PHILADELPHIA chromosome (Ph1)-positive chronic myeloid leukemia (CML) is a well-defined, two-stage, clonal myeloproliferative disease. The initial, chronic phase is characterized by a pronounced but benign leukocytosis. The second, acute phase is characterized by the appearance of a rapidly expanding subpopulation of blasts that fail to differentiate, are unresponsive to chemotherapy, and lead rapidly to death. Ready demonstration of the Ph1 chromosome in multiple hematopoietic cell types, including B cells as well as myeloid cells, suggests that the neoplastic clone originates in a pluripotent stem cell.1 At a molecular level, CML is associated with the creation of a fusion gene between most of c-ABL (from chromosome 9) and some of BCR-1 (on chromosome 22).2 Transcription of this fusion gene leads to the synthesis of a protein with several altered properties relative to p145c-abl, including increased tyrosine kinase activity.3,4

Recently, a CML-like disease has been observed to arise in mice whose hematopoietic cells have been genetically engineered to express various BCR-ABL constructs.5-7 Such findings provide further evidence that this gene product plays a primary role in the pathogenesis of CML in humans. A number of functions have been recently assigned to specific regions of the normal products of BCR-1 and c-ABL and several essential for (or able to contribute to) the transforming potential of BCR-ABL fusion genes have been identified.8-15 In contrast, very little is known about the control of BCR-ABL expression in different primary hematopoietic cell types, particularly progenitor cells, or the biologic consequences of such expression. The most primitive hematopoietic cells are difficult to study because they make up such a small proportion of all the nucleated cells in the blood and marrow. Nevertheless, effects of BCR-ABL expression on their behavior are of key interest because it is these cells that are believed to be responsible for the initial amplification and subsequent maintenance of the leukemic clone in patients with CML.

Such studies require not only specific, quantitative assays for the relevant cells, but also methods for their characterization and ultimately for their isolation as pure populations. The assay for long-term culture-initiating cells (LTC-IC) appears to qualify as one that detects a population more primitive than clonogenic cells10 and that may overlap with totipotent reconstituting cells.17-19 This assay also detects a functionally analogous cell present at low frequency in CML marrow and in markedly elevated concentrations in the blood of CML patients with high white blood cell (WBC) counts.20 A characteristic feature of a variety of primitive Ph1-positive clonogenic cell types is that their proliferation is deregulated both in vivo21 and in vitro22 under conditions in which their normal counterparts are quiescent, suggesting perturbation of a control mechanism also active on the most primitive normal hematopoietic cells. It might therefore be expected that Ph1-positive LTC-IC would also exhibit properties of cycling cells, in
contrast to normal LTC-IC. The present studies were designed to test this prediction. To facilitate the characterization of Ph1-positive clonogenic cells and LTC-IC, peripheral blood (PB) from CML patients with elevated WBC counts was used as starting material. This made laborious genotyping studies (by polymerase chain reaction [PCR] or cytogenetic analysis of their individual progeny) unnecessary, because of the very marked increase in all types of circulating neoplastic progenitors in such patients, thereby increasing both the number of experiments that could be performed and the precision of the measurements made.

**MATERIALS AND METHODS**

**Cells.** Heparinized blood samples were obtained with informed consent from CML patients undergoing routine hematologic assessment. All patients were Ph1-positive and in chronic phase. As shown in Table 1, the number of circulating LTC-IC in all patients studied was abnormally elevated (by a factor of from >400-fold to ~10^8-fold above the average normal value of ~2.9 ± 0.5/mL).

The light-density fraction ( < 1.077 g/mL) was isolated by centrifugation of the blood on ficoll-hypaque (FH) to eliminate the majority of erythrocytes, granulocytes, and platelets and to obtain a preliminary enrichment of progenitor cells. Normal blood samples were obtained with informed consent from normal individuals undergoing platelet/leukapheresis and from these a light-density T-cell-depleted fraction was then isolated by rosetting with sheep erythrocytes and centrifugation on FH as previously described. The number of remaining CD2+ (T cells) detected by FACScan analysis of this T-cell-depleted, light-density fraction of normal blood cells represented, on average, less than 2% of the total. Because the number of T cells in initial CML blood samples was already at or below this level, the T-cell-depletion step was not performed on CML blood samples. Heparinized normal marrow aspirate cells were leftovers obtained with informed consent from allogeneic donors providing marrow for transplantation.

**Staining and flow cytometry.** Cells were washed twice and resuspended in Hank’s solution with 50% FCS and were counted. This made laborious counting was used as starting material. This made laborious laborious genotyping studies (by polymerase chain reaction [PCR] or cytogenetic analysis of their individual progeny) unnecessary, because of the very marked increase in all types of circulating neoplastic progenitors in such patients, thereby increasing both the number of experiments that could be performed and the precision of the measurements made.

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**Table 1. Frequency of Primitive Progenitors in the CML Patients Studied**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>WBC/L (×10^9)</th>
<th>Clonogenic Cells/mL</th>
<th>LTC-IC/mL*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>29,000</td>
<td>17,000</td>
</tr>
<tr>
<td>2</td>
<td>116</td>
<td>704,000</td>
<td>266,000</td>
</tr>
<tr>
<td>3</td>
<td>137</td>
<td>72,000</td>
<td>14,000</td>
</tr>
<tr>
<td>4</td>
<td>62</td>
<td>82,000</td>
<td>7,200</td>
</tr>
<tr>
<td>5</td>
<td>262</td>
<td>1,060,000</td>
<td>145,000</td>
</tr>
<tr>
<td>6</td>
<td>104</td>
<td>161,000</td>
<td>8,300</td>
</tr>
<tr>
<td>7</td>
<td>110</td>
<td>86,000</td>
<td>1,300</td>
</tr>
<tr>
<td>8</td>
<td>142</td>
<td>344,000</td>
<td>12,500</td>
</tr>
<tr>
<td>9</td>
<td>436</td>
<td>1,090,000</td>
<td>10,000</td>
</tr>
</tbody>
</table>

Mean ± SEM 162 ± 40 403,000 ± 145,000 52,000 ± 31,000

*These numbers represent absolute LTC-IC values calculated as described in Materials and Methods.

**RESULTS**

**Phenotype analysis of CML LTC-IC.** To determine the light scattering properties of circulating clonogenic cells and LTC-IC in patients with CML, light-density blood cells were sorted into three fractions (as illustrated in Fig 1A) and the results compared with data for normal bone marrow (BM)16 and blood33 progenitors (Fig 1B). Most of the nucleated cells (~85%) in the light-density fraction of CML blood had a high SSC (fraction III) in contrast to the light-density cells in normal blood, where the proportion of such cells is much lower (<40%, data not shown). The mean number of clonogenic cells and LTC-IC recovered in each sorted fraction was determined and expressed as a percentage of the total number of progenitors present in the starting (light-density) cell suspension of each sample studied. As shown in Fig 1B, the majority of both the
clonogenic cells and LTC-IC in CML blood were consistently found in fraction II (ie, cells with high FSC but low SSC). Cells from this fraction also generally produced more nucleated cells (as well as clonogenic cells) after 5 weeks in LTC (both in the adherent and nonadherent layer) than other fractions on a per cell basis (data not shown). However, a significant proportion of the circulating CML clonogenic cells (~15%) and LTC-IC (~30%) were detected in a population characterized by low FSC and low SSC (fraction I). Some circulating CML clonogenic cells (~5%) were found among the cells with a high SSC (fraction III). These findings suggest subtle differences between circulating CML clonogenic cells and LTC-IC in terms of their overall light scattering properties. This was reinforced by experiments in which fraction II was subdivided further into 2 to 3 fractions. Analysis of these showed that the circulating CML clonogenic cells were more concentrated in fractions containing cells with a slightly higher FSC by comparison to the distribution of LTC-IC in the same fractions (data not shown). The high FSC of circulating clonogenic cells in CML patients differs markedly from the FSC typical of clonogenic cells in the circulation of normal individual but is very similar to the majority of clonogenic cells in normal BM.16 Because very few progenitors were present in fraction III, only cells in fractions I and II were analyzed in all subsequent sorts.

Figure 2 shows representative distributions of light-density normal and CML blood cells gated for low SSC after two-color staining for expression of CD34 and HLA-DR, or expression of CD34 and uptake of Rh-123. A much larger proportion of light-density CML blood cells were found to express readily detectable levels of CD34 than is the case for normal blood cells in the same light scatter window (compare Fig 2D and F with C and E). The CML cells also contained a higher proportion of cells that expressed readily detectable levels of HLA-DR or that retained Rh-123 by comparison to normal blood.23 Figures 3 and 4 show the results obtained when the CD34+SSClow cells were sorted according to their expression of HLA-DR (Fig 3) or Rh-123 uptake (Fig 4) and were then analyzed functionally for clonogenic cell and LTC-IC content. It can be seen that most of the clonogenic cells in CML blood, like most of the clonogenic cells in normal marrow, expressed readily detectable levels of HLA-DR (Fig 3A) and showed positive staining with Rh-123 (Fig 4A). In this respect, however, they both differ markedly from the clonogenic cells found in normal blood, of which very few show a DRhi or Rh-123bright phenotype.23 Further subdivision of the CD34+DRhi fraction of CML blood cells into DR+ and DR++ subpopulations (as defined in Fig 2D) showed the presence of clonogenic cells in both (Tables 2 and 3). Interestingly, a proportion (~10%) of the clonogenic progenitors was also found in the DRlow or Rh-123dim fraction. Although none of these were specifically genotyped, it is unlikely that significant numbers in either of these latter phenotypically defined “normal” subgroups were residual normal progenitors because normal progenitors, even if present at normal levels, would have accounted for less than 10% of the progenitors in the DRlow (Table 3) or Rh-123dim fractions (data not shown) of all patients studied.

When the sorted CML cells were assayed for LTC-IC, the majority (~75%) were also present amongst the CD34+DRhi cells (Fig 3B). This is also in contrast to normal LTC-IC, the majority of which in either blood (~100%) or BM (~55%) express little or no HLA-DR.23 Thus, isolation of CD34+DRhi populations of cells from the PB of CML patients (either DR+ or DR++) yields a highly enriched LTC-IC population (Table 2). As noted for the circulating clonogenic cells in the same CML blood samples, a proportion of the LTC-IC (in this case, ~30%) was also found in the CD34+DRlow fraction. Because of the marked elevation in total LTC-IC numbers in these samples, the number of CD34+DRlow LTC-IC was also consis-
Fig 2. Bivariate contour plots of a single representative sample of normal (A, C, and E) and CML (B, D, and F) light-density blood cells in the low SSC window (fractions I and II in Fig 1A). CD34+ cells (gated as shown by the vertical lines in [C] and [D], or the horizontal lines in [E] and [F]) were subdivided into CD34+DRlow and CD34+DRhigh subpopulations as shown by the horizontal line in (C), or CD34+DRlow, CD34+DR+, and CD34+DR++ subpopulations as shown by the two horizontal lines in (D), or CD34+Rh-123bright and CD34+Rh-123dim populations as shown by the vertical lines in (E) and (F). Unstained cells are shown in (A) and (E).

It can be seen from Table 1 that the initial frequencies of the clonogenic cells and LTC-IC in the CML blood samples studied, although elevated, were quite variable both on a volume and on a per nucleated cell basis. Variability was also encountered after these progenitors were separated into various subpopulations, as shown in Table 2, for light density, CD34+, DRlow or DRhigh cells. However, on average, the purity of circulating CML LTC-IC in the CD34+DRlow and CD34+DR+ fractions was approximately 10% (Table 2). This is approximately fivefold to sixfold higher than the purest populations of normal LTC-IC (1% to 2%) thus far isolated from normal blood or marrow samples. Corresponding values for the frequency of clonogenic cells in the CD34+DRlow and CD34+DR+ fractions were 10% and 20% (Table 2). As for normal blood, recovery of LTC-IC in the light-density, SSClow, CD34+...
fraction of CML blood was high (129%). Recovery of clonogenic cells was lower (73%), suggesting exclusion of some CML clonogenic cells with the gating criteria used.

Sensitivity of CML progenitors to 4-hydroperoxycyclophosphamide (4-HC). We have previously shown that LTC-IC in normal blood, like LTC-IC in normal BM, are relatively resistant to 4-HC, as are circulating clonogenic cells, whereas clonogenic cells in normal BM are more 4-HC-sensitive. Recent clinical findings indicate that reconstitution of hematopoiesis with Ph1-negative cells can be achieved in some CML patients receiving 4-HC-treated autologous BM transplants. This suggests that transplantable Ph1-positive stem cells may be more sensitive to 4-HC than normal stem cells. It was therefore of interest to evaluate the 4-HC sensitivity of circulating CML clonogenic cells and LTC-IC and to compare these with normal clonogenic cells and normal LTC-IC. In this series of experiments, LTC-IC function was assessed in terms of the clonogenic cell content of LTC evaluated after 4 and 8 weeks (rather than after 5 weeks, as in the studies described above), because previous experiments had shown differences in the 4-HC sensitivity of normal LTC-IC measured by these two different endpoints.

Results for light-density CML blood cells exposed to 100 μg/mL of 4-HC under standard transplant exposure conditions (ie, 2 × 10⁷ cells/mL with 7% red blood cells for 30 minutes at 37°C) are shown in Fig 5, together with previous data for normal progenitors tested using the same procedures and reagents. Circulating CML clonogenic cells and clonogenic cells in normal marrow were similarly reduced (to ~10% of initial numbers) by this treatment. LTC-IC in the same CML blood samples appeared only slightly more resistant and were significantly more sensitive (P < .01) than normal LTC-IC from any source.
Differentiative potential of CML LTC-IC. Previous studies have shown that the relative numbers of different types of clonogenic progenitors present in 5-week-old LTC provides a consistent average overall measure of the differentiative behavior of LTC-IC assayed under standard LTC conditions. To assess whether this parameter is altered in LTC of light-density CML blood cells was assessed. As shown in Table 4, after 5 weeks in LTC the proportion of progenitors identified as CFU-GM increased, as documented previously for LTC-IC in the blood and marrow of normal individuals, and this remained constant for an additional 3 weeks (data not shown).

**DISCUSSION**

This report describes a number of features of two functionally distinguished classes of primitive hematopoietic cells (clonogenic cells and LTC-IC) found in the blood of patients with CML. Both of these progenitor types are defined by quantitative assays that measure developmental potential using standardized culture conditions and specific progeny output endpoints, thus allowing comparison with

<table>
<thead>
<tr>
<th>Source of Progenitors</th>
<th>Clonogenic Cells</th>
<th>LTC-IC*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency per 10^6</td>
<td>Enrichment</td>
</tr>
<tr>
<td>Unsorted cells</td>
<td>1,750 ± 940</td>
<td>-</td>
</tr>
<tr>
<td>CD34^+DR^+**</td>
<td>104,000 ± 23,000</td>
<td>120 ± 50</td>
</tr>
<tr>
<td>CD34^+DR^-**</td>
<td>192,000 ± 63,000</td>
<td>230 ± 130</td>
</tr>
<tr>
<td>CD34^+DR^-**</td>
<td>105,000 ± 44,000</td>
<td>80 ± 30</td>
</tr>
</tbody>
</table>

*Measured as the total clonogenic cell output in LTC after 5 weeks (ie, to convert to absolute values, simply divide by 3).
†Relative to all nucleated cells in the population analyzed.
‡Calculated by dividing the frequency per 10^6 sorted cells by the frequency per 10^6 unsorted, light-density cells in each individual experiment, and then deriving the mean ± SEM of these values for the experiments performed.
§Calculated by multiplying the percentages of cells retrieved in the fraction indicated by the corresponding calculated enrichment in each individual experiment, and then deriving the mean ± SEM of these values for the experiments performed, assuming 100% recovery after centrifugation on FH.
||Refers to the light density (presort) population.
*Defined in Fig 2D.

**Table 3. Concentration of Various Subpopulations in CML Blood (n = 4) and Estimates of Potentially Normal (Ph^-negative) Components Assuming These Cells Were Present at the Same Concentration as in Normal Blood**

<table>
<thead>
<tr>
<th>Subpopulation Evaluated</th>
<th>Patient No.</th>
<th>% CD34^+ Cells*</th>
<th>Clonogenic Cells/mL</th>
<th>LTC-IC/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34^+DR^w**</td>
<td>1</td>
<td>17</td>
<td>2,900</td>
<td>9.1</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>62,600</td>
<td>0.42</td>
<td>45,200</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>5,400</td>
<td>4.8</td>
<td>1,300</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>16,400</td>
<td>1.6</td>
<td>5,000</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>17 ± 4</td>
<td>21,800 ± 13,900</td>
<td>4.0 ± 1.9</td>
<td>13,600 ± 10,500</td>
</tr>
<tr>
<td>CD34^+DR^§**</td>
<td>1</td>
<td>65</td>
<td>25,000</td>
<td>0.14</td>
</tr>
<tr>
<td>2</td>
<td>72</td>
<td>507,000</td>
<td>0.0067</td>
<td>190,100</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>24,000</td>
<td>0.14</td>
<td>4,500</td>
</tr>
<tr>
<td>4</td>
<td>39</td>
<td>42,000</td>
<td>0.081</td>
<td>2,200</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>51 ± 10</td>
<td>150,000 ± 119,000</td>
<td>0.09 ± 0.03f</td>
<td>52,300 ± 46,000</td>
</tr>
<tr>
<td>CD34^+DR^++**</td>
<td>1</td>
<td>18</td>
<td>520</td>
<td>6.5</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>134,000</td>
<td>0.026</td>
<td>27,000</td>
</tr>
<tr>
<td>3</td>
<td>62</td>
<td>42,000</td>
<td>0.081</td>
<td>8,600</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>2,400</td>
<td>1.4</td>
<td>36</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>32 ± 11</td>
<td>50,300 ± 24,000</td>
<td>2.0 ± 1.5f</td>
<td>8,900 ± 6,200</td>
</tr>
</tbody>
</table>

*From previous data showing 264 ± 52 CD34^+DR^w** and 34 ± 18 CD34^+DR^§** clonogenic cells/mL, and 2.4 ± 0.4 CD34^+DR^w** and no detectable CD34^+DR^§** LTC-IC/mL of normal blood (n = 9). Zero values indicate not detectable.
*Defined in Fig 2D.
†Absolute values calculated as described in Materials and Methods.
‡Subpopulation of CD34^+DR^w** cells in CML PB that show moderately positive HLA-DR fluorescence, as defined in Fig 2D.
§Subpopulation of CD34^+DR^§** cells in CML PB that show strongly positive HLA-DR fluorescence, as defined in Fig 2D.
¶Based on data for CD34^+DR^w** cells of normal blood, ie, combined data for CD34^+DR^+ and CD34^+DR^++ fractions.
similarly defined normal cells. Because normal (Ph1-
negative) LTC-IC persist in the marrow of many CML
patients (and to a much lesser extent, normal clonogenic
cells), a source of cells that would be reproducibly,
significantly, and preferentially enriched for neoplastic
progenitors was used to avoid the need for laborious
genotyping studies. CML patients with high WBC counts
typically show marked elevations of both of these progeni-
tor populations.20,23 Thus, normal cells, even if present in
such samples at normal levels, would have remained well
below the limit of detectability in any of the separation
experiments performed (as illustrated by the calculations
shown in Table 3). Previous studies aimed specifically at
addressing this question have failed to show evidence of
elevated numbers of circulating normal progenitors in
patients with CML even where these could have been
detected.33,34 In addition, more than 97% of LTC-IC from
CML patients with high WBC counts and markedly ele-
vated LTC-IC concentrations have recently also been
down to exhibit abnormal functional properties (ie, self-
maintenance) in LTC, consistent with a leukemic origin.20

Our results described here show that the majority of both
the clonogenic cells and the LTC-IC in the circulation of
CML patients with high WBC counts are phenotypically
similar to one another with respect to size (FSC), expression
of CD34 and HLA-DR, uptake of Rh-123, and sensitivity to 4-HC. In both cases, the predominant pheno-
type was that expected of proliferating or activated cells (ie,
high FSC, high expression of HLA-DR, high Rh-123
uptake, and relative sensitivity to 4-HC). On the other
hand, subtle differences between circulating clonogenic
(cells more activated) and LTC-IC (less activated) in CML
patients were also consistently noted. This predominant,
"abnormal" phenotype is essentially the opposite of that
previously shown for the majority of clonogenic cells and
LTC-IC in the circulation of normal adults, also shared by
the majority of LTC-IC in normal marrow (ie, low FSC, low
expression of HLA-DR, low Rh-123 uptake, and relative
insensitivity to 4-HC). These findings thus confirm and
extend previous evidence that most of the clonogenic cells
in CML blood are CD34+HLA-DR+,25 and are in agree-
ment with the reported difference in size and HLA-DR
phenotype of normal and neoplastic LTC-IC that coexist
in CML marrow.36 Such observations are consistent with the
likelihood that many Ph1-positive LTC-IC, like their clono-
genic progeny, will be found to be actively proliferating cells
regardless of their location in the CML patient (in blood or
marrow).22 Direct measurements of the cycling status of
LTC-IC are not yet available, but should allow this predic-
tion to be formally tested.

It is of interest that a substantial proportion (10% to 30%)
of the circulating progenitors in the blood of the CML
patients analyzed in this study showed features characteris-
tic of their counterparts in normal individuals. As noted
above, it seems very unlikely that these represented coexist-
ing normal elements, because the numbers involved would
have required the latter to have been greatly increased in
the circulation, a possibility not supported by existing
data.33,34,37 Accordingly, one might anticipate the turnover of
classes of primitive neoplastic progenitors in CML
patients to be increased (ie, LTC-IC as well as clonogenic
cells), although not maximally so, with greater numbers of
quiescent cells occurring among the Ph1-positive LTC-IC
population than among their clonogenic progeny. In this
regard, it is of interest that primitive leukemic progenitors
from patients with CML have been found to be similarly
responsive to the cytokastic, but reversible, effects exerted
by transforming growth factor-β (TGF-β) on primitive
normal hematopoietic cells in vitro.38-40 Alternatively, it is
possible that the phenotypic markers examined may not

Table 4. Relative Numbers (expressed as a percent of the total) of Different Types of Clonogenic Cells Present in CML Blood and Produced by LTC-IC in CML Blood After 5 Weeks in LTC

<table>
<thead>
<tr>
<th>Origin of Samples</th>
<th>No. of Samples</th>
<th>BFU-E</th>
<th>CFU-GM</th>
<th>CFU-GEMM</th>
<th>BFU-E</th>
<th>CFU-GM</th>
<th>CFU-GEMM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CML PB</td>
<td>17</td>
<td>65 ± 3</td>
<td>34 ± 3</td>
<td>1.3 ± 0.2</td>
<td>15 ± 3</td>
<td>83 ± 3</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>Normal PB*</td>
<td>23</td>
<td>74 ± 3</td>
<td>24 ± 2</td>
<td>2.2 ± 0.3</td>
<td>11 ± 2</td>
<td>89 ± 2</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>Normal BM†</td>
<td>10</td>
<td>36 ± 3</td>
<td>62 ± 4</td>
<td>1.2 ± 0.2</td>
<td>9 ± 2</td>
<td>91 ± 2</td>
<td>0.8 ± 0.3</td>
</tr>
</tbody>
</table>

*Data from Udomsakdi et al. 23
†Data from Sutherland et al. 16
PHENOTYPE OF CML STEM CELLS

necessarily be strictly regulated concordantly with changes in cell cycle status.41

By limiting dilution analysis, we previously showed that the total number of clonogenic cells produced on average by LTC-IC in normal blood or BM, or in CML blood is the same after 5 weeks in LTC, although in the same cultures the maintenance of normal LTC-IC is much better.20 Another functional endpoint of LTC-IC behavior in vitro examined here is provided by analysis of the relative numbers of the different types of clonogenic progeny they produce when maintained under LTC conditions. The present studies show that the ratio of erythroid-restricted, granulocyte-macrophage–restricted, and multilineage clonogenic cells generated by LTC-IC in the blood of CML patients with high WBC counts is indistinguishable from that previously documented for normal LTC-IC from all sources thus far analyzed.6,23 These findings reinforce the concept that the LTC-IC assay detects a functionally homogeneous population of primitive hematopoietic cells and that the processes underlying commitment to specific lineages are not substantially affected by the presence of the BCR-ABL gene.

In summary, we have shown that a majority of the most primitive neoplastic progenitors that are currently detectable in the circulation of patients with CML differ from their counterparts in normal individuals with respect to a number of functionally related properties. These differences are suggestive of a deregulation in the control of cell proliferation in CML at the level of the cells initially responsible for maintenance and expansion of the Ph1-positive clone without alteration of their commitment to, or early differentiation down, each of the hematopoietic lineages. These findings may provide a potentially useful theoretical framework for future analysis of the mechanism of BCR-ABL–induced multilineage disease. On the other hand, from a practical viewpoint, the demonstration that, on average, as many as 30% of the circulating LTC-IC in CML patients were phenotypically indistinguishable from normal LTC-IC by the parameters studied (FSC, expression of HLA-DR, and uptake of Rh-123) does not auger well for the use of these properties to selectively isolate primitive normal stem cells from CML marrow harvests for autologous transplantation, particularly because significant contamination of such autografts with PB cells cannot be avoided. Further investigation of the basis of the phenotypic heterogeneity characteristic of primitive circulating cells in patients with CML will clearly be of interest. The procedures described here, which allow populations of these cells to be readily obtained at 10% to 20% purity and in high yield, should provide a useful starting point for such studies.

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Phenotypic heterogeneity of primitive leukemic hematopoietic cells in patients with chronic myeloid leukemia

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