Several studies have previously demonstrated enrichment in primitive progenitor cells in subfractions of CD34+ bone marrow (BM) cells not expressing CD38 or HLA-DR (DR) antigens. However, no studies have directly compared these two cell populations with regard to their content of primitive and more committed progenitor cells. Flow cytometric analysis of immunomagnetic isolated CD34+ cells demonstrated little overlap between CD34+/CD38- and CD34+/DR+ progenitor subpopulations in that only 12% to 14% of total CD34+/DR+ and CD34+/CD38- cells were double negative (CD34+/CD38-). Although the number of committed myeloid progenitor cells (colony-forming units granulocyte-macrophage) was reduced in both subpopulations, only CD34+/CD38- cells were significantly depleted in committed erythroid progenitor cells (burst-forming units-erythroid). In single-cell assay, CD34+/CD38- cells showed consistently poorer response to single as opposed to multiple hematopoietic growth factors as compared with unfractionated CD34+ cells, indicating that the CD34+/CD38- subset is relatively enriched in primitive hematopoietic progenitor cells. Furthermore, CD34+/CD38- and CD34+/DR- cells, respectively, formed 2.2-fold and 1.8-fold more high proliferative potential colony-forming cell (HPP-CFC) colonies than did unfractionated CD34+ cells. Finally, CD34+/CD38- DR- cells were depleted in HPP-CFCs as compared with CD34+/CD38- DR+ cells. The results of the present study suggest that both the CD38- and DR- subfractions of CD34+ bone marrow cells are enriched in primitive hematopoietic progenitor cells, with the CD34+/CD38- subpopulation being more highly enriched than CD34+/DR- cells.

Hematopoiesis involves the continuous production of mature blood cells of different lineages from pluripotent stem cells in the bone marrow (BM). The BM contains a hierarchy of progenitor cells at various levels of maturation with gradual overlap between different compartments. Pluripotent stem cells are defined by the ability of both self-renewal and multilineage differentiation. The estimated frequency of pluripotent stem cells is very low, and such cells have therefore been difficult to identify and characterize. However, during the past decade, major progress has been made in enriching and purifying stem cells or primitive progenitor cells in the mouse. In humans, it has been shown that the CD34 antigen is expressed on committed and primitive hematopoietic progenitor cells, presumably also including the pluripotent stem cells. Thus, enriched CD34+ marrow cells can reconstitute hematopoiesis in vivo in humans and nonhuman primates.

The CD34+ subfraction of human BM, although representing only 1% to 4% of BM mononuclear cells, contains virtually all hematopoietic progenitor cells. However, this highly enriched progenitor cell population is heterogeneous, and the most primitive progenitors detectable in vitro, such as the high proliferative potential colony-forming cells (HPP-CFCs) or the long-term culture-initiating cells (LTC-ICs) are only present in low frequencies in CD34+ BM cells. A number of techniques have been developed to further enrich for the most primitive BM progenitor cells, taking advantage of differences in antigen expression, as well as in rhodamine 123 and Hoechst staining.

Several studies have demonstrated enrichment in primitive progenitor cells in subfractions of CD34+ BM cells not expressing DR or CD38 antigens. Thus, both CD34+DR- and CD34+CD38- BM cells are widely used for studies on primitive BM progenitor cells. However, no studies have directly compared these two populations with regard to their content of primitive and more committed hematopoietic progenitor cells. The present study was undertaken to determine the overlap between these two progenitor cell populations in adult human BM, both with regard to antigen expression and functional capacity. Flow cytometric analysis demonstrated little overlap between the CD34+/DR- and CD34+/CD38- subpopulations, ie, the majority of CD34+/CD38- cells being DR+ and the CD34+/DR- cells being CD38+. Although both populations were enriched in primitive hematopoietic progenitor cells, significant functional differences were observed.

Materials and Methods

Growth factors. Purified recombinant human granulocyte colony-stimulating factor (rHuG-CSF) and rHu stem cell factor (SCF) were generously supplied by Dr Ian K. McNiece (Amgen Inc, Thousand Oaks, CA), rHu granulocyte-macrophage-CSF (rHuGM-CSF), rHu interleukin-3 (rHuIL-3), and rHu PIXY321 (a GM-CSF/IL-3 fusion protein) were generously provided by Dr Steven Gillis (Immunex Corp, Seattle, WA). rHuIL-1α were obtained from Hoffmann-LaRoche (Basel, Switzerland), rHu erythropoietin (rHuEpo) was purchased from Clalg AG (Schafthausen, Switzerland). Unless otherwise indicated, all growth factors were used at predetermined optimal concentrations: rHuG-CSF (20 ng/mL), rHuGM-CSF (50 ng/mL), rHuIL-3 (20 ng/mL), rHuPIXY321 (10 ng/mL), rHuSCF (50 ng/mL), Epo (5 IU/mL), and rHuIL-1α (20 ng/mL).

Cells. BM cells were obtained by iliac crest aspiration from normal adult volunteers with informed consent and the approval of the Ethics Committee of The Norwegian Radium Hospital. Mononuclear cells were isolated by Ficoll-Hypaque gradient centrifugation (Lymphoprep; Nycomed, Oslo, Norway). Positive selection of CD34+ cells and subsequent separation of CD34+DR- and CD34+CD38- subpopulations was performed using monoclonal antibody (mAb) conjugated to magnetic beads (Dynal, Oslo, Norway). Functional capacities of isolated cell populations were measured in single-cell assay, and cell viability was assessed by trypan blue staining.

Results

Flow cytometric analysis of immunomagnetic isolated CD34+ cells demonstrated little overlap between CD34+/CD38- and CD34+/DR+ progenitor subpopulations in that only 12% to 14% of total CD34+/DR+ and CD34+/CD38- cells were double negative (CD34+/CD38-). Although the number of committed myeloid progenitor cells (colony-forming units granulocyte-macrophage) was reduced in both subpopulations, only CD34+/CD38- cells were significantly depleted in committed erythroid progenitor cells (burst-forming units-erythroid). In single-cell assay, CD34+/CD38- cells showed consistently poorer response to single as opposed to multiple hematopoietic growth factors as compared with unfractionated CD34+ cells, indicating that the CD34+/CD38- subset is relatively enriched in primitive hematopoietic progenitor cells. Furthermore, CD34+/CD38- and CD34+/DR- cells, respectively, formed 2.2-fold and 1.8-fold more high proliferative potential colony-forming cell (HPP-CFC) colonies than did unfractionated CD34+ cells. Finally, CD34+/CD38- DR- cells were depleted in HPP-CFCs as compared with CD34+/CD38- DR+ cells. The results of the present study suggest that both the CD38- and DR- subfractions of CD34+ bone marrow cells are enriched in primitive hematopoietic progenitor cells, with the CD34+/CD38- subpopulation being more highly enriched than CD34+/DR- cells.
CD34+CD38− cells were performed according to earlier described methods. Briefly, BM mononuclear cells were resettled with Dynabeads M-450 directly coated with the anti CD34 monoclonal antibody (MoAb) BI-3CS (Product No. 111.10; Dynal, Oslo, Norway) for 45 minutes at 4°C on an apparatus that provided tilting and gentle rotation. The bead to cell ratio was 1:1. Rosetted cells were attracted to a samarium cobalt magnet and washed seven times. Nonrosetting cells were removed by pipetting. Detachment of beads from positively selected cells was performed by incubation with anti-Fab antiserum (DETAChAEBead; Dynal) at a concentration of 35 mg/mL for 1 hour at room temperature. Isolated cells, free of beads, were washed and counted. The purity of cells isolated by this method was reproducibly greater than 90% CD34+ as determined by flow cytometric analysis. CD34+ cells with undetectable or very low expression of DR (for convenience called CD34+DR− cells) were obtained by incubation of CD34+ cells with the anti-DR MoAb L243 (American Type Culture Collection, Rockville, MD; 2.5 μg antibody/106 cells) for 30 minutes at 4°C. After washing, Dynabeads M-450 coupled with sheep antirabbit IgG were added (bead to cell ratio 25:1) and incubated in a volume of 0.5 mL for 30 minutes at room temperature on an apparatus that provided gentle rotation. Rosetted cells were then attracted to a magnet, and nonrosetting cells were collected. This cycle was repeated once. CD34+ cells with undetectable or very low expression of CD38 (called CD34+CD38− cells) were obtained by incubation of isolated CD34+ cells with the anti-CD38 MoAb MAS-224 (Serotec, Oxford, UK; 5 μg antibody/106 cells), followed by the same procedure as for isolation of CD34+DR− cells.

Morphologic classification of isolated cells was performed by differential counts on May-Grünwald-Giemsa-stained cytocentrifuge cell preparations.

Colony formation in semisolid media. Cells (1 × 105CD34+CD38−, or CD34+DR− cells per tissue-culture grade 35-mm Petri dish) were plated in a volume of 1 mL Iscove’s modified Dulbecco’s medium (IMDM; GIBCO, Paisley, UK) containing 20% fetal calf serum (FCS; Sera-lab, Sussex, UK) 1.2% methylcellulose (Methodol 4,000,000 s-1; Fluka AG, Buchs, Switzerland), 5 × 10−5 mol/L 2-mercaptoethanol, 300 mg/L glutamine, 66 μg/L penicillin, and 100 μg/L streptomycin, and recombiant human growth factors as indicated. After 2 weeks of incubation at 37°C and 5% CO2 in air, colonies (>40 cells) derived from granulocyte-macrophage colony-forming units (CFU-GM), erythroid burst-forming units (BFU-E), and mixed granulocyte-megakaryocyte colony-forming units (CFU-Mix) were assessed according to established criteria and counted using an inverted microscope.

HPP-CFCs. The assay for HPP-CFCs was performed in double-layer nutrient Seaplaque agarose culture as previously described. Modified minimum essential medium (MEMα) (GIBCO) was used, supplemented with vitamins, L-glutamine, penicillin/streptomycin, and 20% FCS (Sera-lab). Growth factors were included in a 0.5-mL overlayer with a final agarose concentration of 1% (the adherent layer was trypsinized) were seeded in methylcellulose cultures in presence of predetermined optimal concentrations of G-CSF, PIXY321, SCF, and Epo. Colonies were scored after 10 to 14 days of incubation at 37°C and 5% CO2 in air.

Flow cytometry and cell sorting. Three-color staining of immunomagnetically isolated CD34+ cells was performed with anti-CD34 MoAb (HPCA-2; Becton Dickinson, San Jose, CA) directly conjugated to phycoerythrin (PE), anti-CD38 MoAb (I0B6; Immunotech, Marseilles, France) directly conjugated to fluorescein isothiocyanate (FITC), and anti–HLA-DR MoAb (L243; Becton Dickinson) directly conjugated to peridinin chlorophyll protein (PerCP). Two-color staining of negatively selected subfractions of CD34+ cells was performed with PE-conjugated anti-CD34 (Becton Dickinson) and FITC-conjugated anti–HLA-DR (CR3/43; Dakopatts, Copenhagen, Denmark) or FITC-conjugated anti–CD38 (Immunotech). Isootype-matched, FITC−, PerCP−, and PE-conjugated irrelevant mouse MoAbs served as controls. Cells were analyzed on a FACScan flow cytometer (Becton Dickinson) with an argon-ion laser tuned at 488 nm. Data acquisition and analysis were performed using Lysis II and Paint-A-Gate software (Becton Dickinson, San Jose, CA).

To obtain cells with the CD34+CD38−DR− or CD34+CD38+DR− phenotypes by cell sorting, positively selected CD34+ cells were stained with PE-conjugated anti-CD34 (Becton Dickinson), FITC-conjugated anti-CD38 (Immunotech), and FITC-conjugated anti–HLA-DR (Dakopatts). Cell sorting was performed on an Epics Elite cell sorter (Coulter Electronics, Hialeah, FL). A sort gate within a dual-parameter cytogram of forward light scatter against 90° side scatter was drawn. A second amorphous gate was drawn on two-color cytograms, and sort equations were set to positively sort cells satisfying both gates.

Statistical analysis. The data on HPP-CFC colony formation was binomially distributed as assessed by an ordinary χ2 variance test. Thus, the probability of significant differences between unfraccionated CD34+ cells, CD34+CD38− cells, and CD34+DR− cells in this regard was determined using simple contingency tests. Otherwise, after assessing approximate normality of the distributions, the probability of significant differences when comparing multiple groups was examined by using analysis of variance in the Student-Newman-Keuls approximation. Whenever significant deviations from the hypothesis of equality was detected, groups were tested in pairs by binomial tests or by two-sample t-tests, respectively, for mutually significant differences. P values <.05 were considered significant.

RESULTS

Flow cytometric analysis of positively selected CD34+ cells. We have previously reported isolation of human BM CD34+ cells by immunomagnetic beads resulting in a greater than 90% pure population of CD34+ cells. These cells have...
an intact antigenic profile and can easily be stained with a number of MoAbs. In the present study, double staining of positively selected CD34+ cells with PE-conjugated anti-CD38 (Becton Dickinson) and FITC-conjugated anti-DR (Dakopats) demonstrated consistently little overlap between CD38 and DR+ subpopulations. In eight experiments, a mean (SD) of 2.6% (1.9%) of CD34+ cells was CD38 'DR+', 2.3% (1.9%) CD38+DR-, 94.3% (3.3%) CD38-DR-, and 0.8% (0.6%) CD38-DR+. Thus, double-negative (CD38-DR-) cells constituted only 14% of total CD38+ and DR- cells.

To confirm that the majority of CD38- and DR- cells indeed expressed the CD34 antigen, three-color staining was next performed (n = 3) to examine the simultaneous expression of CD34, DR, and CD38 antigens on the isolated CD34+ cells. A representative experiment is presented in Fig 1. As can be seen, contaminating CD34+ cells were almost exclusively DR+, whereas 71% of the CD34+ cells expressed the CD38 antigen. Similar results were observed in two other experiments. Flow cytometric analysis of the CD34- subset staining weakly positive or negative for CD38 (Fig 1) demonstrated that these cells fell into the lymphocyte light scatter gate (data not shown) and expressed high levels of DR, suggesting that they in fact were lymphocytes. Furthermore, only 12% of total CD34-CD38- and CD34+DR- cells were CD34+CD38-DR-, confirming the observation that most of the CD34-CD38- cells in human BM express DR antigens, and that most of the CD34+DR- cells express CD38. Finally, no or low expression of CD38 was associated with high CD34 antigen density, whereas cells with high expression of CD38 stained heterogeneously for CD34 (Fig 1).

Isolation of CD34+CD38- and CD34+DR- cells by immunomagnetic selection. CD34+CD38- and CD34+DR- cells were obtained from CD34+ cells by negative selection using immunomagnetic beads. The median yield of CD34+DR- cells was 2.7% (range, 0% to 18%; n = 15) and of CD34+CD38- cells 4.0% (range, 1.1% to 16%; n = 13) of unfractionated CD34+ BM cells. In two experiments, morphologic examination of cytospin preparations showed that 81% to 87% of the cells in both subfractions were primitive blasts, the contaminating cells being mainly mature lymphocytes, but some normoblasts and mature myeloid cells were also present. The CD34+CD38- cells were strikingly homogeneous with regard to size, whereas CD34+DR- cells showed considerable heterogeneity in this respect (Fig 2).

Flow cytometric analysis of negatively selected CD34+ and DR- subpopulations of CD34+ cells. We next performed flow cytometric analysis to examine the pattern of CD34 and DR expression on isolated CD34+ cells, as well as CD34+CD38- cells in Fig 1). negatively selected CD34+CD38- cells were almost exclusively DR+, whereas the majority of CD34+DR- cells expressed high levels of CD38 (Fig 1), supporting little overlap between CD34+CD38- and CD34+DR- subpopulations. This was further substantiated by CD34+CD38- cells having uniformly high expression of CD34, whereas increasing expression of CD38 on CD34+DR- cells was associated with decreased CD34 antigen density (Fig 3). Finally, CD34+DR- cells stained more heterogeneously for CD38 than did CD34+CD38- cells for DR.
Fig 2. Morphology of cells from the CD38- (A) and DR- (B) subsets of CD34+ BM cells. The cells were isolated, prepared, and stained as described in Materials and Methods. Original magnification × 1,000.

Fig 3. Flow cytometric analysis of negatively selected CD38- (A and B) and DR- (C and D) subfractions of CD34+ BM cells; two-color fluorescence staining with PE-conjugated anti-CD34, FITC-conjugated anti-DR, or FITC-conjugated anti-CD38 MoAbs (Materials and Methods). The control cells were stained with irrelevant mouse MoAbs as described in Materials and Methods. Gates were set to include 97.5% of the control cells in the double-negative population. The dot plots represent the collection of 5,000 (B) or 10,000 (D) events. Data show one representative experiment of three.
CD38- and DR- SUBFRACTIONS OF CD34+ BM CELLS

Table 1. Colony Formation of DR- and CD38-

Subfractions of CD34+ BM Cells

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Cells</th>
<th>CFU-GM</th>
<th>BFU-E</th>
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<tbody>
<tr>
<td>1</td>
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<td>44</td>
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</tr>
<tr>
<td></td>
<td>CD34+CD38-</td>
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<td>76</td>
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<td>11</td>
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<td>7</td>
</tr>
<tr>
<td></td>
<td>CD34+DR-</td>
<td>19</td>
<td>62</td>
</tr>
</tbody>
</table>

Cells were isolated and plated in methylcellulose cultures as described in Materials and Methods at 1 × 10^5 cells/plate. Individual cultures were supplemented with PIXY321 (2 ng/mL), SCF (100 ng/mL), and Epo (5 IU/mL). Cultures were scored for colony growth (>40 cells) after 14 days of incubation at 37°C and 5% CO2 in air. Results are presented as the median number of colonies per 1 × 10^4 cells from three independent experiments with triplicate determinations.

In contrast, the BFU-E content of the CD34+DR- subset was not significantly different from that of the unfracti-
nated CD34+ cells (P = .15). Thus, the CD34+DR- sub-
population is enriched in committed erythroid progenitors as compared with the CD34+CD38- subpopulation. The relative number of committed myeloid progenitor cells (CFU-GM) in the CD34+CD38- fraction was reduced by 75% (P < .0001) when compared with the unfracti-nated CD34+ BM cells (Table 1). In contrast to the BFU-E content, the number of CFU-GM was significantly reduced (61%; P < .0001) also in the CD34+DR- population.

Committed hematopoietic progenitors respond well to single hematopoietic growth factors (HGFs), whereas more primitive progenitors typically require multiple HGFs for optimal proliferation. Thus, the differential responses to single as opposed to multiple HGFs can be used as an indication of the relative proportion of mature and primitive cells in given cell populations. Therefore, the direct proliferative effects of single as well as multiple HGFs were investigated on single CD34+CD38-, CD34+DR-, and unfracti-
nated CD34+ BM cells (Fig 4). Thirty-one percent of individually plated unfracti-nated CD34+ cells proliferated in response to a potent synergistic growth factor combination (G-CSF + PIXY321 + SCF), whereas 24% and 19% of CD34+CD38- and CD34+DR- cells, respectively, responded to the same combination. Although G-CSF alone stimulated the growth of 16% of unfracti-nated CD34+ cells, only 7% of the CD34+CD38- and 6% of the CD34+DR- cells re-
sponded to G-CSF (Fig 4). Thus, the relative response to G-

CSF as opposed to multiple HGFs was significantly higher for unfracti-nated CD34+ cells than for CD34+CD38- cells (P = .012), and marginally higher for CD34+DR- cells (P = .06). Ten percent of unfracti-nated CD34+ cells proliferated in response to IL-3, whereas 4% of CD34+DR- and 3% of CD34+CD38- cells responded to IL-3. The relative response to IL-3 versus multiple HGFs was significantly lower for CD34+CD38- cells than for both unfracti-nated CD34+ cells (P = .009) and CD34+DR- cells (P = .02), whereas the latter two did not differ in this respect. Thus, both the CD34+DR- and CD34+CD38- subfractions con-
tained relatively less clonogenic cells than unfracti-nated CD34+ cells, and CD34+CD38- cells showed consistently poorer response to single growth factors as opposed to multi-
ple HGFs when compared with unfracti-nated CD34+ cells, indicating that the CD34+CD38- subset is relatively en-
riched in primitive hematopoietic progenitor cells.

To better compare the content of primitive progenitor cells in the CD34+CD38- and CD34+DR- subsets of CD34+ BM cells, we next investigated the number of HPP-CFCs and LTC-ICs in these populations, both of which have been shown to be among the most primitive progenitors that can be grown in vitro. In response to a five-factor combi-
nation of HGFs (G-CSF + IL-3 + GM-CSF + SCF + IL-1α), CD34+CD38- and CD34+DR- BM cells, respectively, formed 3.2-fold and 1.6-fold more HPP-CFC colonies than unfracti-nated CD34+ cells, demonstrating a significant enrich-
ment in HPP-CFCs in the CD34+CD38- subset as com-
pared with unfracti-nated CD34+ cells (P < .001), as well as in the CD34+CD38- subset versus CD34+DR- cells (P < .001; Fig 5). Furthermore, the results of two experiments shown in Table 2 indicate a similar enrichment in LTC-ICs in the CD34+CD38- and CD34+DR- subfractions, which is equivalent to a 200-fold to 400-fold enrichment in LTC-ICs as compared with BM mononuclear cells (data not shown). With a prolonged culture period (8 weeks), the CD34+CD38- subset had greater potential for generating clonogenic cells in LTC than did CD34+DR- cells. Combined, the results
from the HPP-CFC and LTC-IC assays suggest that both the CD34+CD38- and CD34+DR- subfractions of BM cells are enriched in primitive hematopoietic progenitor cells, with the CD34+CD38- subpopulation being more highly enriched than CD34+DR- cells.

In vitro culture of CD34+CD38-DR- cells. In light of a previous study suggesting that very primitive stem cells are contained within the CD34+CD38-DR- subpopulation of fetal BM cells,35 we finally performed experiments comparing the ability of sorted cells with this phenotype to form HPP-CFC colonies as compared with sorted CD34+CD38-DR- cells as well as un fractionated CD34+ cells. As shown in Fig 6, CD34+CD38-DR- cells formed 1.9-fold more HPP-CFC colonies than CD34+CD38-DR- cells (P < .05), suggesting that the latter cell type is depleted in HPP-CFCs. Furthermore, single-cell experiments showed that 12% of CD34+CD38-DR- cells proliferated in response to G-CSF + PIXY321 + SCF, whereas 4% responded to G-CSF and 3% responded to IL-3 (Fig 7). In contrast, 47% of CD34+CD38-DR+ cells proliferated in response to G-CSF + PIXY321 + SCF, 19% responded to G-CSF, and 14% responded to IL-3. Finally, whereas 12% of individually plated CD34+CD38-DR- cells formed erythroid colonies (>40 cells) in response to SCF + Epo, only 1% of SCF + Epo-stimulated CD34+CD38-DR- cells had erythroid colony-forming ability (Fig 7).

DISCUSSION

Both CD34+CD38- and CD34+DR- human BM cells have been shown to be enriched in primitive hematopoietic progenitor cells. Thus, both populations, and in particular CD34+DR- cells, have been widely used as a source of primitive progenitors.2,15,16,21,22,36,37 In this regard, the enrichment in primitive progenitor cells in the CD38- and DR-
cells would be removed in the negative selection procedure, the vast majority of CD34- primitive progenitors than the conclusion that this cell population is more enriched in primitive progenitors and depleted in committed myeloid progenitors. Evaluation of light scatter profile (data not shown) here that, although both subpopulations determined optimal concentrations of the growth factors indicated. Cultures were scored for proliferation (>10 cells) or SCF + Epo-induced erythroid colony growth (Materials and Methods) after 14 days at 37°C and 5% CO2 in air. Each group consisted of 300 wells. Results are presented as the mean number of positive wells in three separate experiments; error bars show the SEM.

Subfractions of CD34+ cells demonstrated in the present study was comparable to previous reports. We show here that, although both subpopulations are enriched in primitive progenitors and depleted in committed myeloid progenitors, there are significant differences between the two as well. First, three-color flow cytometric analysis of isolated CD34+ cells demonstrated little overlap between CD34+CD38- and CD34+DR- subpopulations. Second, evaluation of light scatter profile (data not shown) as well as pattern of CD34 expression and morphologic examination demonstrated that CD34+CD38- cells represent a more homogeneous population than do CD34+DR- cells. Third, CD34+DR- cells were enriched in BFU-E as compared with CD34+CD38- cells. Finally, although both populations were enriched in primitive progenitors as compared with unfractionated CD34+ BM cells, the CD34+CD38- population contained relatively more HPP-CFCs and LTC-ICs than did CD34+DR- cells.

Increasing density of the CD38 antigen as well as decreasing levels of CD34 expression have been described as features of progenitor cell differentiation. In the present study, CD34+DR- cells displayed considerable heterogeneity with regard to CD34 expression. In contrast, the uniformly high expression of CD34 on CD34+CD38- cells might support the conclusion that this cell population is more enriched in primitive progenitors than CD34+DR- cells.

It has previously been shown that the contaminating CD34+ cells in this system mainly consist of CD19+ mature B lymphocytes. In agreement with this, we found that the vast majority of CD34- cells were DR+. Thus, these cells would be removed in the negative selection procedure, ensuring high purity of the resulting CD34+DR- cells. Although a majority of CD34- cells expressed the CD38 antigen, a subpopulation of contaminating lymphocytes was CD38+. However, this potential impurity of negatively selected CD34+CD38- cells should not influence the validity of our results, because practically all hematopoietic progenitor cells reside in the CD34+ compartment, and because experiments were also performed in single-cell assays to avoid indirect effects. If anything, the enrichment in primitive hematopoietic progenitors in the CD34+CD38- subpopulation has been underestimated, whereas the relative response to single versus multiple HGFs in single-cell assay should not be significantly affected.

The depletion in committed erythroid progenitors in the CD34+CD38- subset observed in the present study is in agreement with previous reports demonstrating that the vast majority of committed erythroid progenitors expresses the CD38 antigen. Using both cell sorting and complement-dependent cytotoxicity assays, several authors have previously reported the expression of monomorphic DR determinants on committed erythroid progenitor cells (BFU-E). However, the existence of BFU-E not expressing DR antigens is supported by the present study as well as by recent studies of Verfaillie et al demonstrating growth of a substantial number of BFU-E colonies from sorted lineage-negative CD34+DR- BM cells.

The expression of both DR and CD38 antigens is known to increase on the cell surface of B and T lymphocytes after activation. Similarly, it has been suggested that upregulation of DR and CD38 antigens are early events in the differentiation of primitive BM progenitor cells. Several studies have shown that greater than 95% of unfractionated CD34+ BM cells express the CD38 antigen, which is in agreement with our results. However, conflicting data have been reported with regard to expression of DR antigens on CD34+ BM cells, ranging from 75% to 97.5%. This variation might reflect various levels of detection of different methods. Alternatively, because DR is known to be an activation antigen, it could be explained by cell separation procedures influencing the activation status of the cells differently. Furthermore, as observed in this study, interindividual variations in expression of DR antigens on hematopoietic progenitor cells exist as well.

The enrichment in HPP-CFCs and LTC-ICs in the CD34+CD38- population when compared with CD34+DR- cells demonstrates quantitative differences between these two subpopulations with regard to primitive progenitor cell content. Whether qualitative differences exist between primitive progenitors within these two populations as well remains to be determined. In this regard, Huang and Terstappen recently reported that CD34+CD38- DR- but not CD34+CD38- DR+ fetal human BM cells have features of primitive hematopoietic stem cells in that they can give rise to progeny of all hematopoietic lineages in vitro. However, Srour et al found that CD34+DR-, but not CD34+DR+, adult human BM cells could engraft in fetal sheep and give rise to multi-lineage progeny and possibly exhibit self-renewal capabilities, demonstrating the presence of primitive stem cells also within the CD34+DR- subset.
Recently, much interest has been focused on the CD34+CD38−DR− subset, because studies on fetal BM cells suggested that very primitive stem cells were contained within this progenitor cell population. In the present study, only 12% to 14% of total CD34+DR− and CD34+CD38− BM cells were CD34+CD38−DR−. The presence of HPP-CFCs, as well as of progenitors responding to G-CSF alone, showed heterogeneity within the CD34+CD38−DR− cell compartment. Interestingly, the CD34+CD38−DR− subpopulation was depleted in primitive HPP-CFCs as compared with CD34+CD38−DR− cells. The limited in vitro proliferation of CD34+CD38−DR− cells could potentially be caused by the presence of very primitive progenitors not capable of responding in available in vitro assays. However, our data are in agreement with other recent studies suggesting that the CD34+CD38−DR− subset contains few hematopoietic progenitor cells.

In conclusion, there is little overlap between CD34+CD38− and CD34+CD38−DR− subfractions of CD34+ BM cells. The CD34+CD38− subset contains a higher frequency of primitive progenitor cells such as HPP-CFCs and LTC-ICs, whereas the CD34+CD38−DR− subset is enriched in committed erythroid progenitors. Whether CD34+CD38− cells also result in better long-term marrow reconstitution than CD34+DR− cells remains to be determined.

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Functional differences between CD38- and DR- subfractions of CD34+ bone marrow cells

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