Cytogenetic Studies of Early Myeloid Progenitor Compartments in Ph-positive Chronic Myeloid Leukemia. II. Long-Term Culture Reveals the Persistence of Ph-negative Progenitors in Treated as Well as Newly Diagnosed Patients

By Ian D. Dubé, Dagmar K. Kalousek, Laure Coulombel, Chander M. Gupta, Connie J. Eaves, and Allen C. Eaves

We recently showed that long-term marrow cultures can be used to demonstrate the presence of Philadelphia (Ph') negative progenitors in patients with newly diagnosed Ph'-positive chronic myeloid leukemia (CML). We now report results for 6 chronic phase patients studied 5–83 mo postdiagnosis and an additional 3 newly diagnosed patients. Marrow metaphases were exclusively Ph'-positive. Clonogenic assays revealed a minor population of Ph'-negative progenitors in 3 cases (1 treated, 2 untreated). Long-term marrow culture adherent layers contained Ph'-negative progenitors in 6 cases (3 treated, 3 untreated). Whenever this occurred, the Ph'-negative population had become the only one detectable within 3–4 wk, and this was always associated with a rapid decline of the Ph'-positive population. For 2 of the 3 cases where Ph'-negative progenitors were not detected, there was a similar rapid decline in the Ph'-positive population in culture. In the other case, Ph'-positive progenitors were maintained at levels typically seen in normal long-term marrow cultures. These results suggest that chromosomally normal stem cells may persist for a considerable period in the marrow of some, but perhaps not all, patients with CML, even in the face of maintenance chemotherapy. In addition, they provide new evidence of heterogeneity in this disease, as shown by the variable ability of Ph'-positive progenitor populations to be maintained in vitro.

Chronic Myeloid Leukemia (CML) is a clonal disorder that arises in the pluripotent hemopoietic stem cell compartment. Typically, all primitive progenitor compartments are enlarged by the abnormally expanding clone so that, by the time of diagnosis, circulating BFU-E and CFU-C progenitor numbers may be increased more than 1,000-fold. A dramatic expansion of the terminally differentiating compartments is usually limited to the granulocyte-monocyte lineages, although significant in platelet production frequently also occur. In 85%-90% of cases, the clone is marked by the presence of the Philadelphia (Ph) chromosome, a readily visible and uniquely altered chromosome 22 resulting from a balanced chromosomal translocation. Thus, at presentation, the Ph1 chromosome is usually seen in all marrow metaphases, and Ph1-positive BFU-E and CFU-C also predominate. Nevertheless, Ph1-negative progenitors may be found if sufficient numbers of colonies are examined, and restoration of Ph1-negative hemopoiesis from residual normal stem cells can be obtained with aggressive treatment protocols.

Recently, we found that the dominance of the Ph1-positive population over the Ph1-negative population in vivo was reversed in the adherent layer of long-term marrow cultures initiated with cells from newly diagnosed patients. This provided a sensitive method for demonstrating the existence of Ph1-negative progenitors in cases where these were otherwise undetectable. In this study, we have extended this approach to other newly diagnosed patients and have evaluated its applicability to the assessment of progenitor populations in patients with established CML treated with conventional therapy. This latter group of patients is of particular interest, because such treatment does not generally alter the ability of the Ph1-positive clone to dominate all levels of hemopoiesis, and the fate of the initial reservoir of Ph1-negative stem cells under these conditions is unknown. Our findings suggest that, in most cases, Ph1-negative progenitors do not rapidly disappear or lose their functional integrity, even after cytotoxic therapy. They also indicate that the usual failure of Ph1-positive progenitors to survive in long-term culture may not be an intrinsic property of this genotype, but may rather be the net result of several factors that determine early on the composition of the adherent layer.

MATERIALS AND METHODS

Patients

Clinical and hematologic data for the 9 patients included in this study are shown in Table I. There were 5 males and 4 females, and the average age was 37 yr (range 20–39). All patients were in the chronic phase of CML. Three were studied within 1 mo of diagnosis.
albumin (BSA), I0 concentration in 0.8% methylcellulose in Iscove's medium supple-
at 37°C in the presence of 0.1% collagenase, as previously
or partial replacement of the growth medium. 
seventh day after initiation and at weekly intervals thereafter by
at this time by centrifugation of the nonadherent cells over Ficoll-
glutamine. Up to 10 cultures were initiated from each specimen.
Adherent layer cells were harvested after a nontoxic 3-hr incubation
were also evaluated. This required sacrificing the entire culture.
tored weekly. At varying intervals, progenitors in the adherent layer
removal of half of the nonadherent cell fraction and either complete
the cultures in new growth medium. Cultures were fed on the
3-5 M (lot 412024 or 29211015, also from Flow Laboratories), I0
medium. This consisted of 12.5% fetal calf serum (lot 2910111, Flow
Laboratories, Mississauga, Ontario, Canada), I2.5% horse serum
7
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Ph'-VE CELLS FROM LONG-TERM CML CULTURES

Table 1. Clinical and Hematologic Data for the Nine Cases Included in This Study

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Sex</th>
<th>Months Since Presentation</th>
<th>WBC (per cu mm)</th>
<th>Hb (g/dL)</th>
<th>Platelets (per cu mm)</th>
<th>Marrow BP-E† CFU-C</th>
<th>BFU-E† CFU-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated patients</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1(a)</td>
<td>38</td>
<td>F</td>
<td>0</td>
<td>18,000</td>
<td>16.3</td>
<td>590,000</td>
<td>24</td>
<td>26</td>
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<tr>
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<td>13,000</td>
<td>14.9</td>
<td>580,000</td>
<td>20</td>
<td>40</td>
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<tr>
<td>2</td>
<td>60</td>
<td>F</td>
<td>0</td>
<td>27,000</td>
<td>14.4</td>
<td>660,000</td>
<td>2</td>
<td>102</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>M</td>
<td>0</td>
<td>142,000</td>
<td>14.8</td>
<td>205,000</td>
<td>14</td>
<td>100</td>
</tr>
<tr>
<td>Treated patients</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>M</td>
<td>5</td>
<td>25,000</td>
<td>12.4</td>
<td>224,000</td>
<td>52</td>
<td>147</td>
</tr>
<tr>
<td>5</td>
<td>39</td>
<td>M</td>
<td>9</td>
<td>16,000</td>
<td>13.4</td>
<td>249,000</td>
<td>17</td>
<td>74</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>F</td>
<td>14</td>
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<td>13.0</td>
<td>600,000</td>
<td>6</td>
<td>105</td>
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<td>7</td>
<td>39</td>
<td>M</td>
<td>43</td>
<td>32,000</td>
<td>15.4</td>
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<td>10</td>
<td>180</td>
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<td>8</td>
<td>38</td>
<td>F</td>
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<td>4,600</td>
<td>13.5</td>
<td>198,000</td>
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<td>27</td>
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<tr>
<td>9</td>
<td>33</td>
<td>M</td>
<td>83</td>
<td>53,000</td>
<td>14.7</td>
<td>338,000</td>
<td>8</td>
<td>58</td>
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<td>Controls†</td>
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<tr>
<td>Mean - 28; Mean - 64; Mean - 53; Mean - 26;</td>
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</tr>
<tr>
<td>1-284</td>
<td>19-218</td>
<td>10-284</td>
<td>4-147</td>
<td></td>
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</tr>
</tbody>
</table>

*Per 200,000 marrow cells plated or per milliliter blood.
†More than 8 clusters.
‡Values from reference 25. Range shown is that delimited by the geometric mean ± SEM.

and before the initiation of any therapy. The remaining 6 were
studied 5–83 mo after presentation and had been treated with
busulfan and/or hydroxyurea for a similar period. Marrow cells used
for culture were part of aspirates taken for diagnostic purposes and
were obtained with informed consent.

Culture Procedures

A detailed description of the procedures used to initiate and
maintain long-term marrow cultures has been described previously. Briefly, aliquots of the unprocessed marrow aspirate containing
2–2.5 x 10^7 nucleated cells were placed in 60 x 15 mm Falcon tissue
culture dishes, each containing 8 ml of long-term culture growth
medium. This consisted of 12.5% fetal calf serum (lot 2910111, Flow Laboratories, Mississauga, Ontario, Canada), 12.5% horse serum (lot 412024 or 29211015, also from Flow Laboratories), 10^-4 M 2-mercaptoethanol, 10^-4 M hydrocortisone sodium hemisuccinate, and alpha-medium supplemented with extra inositol, folic acid, and glutamine. Up to 10 cultures were initiated from each specimen. Cultures were incubated for the first 3–5 days at 37°C and then transferred to 33°C. In some instances, excess red cells were removed at this time by centrifugation of the nonadherent cells over Ficoll-
Hypaque and subsequent return of the washed light density cells to
the cultures in new growth medium. Cultures were fed on the
seventh day after initiation and at weekly intervals thereafter by
removal of half of the nonadherent cell fraction and either complete
or partial replacement of the growth medium.

Myeloid progenitor cells in the nonadherent fraction were monitored weekly. At varying intervals, progenitors in the adherent
layer were also evaluated. This required sacrificing the entire culture. Adherent layer cells were harvested after a nontoxic 3-hr incubation at 37°C in the presence of 0.1% collagenase, as previously
described. Colony assays were performed by plating cells at an appropriate concentration in 0.8% methylcellulose in Iscove's medium supple-
mented with 30% fetal calf serum, 1% deionized bovine serum albumin (BSA), 10^-4 M 2-mercaptoethanol, 292 mg/ml glutamine,
5.0 U/ml of burst-promoting activity (BPA) and colony-stimulating activity (CSA) free human urinary erythropoietin (purified in our laboratory to a specific activity of >100 U/mg"
), and optimal concentrations of human leukocyte conditioned media.13 Pure and mixed colonies were carefully scored in the living state using
standard criteria. Values for the CFU-C and primitive BFU-E (>8 erythroblast clusters per colony) content of the marrow and
peripheral blood at the time of study are shown in Table 1.

Cytogenetic Studies

An aliquot of marrow from all patients studied was used to obtain simultaneous direct preparations of Giemsa-banded bone marrow
metaphases. Cytogenetic analysis of these preparations revealed the presence of the standard Ph' translocation, t(9;22)(q34;q11), in all
cases at the time of study, and in no patient was there any
evidence for the proliferation of cytogenetically normal bone marrow
cells (Table 2).

To determine the relative proportions of Ph'-positive and Ph' negative progenitor cells in a given suspension, large, well isolated, and readily identifiable erythroid (primitive BFU-E), granulocyte
(CFU-C), and mixed granulocyte/erythroid (CFU-G/E) colonies
arising in methylcellulose cultures were selected, plucked, and processed individually for cytogenetic analysis. This procedure has been
described previously in detail. A minimum of two G-banded metaphases were analyzed per colony and, in most cases, karyotypes
were prepared. The genotypes of the progenitors from which the
colonies arose were thus inferred by cytogenetic analysis of their
differentiating descendants. The validity of this approach is based on
the assumption that each colony is derived from a single progenitor
cell, either Ph'-positive or Ph' negative. Support for this assumption
was already been reported. Whenever possible, at least 14 colonies from a given specimen were analyzed, as such a sample size permits
detection of a second component of a mixed population with 95% confidence if it comprises 20% or more of the total. Occasionally, 2
or more colonies were pooled for analysis. (These are indicated as
"pooled colonies" in Table 2.)
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Table 2. Ratios of Cytogenetically Normal: Ph'-Positive Metaphases in Direct Bone Marrow Aspirates and Ratios of Cytogenetically Normal: Ph'-Positive Colonies in Methylcellulose Assays Before and After Long-Term Culture of Progenitors

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Normal: Ph'-Positive Direct Bone Marrow</th>
<th>Normal: Ph'-Positive Initial Colony Assay</th>
<th>Long-Term Culture Adherent Layer</th>
<th>Time of Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Un-treated patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1(a)</td>
<td>0:30</td>
<td>3:7</td>
<td>11:0</td>
<td>1:0</td>
</tr>
<tr>
<td>1(b)</td>
<td>0:13</td>
<td>7:17</td>
<td>10:0</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>0:25</td>
<td>1:1</td>
<td>5:0</td>
<td>4:0</td>
</tr>
<tr>
<td>3</td>
<td>0:16</td>
<td>0:14</td>
<td>7:0</td>
<td>6:0</td>
</tr>
<tr>
<td><strong>Treated patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0:21</td>
<td>6:21</td>
<td>13:0</td>
<td>1:0</td>
</tr>
<tr>
<td>5</td>
<td>0:25</td>
<td>0:10</td>
<td>10:0</td>
<td>24:1†</td>
</tr>
<tr>
<td>6</td>
<td>0:9</td>
<td>0:16</td>
<td>1:0</td>
<td>13:0</td>
</tr>
<tr>
<td>7</td>
<td>0:25</td>
<td>0:25</td>
<td>0:13</td>
<td>0:46†</td>
</tr>
<tr>
<td>8</td>
<td>0:31</td>
<td>0:11</td>
<td>0:1</td>
<td>0:5</td>
</tr>
<tr>
<td>9</td>
<td>0:10</td>
<td>0:10</td>
<td>—†</td>
<td>—</td>
</tr>
</tbody>
</table>

*More than 8 clusters.
†Metaphases from pooled colonies.
‡No colonies that could be cytogenetically analyzed were derived from these long-term cultures at any time.

RESULTS

The differential effects of long-term marrow culture on the maintenance of Ph'-positive and Ph'-negative progenitors from the nine different chronic phase CML patients included in this study are summarized in Table 2. The initial progenitor data for two of the three untreated patients and two of the six treated patients have been presented elsewhere.9 They are shown again here to facilitate comparison with the results obtained after 1–6 wk of long-term culture.

Untreated Patients

Two of the three newly diagnosed patients studied had low peripheral WBC counts at presentation, and assessment of their blood levels of CFU-C and primitive BFU-E suggested that these compartments had not yet expanded markedly (see Table 1). Consistent with this finding was the demonstration of a significant degree of Ph'-negative/Ph'-positive mosaicism among their numbers, in spite of the absence of Ph'-negative metaphases in direct marrow preparations from these same two patients. The third untreated patient (case 3) presented with a peripheral WBC count of 142,000 and blood progenitor levels that were increased approximately 200-fold above normal. In this patient, a Ph'-negative population was not detected in initial methylcellulose assays.

Long-term marrow cultures from all three patients yielded results similar to those previously reported for untreated CML.12 This included a tendency for supernormal numbers of nucleated cells to be present in the nonadherent fraction during the first 3 wk, although normal or reduced numbers were measured thereafter. At all times the progenitor content of both adherent and nonadherent fractions was also either within normal limits or reduced, and as found previously, the majority of primitive (high proliferative potential) progenitors were concentrated in the adherent layer.

Previous studies had also shown that most of the primitive progenitors in the adherent layer were usually Ph'-negative by 4 wk.13 In the present study, cytogenetic analysis of colonies from long-term culture assays was therefore performed initially after 3 or 4 wk. For the three newly diagnosed patients studied here, all progenitors analyzed at this time proved to be Ph'-negative (Table 2).

An increase in the proportion of Ph'-negative progenitors present after 3–4 wk implies that there must have been a significant difference in the maintenance of Ph'-negative and Ph'-positive progenitors during this interval. Table 3 shows the number of primitive Ph'-positive BFU-E present initially and the maximum number that could have been present in the adherent layer 3–4 wk later and still have escaped detection. Changes in primitive Ph'-negative BFU-E numbers between 0 and 3–4 wk are also shown. The dramatic decline in Ph'-positive BFU-E in all three experiments is readily seen and contrasts with the much slower decline of Ph'-negative BFU-E. The kinetics of the Ph'-negative BFU-E in these experiments closely resembles that of BFU-E in normal long-term marrow cultures.15

To investigate the possibility that this abnormal behavior of the Ph'-positive progenitors might be related to their exclusion from or inactivation in the
adherent layer during the initial phases of its formation, an experiment to evaluate 1-wk-old cultures was undertaken. The marrow used was a repeat aspirate from case 1 that was obtained 1 mo after the first. As can be seen in Table 2, only Ph'-negative primitive BFU-E were detected in the adherent fraction of these 1-wk-old cultures.

**Treated Patients**

To evaluate the effect of conventional treatment on the subsequent behavior of Ph'-positive progenitors in long-term marrow cultures, and to determine how long after diagnosis Ph'-negative stem cells might still be detected in such cultures, six patients with established treated disease were studied. In all six cases, treatment was initiated soon after diagnosis. The interval from diagnosis to the time of study varied from 5 to 83 mo (Table 1). Cytogenetic analysis of hemopoietic colonies generated in methylcellulose assays of the same marrow specimens used to initiate long-term cultures failed to detect the presence of Ph'-negative progenitors in five of the six treated patients (Table 2). In the remaining treated patient (case 4), 22% of marrow progenitors were Ph'-negative at the time of study, and a similar distribution of progenitor genotypes was found in the peripheral blood.

Long-term cultures established from marrow aspirates from all six patients were monitored for up to 6–8 wk, and the number of primitive hemopoietic progenitors detected was sufficient to permit cytogenetic data to be collected in all but one experiment. Long-term cultures from this patient’s marrow (case 9) failed to develop a typical adherent layer, remained hypocellular, and yielded few colonies, none of which could be cytogenetically analyzed. Long-term cultures from the other five treated patients were similar in appearance, behavior, and selective concentration of primitive progenitors in the adherent layer to cultures of untreated CML marrow.

Cytogenetic analyses were performed on colonies generated in assays of adherent layers harvested initially after 3–4 wk, and in some instances, again after 6 wk. For three of the treated patients (cases 4, 5, and 6), the results were similar to those obtained for the three untreated patients, i.e., virtually all progenitors present after 3 wk were Ph'-negative. In the other two experiments, only Ph'-positive progenitors were detected (Table 2).

As shown above for cultures established with marrow from untreated patients, conversion to Ph'-negativity in long-term cultures of treated CML marrow was associated with an abnormally rapid decline of the originally dominant Ph'-positive population (see Table 3). In one of the two treated CML experiments where Ph'-negative progenitors were not detected (case 8), this abnormal behavior of the Ph'-positive population was also observed. However, in the other (case 7), a different pattern was seen. In this experiment, the number of primitive BFU-E (all of which were consistently Ph'-positive) present in the adherent layer was maintained in the range previously determined for a series of normal marrow cultures.

**DISCUSSION**

Although it is now clear that many patients with CML may still have a significant population of chromosomally normal progenitors in their marrows at the time when their disease is first recognized, very little is known about what happens to these cells as the disease progresses. We have previously shown that, in direct marrow preparations, where the majority of the dividing cells can be assumed to be terminally differentiating granulocytic and erythroid cells, the proportion of Ph'-negative metaphases is lower than that predicted by the degree of mosaicism in the primitive progenitor compartments from which these lineages derive. This is consistent with the concept that the presence of an expanded neoplastic clone may have a suppressive effect in vivo on the differentiative capacity of normal progenitor cells. If this suppression also affects the self-maintaining ability of residual normal stem cells, then with time, this population also would be expected
to disappear, although this might occur relatively slowly.

In this study, we have made use of the long-term culture system to explore this question. Our results show that a readily detectable Ph<sup>−</sup>-negative hematopoietic progenitor population, capable of normal growth and differentiation in vitro, may frequently persist in the marrow of CML patients even after more than a year of conventional treatment. Such cells become detectable in long-term marrow cultures because the Ph<sup>+</sup>-positive population usually declines rapidly (Table 3).

To date, a total of 13 patients have been studied—9 in this report and 4 in a previous one. In only one case (an untreated patient) was there evidence for the proliferation of Ph<sup>−</sup>-negative cells in the direct marrow preparation (case 1, experiment 1 of reference 12). Analysis of hematopoietic colonies produced in assays of fresh marrow revealed the presence of Ph<sup>−</sup>-negative progenitors in four cases (cases 1, 2, and 4 in this report and case 1 in reference 12). Three of these were cases who presented with low WBC counts (<27,000) and were studied at diagnosis. The fourth was a patient who, at diagnosis 5 mo previously, was also in this category (WBC was 23,000 at that time.) Analysis of hematopoietic colonies produced in assays of long-term culture adherent layers showed complete conversion to Ph<sup>−</sup>-negativity in six of the seven newly diagnosed patients studied (cases 1–3 in this report and cases 1–3 in reference 12), and in three of the six treated patients studied (cases 4–6 in this report). No Ph<sup>−</sup>-negative progenitors were detected in the adherent layer of long-term cultures from three of the remaining four patients. One of these was an untreated patient (case 4 in reference 12). Two were treated patients whose disease had been diagnosed and treated the longest (cases 7 and 8 in this report). In the remaining experiment (case 9), the number of progenitors recovered was very low, and none of these could be cytogenetically analyzed. No examples of persisting progenitor mosaicism in long-term culture have yet been found.

If marrows from nine patients were able to maintain a Ph<sup>−</sup>-negative population in the long-term culture system, why were such cells not detected in cultures from the other four patients studied? The simplest explanation is that, in each of these four cases, the initial aspirate was devoid of Ph<sup>−</sup>-negative progenitors. However, it could also be argued that the composition of the adherent layers obtained might have been insufficient to support Ph<sup>−</sup>-negative progenitors or may have led to their suppression, as occurs in vivo.

In two cases where Ph<sup>−</sup>-negative progenitors were not detected (case 8 in this report and case 4 in reference 12), the number of progenitors recovered from both adherent and nonadherent fractions was low, albeit still detectable. The actual values measured were not, however, lower than the maximum numbers of Ph<sup>+</sup>-positive progenitors that could have been in the other long-term CML marrow cultures where most of the persisting progenitors were Ph<sup>−</sup>-negative (Table 3). There is thus no reason, a priori, to assume that, in these two cases, Ph<sup>−</sup>-negative progenitors would not have been maintained had they been present at the outset. In the third case, no progenitors were recovered. This patient had been treated for approximately 8 yr, and because of this, it seems likely that his marrow was no longer able to establish a competent adherent layer.

The fourth case where Ph<sup>−</sup>-negative progenitors were not detected (case 7) is perhaps the most interesting. High numbers of primitive Ph<sup>+</sup>-positive progenitors were detected in the adherent layer of long-term cultures established from this patient’s marrow, in contrast to the very poor recoveries of Ph<sup>+</sup>-positive progenitors in all other long-term CML marrow culture experiments (Table 3). At present, we cannot exclude the possibility that, in this case, significant numbers of Ph<sup>−</sup>-negative cells were also present, but were not detected because they failed to achieve a sufficient survival advantage over the Ph<sup>+</sup>-positive population due to the unusual establishment in vitro of conditions similar to those prevailing in vivo. Nevertheless, from this limited series, it would appear that the frequency of patients with detectable Ph<sup>−</sup>-negative progenitors tends to decrease as the time from diagnosis and initiation of therapy increases. To establish whether this trend has general validity will clearly require assessment of a much larger number of patients and/or repeated studies of the same patients after varying periods of time.

Previous studies of long-term marrow cultures of both human and murine origin have indicated that progenitor maintenance is a function of the primitive cells that early on become a part of the adherent layer. The abnormally low numbers of primitive Ph<sup>+</sup>-positive progenitors typically found in the adherent layer of 3–4-wk-old cultures may be accounted for, at least partially, by a relatively decreased ability of Ph<sup>+</sup>-positive cells to adhere to the bottom of the culture flask either directly or indirectly via other adherent cell types. Alternatively, they may adhere but then be unable to survive or proliferate. The results of the second experiment set up with marrow cells from case 1 indicated that Ph<sup>+</sup>-positive progenitors were not part of the adherent fraction recovered after 7 days. This is consistent with both hypotheses. However, this abnormal behavior is not a universal property of all Ph<sup>+</sup>-positive progenitors, as exceptions can be found (e.g.,
case 7). This abnormal behavior is also not unique to Ph\(^1\)-positive cells. We have recently documented a similar conversion to cytogenetic and functional normalcy in some, but not all, long-term cultures established with cells from newly diagnosed AML patients.\(^{24}\)

The usefulness of the long-term culture method in facilitating the detection of Ph\(^1\)-negative progenitors is thus not always predictable. At least two contributing factors are involved. On the one hand, the number of Ph\(^1\)-negative progenitors in the initial inoculum is of prime importance. On the other hand, the composition of the adherent layer also seems to play a major role. It appears that heterogeneity may exist among CML patients with respect to both of these factors, and, in the final analysis, it is their interaction that determines whether or not Ph\(^1\)-negative progenitors can be demonstrated in a given patient.

ACKNOWLEDGMENT

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Cytogenetic studies of early myeloid progenitor compartments in Ph1-positive chronic myeloid leukemia. II. Long-term culture reveals the persistence of Ph1-negative progenitors in treated as well as newly diagnosed patients

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