progenitor cells. Expression of KR (SR-1+) on hematopoietic progenitor cells identified subpopulations of cells expressing CD34 (CD34+). While one-half of the CD34+ and HLA-DR+ expressing cells (CD34+ HLA-DR+) expressed the KR (SR-1+), one-third of the CD34+ cells that lacked HLA-DR expression (CD34+ HLA-DR-) were SR-1+. The CD34+ HLA-DR+ SR-1+ cell population contained the vast majority of the more differentiated progenitor cells, including the colony-forming unit (CFU) granulocyte-macrophage; burst-forming unit-erythrocyte; CFU-granulocyte, erythrocyte, macrophage, megakaryocyte; and the CFU-megakaryocyte. The overall progenitor cell cloning efficiency of this subpopulation was greater than 31%. By contrast, the CD34+ HLA-DR- SR-1+ cell population contained fewer of these more differentiated progenitor cells but exclusively contained the more primitive progenitor cells, the BFU-erythro, macrophage; burst-forming unit-erythrocyte; CFU-granulocyte, erythrocyte, macrophage, megakaryocyte; and the CFU-megakaryocyte. The overall progenitor cell cloning efficiency of this subpopulation was greater than 7%. Both the CD34+ HLA-DR- and CD34+ HLA-DR+ cell subpopulations lacking KR expression contained few assayable hematopoietic progenitor cells. Long-term bone marrow cultures initiated with CD34+ HLA-DR+ SR-1+ but not CD34+ HLA-DR+ SR-1- cells, which were repeatedly supplemented with c-kit ligand (KL) and interleukin-3, generated assayable progenitor cells of at least 2 lineages for 10 weeks. These experiments demonstrate the expression of the KR throughout the hierarchy of human hematopoietic progenitor cell development. We conclude from our data that the KL and KR play a pivotal role in cytokine regulation of both the primitive and more differentiated human hematopoietic progenitor cells.

HUMAN BONE MARROW (BM) is composed of a heterogeneous population of hematopoietic precursor and progenitor cells. This broad array of cells, with differing phenotypic and functional properties, is thought to ultimately originate from primitive hematopoietic cells termed stem cells.1-6 Hematopoietic stem cells (HSCs) have not only the ability to supply mature hematopoietic cells for prolonged periods of time, but are also able to generate a continuous supply of progeny stem cells, a process referred to as self-renewal.7,8

To directly examine the properties of the human HSC, several laboratories, including our own, have attempted to develop multistep purification procedures.1,4-6,13 If this goal is accomplished, for the first time the cellular and molecular nature of HSCs may be directly examined. Several investigators, using a variety of in vitro and in vivo assays, have shown that an infrequent population of BM cells expressing CD34 and undetectable amounts of the class II major histocompatibility antigen, HLA-DR, possess many of the properties associated with HSCs.12-15 The primitive progenitor cells in this population lack expression of various other antigens associated with differentiated myeloid and lymphoid cells, have minimal uptake of Rhodamine 123 (R 123), a positively charged rhodamine analog that accumulates preferentially in cycling cells, and are resistant to in vitro treatment with 5-fluorouracil (5-FU).6,14 This phenotype is quite different from more differentiated progenitor cells that express both CD34 and HLA-DR, possess antigens associated with particular lineages, and are sensitive to in vitro 5-FU treatment.6,14

The ligand for the proto-oncogene product, c-kit, is produced by marrow stromal cells, and is capable of influencing the proliferation and development of multiple hematopoietic lineages.16-29 When acting in synergy with a number of cytokines, the c-kit ligand (KL) is capable of profoundly promoting the proliferation of several classes of the most primitive human hematopoietic progenitor cells, including the burst-forming unit-megakaryocyte (BFU-MK), high proliferative potential-colony forming cell (HPP-CFC), and long-term BM culture-initiating cell (LTBMC-IC).30-33

Recently, murine monoclonal antibodies (MoAbs) to the human and murine c-kit receptor (KR) have been developed.34-36 Ogawa et al used MoAbs to the extracellular domain of murine KR to show that most hematopoietic progenitor cells in adult mouse marrow express KR.36 This group also reported that the injection of such an MoAb into mice led to the disappearance of almost all hematopoietic progenitor cells, including the colony forming unit-spleen (CFU-S).36 An MoAb referred to as stem cell factor receptor-1 (SR-1) has been shown to block the binding of human KR to the KR.35 Normal human hematopoietic cells reactive with SR-1 morphologically resemble hematopoietic blast cells.33 Nearly 60% of the normal human hematopoietic cells that express CD34 also express the KR.35 Papayannopoulou et al have already used immunoadherence to SR-1 as a means of obtaining enriched populations

From the Hematology/Oncology Section, Department of Medicine, Indiana University School of Medicine, Indianapolis; and the Division of Hematology, Department of Medicine, University of Washington Medical School, Seattle.

Submitted December 13, 1991; accepted February 13, 1992.

Supported in part by grants from both the National Institutes of Health and the American Cancer Society.

Address reprint requests to Ronald Hoffman, MD, Indiana University School of Medicine, Department of Medicine, Hematology/Oncology Section, Medical Research and Library Building, Room 442, 975 W Walnut St, Indianapolis, IN 46202-5121.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1992 by The American Society of Hematology.

From www.bloodjournal.org by guest on August 30, 2017. For personal use only.
of human hematopoietic progenitor cells.\textsuperscript{37} Furthermore, Ashman et al, using an immune rosetting technique and a MoAb to the human KR, have also shown that the human CFU-erythrocyte (CFU-E), BFU-E, CFU-granulocyte-macrophage (CFU-GM),\textsuperscript{38} and CFU-granulocyte, erythrocyte, macrophage, megakaryocyte (CFU-GEMM) express KR.\textsuperscript{39} We used a multistep MoAb staining protocol, including SR-1, and fluorescence-activated cell sorting (FACS) to further characterize the more primitive classes of human hematopoietic progenitor cells. These results indicate that the KR is expressed on both primitive and more differentiated human hematopoietic progenitor cells and that SR-1, in conjunction with CD34 and HLA-DR antibodies, may serve as a valuable tool in identifying and purifying both of these types of human hematopoietic progenitor cells.

**MATERIALS AND METHODS**

BM aspirates were obtained under local anesthesia from the posterior iliac crests of hematologically normal volunteers. Informed consent was obtained from the donors according to guidelines previously established by the Human Investigations Committee of the Indiana University School of Medicine, which adheres to the principles of the Declaration of Helsinki.

**Cell Separation Techniques**

BM aspirates were immediately diluted 1:1 with Iscove’s Modified Dulbecco’s Media (IMDM; GIBCO Laboratories, Life Technologies, Inc; Grand Island, NY) containing 20 U sodium-heparin/mL. Low-density mononuclear cells (LDMC) were obtained by density centrifugation over Ficoll-Paque (Pharmacia LKB Biotechnology Inc, Piscataway, NJ) at 750 g. We further separated LDMC by counterflow centrifugal elutriation (CCE) to obtain those cells eluting at flow rates between 12 and 14 mL/min (FR 12-14). The FR 12-14 cell population contained greater than 70% of progenitor cells as previously described by our laboratory.\textsuperscript{35}

**Mouse MoAbs**

The following three purified mouse antihuman (m α h) MoAbs were used as immunologic probes for cell sorting experiments: (1) m α h CD34, IgG\textsubscript{1} (Becton Dickinson Immunocytometry Systems, Mountain View, CA); (2) m α h HLA-DR conjugated with phycocyanin (HLA-DR*PE), IgG\textsubscript{2a} (Becton Dickinson); (3) m α h SR-1, IgG\textsubscript{1}.\textsuperscript{35}

**Cell Labeling Techniques**

FR 12-14 cells were centrifuged at 750 g to obtain a pellet to which 1 μg m α h CD34/10\textsuperscript{6} FR 12-14 cells and 1 μg SR-1/10\textsuperscript{6} FR 12-14 cells were added for 20 minutes of incubation at 4°C. Control FR 12-14 cells were incubated with 1 μg mouse IgG\textsubscript{1}/10\textsuperscript{6} FR 12-14 cells and 1 μg mouse IgG\textsubscript{2a}/10\textsuperscript{6} FR 12-14 cells concurrently.

FR 12-14 cells were washed with 1% wt/vol bovine serum albumin (BSA; Calbiochem Corporation, La Jolla, CA) in phosphate-buffered saline (PBS). Both 1 μg goat antimouse (g α m) IgG\textsubscript{1} conjugated with Texas Red (TR; Southern Biotechnology Associates, Inc, Birmingham, AL)/10\textsuperscript{6} FR 12-14 cells and 1 μg g α m IgG\textsubscript{2a} conjugated with fluorescein isothiocyanate (FITC; Southern Biotechnology)/10\textsuperscript{6} FR 12-14 cells were added concurrently for both experimental and control cell samples for 20 minutes of incubation at 4°C.

FR 12-14 cells were washed with 1% wt/vol BSA/PBS and 10 μL mouse serum (Sigma Chemical Company, St Louis, MO)/10\textsuperscript{6} FR 12-14 cells was added to both experimental and control cell samples for 10 minutes to block any free binding sites on the two g α m second-step antibodies.

One microgram of HLA-DR*PE/10\textsuperscript{6} FR 12-14 cells was added for 20 minutes of incubation at 4°C to experimental cells while 1 μg mouse IgG\textsubscript{2a}*PE (Becton Dickinson)/10\textsuperscript{6} FR 12-14 cells was added to control cells.

Cells were washed with 1% wt/vol BSA/PBS and resuspended in this same buffer at a concentration of 10\textsuperscript{7}/mL.

**Cell Sorting Techniques**

Cells were sorted on a Coulter Epics 753 dual laser flow cytometry system (Coulter Electronics, Hialeah, FL). Sorting gates were established for both forward angle light scatter (FALS) and TR fluorescence-positive events (CD34*). A dual-parameter histogram displaying FITC (SR-1) and phycocyanin (PE) (HLA-DR) fluorescence was then generated from gated CD34* events. Using this gated histogram, sorting windows were established for both positive and negative FITC and PE fluorescence. This gating procedure allowed for the isolation of four different cell populations: CD34* HLA-DR* SR-1*; CD34* HLA-DR* SR-1*; CD34+ HLA-DR* SR-1*; and CD34* HLA-DR* SR-1+. The phenotypic purity of all four populations as determined by postsort flow cytometric analysis exceeded 95%.

The cells from each population were classified by performing differential cell counts on Wright-Giemsa-stained cytospin centrifuge cell preparations using established morphologic criteria. Each population of cells was also assayed for various classes of human hematopoietic progenitor cells using procedures described below.\textsuperscript{6,9,39,40}

**Recombinant Human Hematopoietic Cytokines**

The following three purified recombinant human hematopoietic cytokines were used as colony-stimulating factors in these experiments: (1) interleukin-3 (IL-3): specific activity (sp act) 1.0 × 10\textsuperscript{8} U/mg protein determined from mixed colony formation by human BM cells (Genzyme Corporation, Boston, MA); (2) KL: sp act 1.0 × 10\textsuperscript{8} U/mg protein determined by proliferative effects on MC6 cells (kindly provided by Immunex Corporation, Seattle, WA); (3) erythropoietin (Epo): sp act 1.0 × 10\textsuperscript{8} U/mg protein after formulation in BSA as determined by the exsypenos, polyethylyme mouse assay (Amgen Biologicals, Thousand Oaks, CA); (4) granulocyte-macrophage colony-stimulating factor (GM-CSF): sp act 5.0 × 10\textsuperscript{7} U/mg protein determined by granulocyte-macrophage colony formation from human BM cells (Genzyme); (5) GM-CSF:IL-3 fusion protein (FP): sp act 2.0 × 10\textsuperscript{8} U/mg protein determined by proliferative effects on AML193 cells (kindly provided by Immunex Corporation).

**LTBMC System**

LTBMCs lacking preestablished stromal cell layers were initiated and maintained as previously described.\textsuperscript{9,31,41} Briefly, 35-mm polystyrene tissue culture dishes containing 1 mL IMDM with 10% vol/vol fetal bovine serum (Hyclone, Logan, UT) were inoculated with 10\textsuperscript{6} of either the CD34* HLA-DR* SR-1* or the CD34* HLA-DR* SR-1* marrow subpopulations obtained after FACS and incubated at 37°C in 100% humidified 5% CO\textsubscript{2} in air. At this time, and every 48 hours thereafter, 1 mL cultures received both 1.0 ng IL-3 and 10.0 ng KL. To address the possibility that CD34* HLA-DR* SR-1* cells might be responsive to other cytokines, we established LTBMCs initiated with these cells to which either GM-CSF/KL or FP/KL were added at 48-hour intervals. These two combinations proved to be inferior to the IL-3/KL combination in progenitor cell production and length of viable culture duration from LTBMCs. At weeks 2, 4, 6, 8, 10, and 12, the cultures
were demidepopulated by removal of one-half the culture volume, which was replaced with fresh media. Cells in the harvested media were counted, divided, and assayed in both of the subsequently described progenitor cell assay systems.

**Progenitor Cell Assay Systems**

**Serum-containing methylcellulose assay system.** Sorted cells, or cells harvested after the demidepopulation of LTBMCs, were assayed for their ability to produce CFU-GM-, BFU-E-, CFU-GEMM-, and HPP-CFC-derived colonies in a serum-containing methylcellulose assay system. One nanogram of IL-3, 10.0 ng KL, and 250.0 ng Epo/mL culture were used as sources of colony-stimulating activity (CSA). Cultures initiated were incubated for 28 days at 37°C in 100% humidified 5% CO2 in air. CFU-GM-, BFU-E-, and CFU-GEMM-derived colonies were scored after 14 days, while HPP-CFC-derived colonies were scored after 28 days of culture, according to previously established morphologic criteria. HPP-CFC-derived colonies are defined both by the colony size and time of appearance in culture as previously described.

Human HPP-CFC have been shown by Sour et al to be capable of self-renewal and differentiation to multiple progenitor cells, indicative of their primitive nature.

**Serum-depleted fibrin clot assay system.** Sorted cells or cells obtained from LTBMCs were assayed for their ability to produce CFU-MK-, BFU-MK-derived colonies in a serum-depleted fibrin clot assay system. One nanogram of IL-3 and 10.0 ng KL/mL culture were used as sources of CSA. Cultures initiated were incubated for 14 or 21 days, at 37°C in 100% humidified 5% CO2 in air, to quantitate CFU-MK- and BFU-MK-derived colonies, respectively. After incubation, fibrin clots were fixed in situ in methanol:acetone (1:3) for 20 minutes, washed with PBS, and air dried.

10E5 monoclonal mouse IgG2a antibodies recognizing the human platelet glycoprotein IIb-IIIa complex (graciously provided by Dr Barry S. Coller, State University of New York, Stony Brook, Stony Brook, NY) were used as immunologic probes for identifying human megakaryocytes (MKs). 10E5 was subsequently tagged with a polyclonal, affinity-purified FITC-labeled g alpha m IgG (H + L) antiserum (Kirkegaard and Perry Laboratories, Inc, Gaithersburg, MD). The 35-mm petri dishes were inverted, and their bases were completely scanned at 100x, using an inverted microscope with reflected fluorescent light attachment (Olympus Corporation, Lake Success, NY). A CFU-MK-derived colony was defined as a cluster of three or more fluorescent cells. A BFU-MK-derived colony was described by criteria established by Long et al. These colonies appeared in marrow cultures as clusters > 42 fluorescent cells usually distributed in multiple foci of development. Human CFU-MK-derived colonies are distinguished from human BFU-MK-derived colonies by duration of incubation required for their appearance in vitro (12 days vs 21 days, respectively). Colony size (11.2 ± 1.2 cells/c colony vs 108.6 ± 4.4 cells/c colony, respectively), and foci of development (1.2 ± 0.1 foci/c colony vs 2.3 ± 0.4 foci/c colony, respectively). Statistical Analysis

Results are expressed as the mean ± SD of data obtained from multiple, separate experiments. Statistical significance was determined using the Student’s t-test.

**RESULTS**

Initially, the FR 12-14 cells obtained after CCE were analyzed for expression of CD34, HLA-DR, and the KR. Data obtained from three separate marrow specimens showed that 6.5% ± 1.4% of these FR 12-14 cells expressed CD34 (CD34+). In Fig 1, a representative flow cytometric analysis of HLA-DR and KR expression by these CD34+ cells is shown. In this particular marrow specimen, nearly 80% of the CD34+ cells expressed HLA-DR (HLA-DR+) while over 50% of the CD34+ cells expressed detectable
were SR-1+ (Table 1).

In Fig 2A, it is shown that levels of the KR (SR-1+) (Fig 1). In Fig 2B, it is shown that CD34+ HLA-DR- SR-1+ cells (Table 2) contained significant numbers of MK elements present in any of the populations analyzed (data not shown).

A morphologic analysis of the four CD34+ marrow subpopulations from two separate BM donors, fractionated on the basis of HLA-DR and KR expression, is provided in Table 2. Cell populations expressing the KR (CD34+ HLA-DR- SR-1+ and CD34+ HLA-DR+ SR-1+) largely resembled blast cells (Table 2). Cell populations not expressing the KR (CD34+ HLA-DR+ SR-1-) contained more differentiated myeloid elements (Table 2). There were no observable MK elements present in any of the populations analyzed (data not shown).

All four cell populations were directly assayed for hematopoietic progenitor cell enrichment (CFU-GM, BFU-E, CFU-GEMM, CFU-MK, BFU-MK, HPP-CFC). In Table 3, the progenitor cell enrichment of each population is shown. The majority of the most primitive progenitor cells, the BFU-MK and HPP-CFC, were detected in the CD34+ HLA-DR- SR-1+ population (Table 3). The majority of the more differentiated progenitor cells, the CFU-GM, BFU-E, CFU-GEMM, and CFU-MK, were present in the CD34+ HLA-DR+ SR-1+ cell population (Table 3). CD34+ HLA-DR+ SR-1+ cells had a cloning efficiency of 31.4%, whereas the CD34+ HLA-DR- SR-1+ cell population had an overall 7.3% cloning efficiency. Although the CD34+ HLA-DR+ SR-1+ cell population contained significant numbers of progenitor cells (16.9% cloning efficiency), the numbers were far less than its SR-1- counterpart.

Our laboratory has previously shown that CD34+ HLA-DR- cells are responsible for initiating LTBCMs. We attempted to determine whether the CD34+ HLA-DR- cells possessing this ability express the KR. The cellular production by each type of LTBCM (CD34+ HLA-DR- SR-1-, CD34+ HLA-DR+ SR-1+) over a 12-week period is shown in Fig 3. LTBCMs initiated with CD34+ HLA-DR- cells expressing the KR produced greater than 25 times the number of cells (2.5 × 10^6) as LTBCMs initiated with cells lacking KR expression (1.0 × 10^5). This number represents a 250-fold increase in cell number when compared with the starting cellular inoculum (1.0 × 10^5) (Fig 4).

The ability of these LTBCMs to produce assayable progenitor cells is shown in Figs 4, 5, and 6. The CD34+ HLA-DR- SR-1+-initiated LTBCMs produced greater than 6.0 × 10^5 CFU-GM during the period of culture, indicating a greater than 10-fold increase in the number of these progenitor cells from the initial cellular inoculum (550.0 ± 50.0) (Fig 4). In Fig 5A and B, progenitor cell production of other lineages is shown for both LTBCMs. Far greater production of BFU-E, CFU-GEMM, and CFU-MK was observed in the LTBCM initiated with CD34+ HLA-DR- SR-1+ cells (Fig 5A and B). BFU-E and CFU-GEMM production by these cultures was sustained for 4 weeks, whereas CFU-MK generation persisted for 10 weeks. In Fig 6, the number of assayable HPP-CFC generated during the period of culture is shown. The CD34+ HLA-DR- SR-1+ population was solely responsible for HPP-CFC production (Fig 6). In fact, the CD34+ HLA-DR- SR-1+-initiated LTBCMs generated nearly a threefold increase in HPP-CFC numbers over the initial cellular inoculum (708.0 ± 70.0) (Fig 6).

**DISCUSSION**

The anatomy and physiology of human hematopoiesis is extremely complex. To further dissect the central mechanisms that lead to effective, sustained blood cell production, the properties of the human HSC and HSC-derived progen-
stem cells express c-kit receptor

Various classes of the most primitive human hematopoietic and progenitor cells require direct examination. With this goal in mind, significant progress has been made toward the isolation of these cells. Previously, isolation of BM subpopulations has largely relied on the use of physical properties, namely the uptake of photosensitive dyes or reactivity to MoAbs directed to lineage-specific surface antigens. Such efforts in vitro and in vivo assays have been developed to assess the capabilities of the most primitive progenitor cells. Previouisly, isolation of BM subpopulations has largely relied on the use of physical properties, namely the uptake of photosensitive dyes or reactivity to MoAbs directed towards lineage-specific surface antigens. Such efforts have resulted in important strides being made in both stem and progenitor cell isolation.

A growing number of ligand-receptor relationships are now known to play a pivotal role in the regulation of hematopoiesis. Receptors for several hematopoietic growth factors have been characterized initially using human leukemia cell lines. These receptors have eventually been shown to be present on various normal hematopoietic precursor, progenitor, and terminally differentiated cells.

The KL is known to augment the proliferative signals of a number of cytokines when acting on both the primitive and more differentiated human hematopoietic progenitor cells. Several groups have shown that the action of KL is, in part at least, a direct one because marrow accessory cells are not required for this synergistic action. The breadth of response of KL on progenitor cells of various lineages, in addition to its action on the HPP-CFC and LTBMIC, suggests that the true human HSC might express the KR. This hypothesis does not eliminate the possibility of the existence of an even more primitive cell, a human pre-HSC that lacks KR expression. Our ability to define the existence of this cell might potentially be limited by the availability of assays to detect such a cell.

With these reservations in mind, we and others have proposed to use anti-KR antibodies to further isolate human hematopoietic progenitor cells. The availability of MoAbs to the human KR have only now made this approach feasible. Ashman et al and Papayannopoulos et al have already used such reagents to initially characterize KR-expressing cells present in normal adult and fetal hematopoietic tissues. In the present report, additional information concerning the KR status on human BM cells is provided. These data indicate the scope of expression of KR on both primitive and differentiated human hematopoietic progenitor cells and indicate the potential use of such antireceptor antibodies as a means of further purifying candidate stem and progenitor cells.

Table 3. Hematopoietic Progenitor Cells Present in Various Cell Populations

<table>
<thead>
<tr>
<th>Population</th>
<th>CFU-GM*</th>
<th>BFU-E*</th>
<th>CFU-GEMM*</th>
<th>CFU-M0*</th>
<th>BFU-M0*</th>
<th>HPP-CFC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34+ HLA-DR- SR-1-</td>
<td>9.2 ± 3.0</td>
<td>3.2 ± 1.0</td>
<td>0.5 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>5.0 ± 1.0</td>
<td>8.2 ± 2.0</td>
</tr>
<tr>
<td>CD34+ HLA-DR+ SR-1-</td>
<td>55.0 ± 6.0</td>
<td>24.0 ± 10.0</td>
<td>5.2 ± 1.0</td>
<td>0.0 ± 0.0</td>
<td>22.0 ± 2.0</td>
<td>70.8 ± 7.0</td>
</tr>
<tr>
<td>CD34+ HLA-DR- SR-1-</td>
<td>32.8 ± 15.0</td>
<td>23.2 ± 10.0</td>
<td>2.8 ± 2.0</td>
<td>5.0 ± 1.0</td>
<td>0.0 ± 0.0</td>
<td>5.0 ± 3.5</td>
</tr>
<tr>
<td>CD34+ HLA-DR+ SR-1-</td>
<td>170.0 ± 10.0</td>
<td>99.8 ± 4.0</td>
<td>29.0 ± 0.5</td>
<td>25.0 ± 7.0</td>
<td>0.0 ± 0.0</td>
<td>12.2 ± 8.0</td>
</tr>
</tbody>
</table>

Values are the mean number of derived colonies ± SD of data obtained from duplicate assays performed on two separate occasions.

*Sorted cells were cultured at a concentration of 10^3/mL in a serum-containing methylcellulose assay system with 1.0 ng IL-3/mL, 10.0 ng KL/mL, and 250.0 ng Epo/mL.

†Sorted cells were cultured at a concentration of 10^3/mL in a serum-depleted fibrin clot assay system with 1.0 ng IL-3/mL and 10.0 ng KL/mL.
The presence of KR on the BFU-MK, HPP-CFC, and LT-BMC-IC was also demonstrated. The BFU-MK and HPP-CFC are already known to be CD34+ HLA-DR- 9,30 Between 80% and 90% of these progenitors were shown to express the KR (Table 2). Whether functional differences exist between the SR-1- and SR-1+ BFU-MK or HPP-CFCs or whether the SR-1- BFU-MK or HPP-CFCs precede their SR-1+ counterparts in the hierarchy of stem cell development is unknown.

These experiments demonstrate the expression of KR on human hematopoietic progenitor cells present throughout the hierarchy of hematopoietic development. We conclude from our data that KL and KR not only are important regulators of more differentiated classes of human hematopoietic progenitor cells but also play a pivotal role in cytokine regulation of the most primitive classes of human hematopoietic progenitor cells.

ACKNOWLEDGMENT

The authors thank Stephanie McGillem and Deborah Navarro for their excellent secretarial assistance in the preparation of this manuscript.

REFERENCES


27. Copeland NG, Gilbert DJ, Cho BC, Donovan PJ, Jenkins NA, Cosman, D, Anderson D, Lyman SD, Williams DE: Mast cell growth factor maps near the steel locus on mouse chromosome 10 and is deleted in a number of steel alleles. Cell 63:175, 1990
37. Papayanopoulos T, Brice M, Broudy VC, Zeebo KM: Isolation of c-kit receptor-expressing cells from bone marrow.
Expression of the YB5, B8 antigen (c-kit proto-oncogene product) in normal human bone marrow. Blood 88:371, 1988


67. Poelmacher RE, Brons NHC: Cells with marrow and spleen repopulating ability and forming spleen colonies on day 16, 12 and 8 are sequentially ordered on the basis of increasing rhodamine-123 retention. J Cell Physiol 136:531, 1988


75. Broudy VC, Lin N, Egrie J, deHaen C, Weiss T, Papayannopoulou T, Adamson JW: Identification of the receptor for erythro-
poietin on human and murine erythroleukemia cells and modulation by phorbol ester and dimethylsulfoxide. Proc Natl Acad Sci USA 85:6513, 1988


79. Bernstein ID, Andrews RG, Zsebo KM: Recombinant human stem cell factor (SCF) enhances the formation of colonies by CD34+ and CD34+ line cells, and the generation of colony-forming cell progeny from CD34+ lin− cells cultured with IL-3, G-CSF, or GM-CSF. Blood 77;2316, 1991
Further phenotypic characterization and isolation of human hematopoietic progenitor cells using a monoclonal antibody to the c-kit receptor

RA Briddell, VC Broudy, E Bruno, JE Brandt, EF Srour and R Hoffman