Mitotic Ability of Leukemic Leukocytes in Chronic Myelocytic Leukemia*

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SUMMARY

In vitro culture of leukemic blood leukocytes from patients with chronic myelocytic leukemia (CML) showed them to be capable of division in the absence of phytohemagglutinin. In the presence of the latter, lymphocytes in the blood of these subjects were preferentially stimulated into mitosis. In the absence of the mitogenic stimulant only the immature leukemic granulocytic cells underwent division. The capacity of the leukemic leukocytes in CML to divide in vitro without an exogenous mitogenic agent was a characteristic of both Ph'1-positive and Ph'1-negative CML cells. The results indicate that immature granulocytic cells in the blood of patients with CML, and possibly in the blood of nonleukemic subjects, are capable of division without exogenous mitogenic substances.

MATERIALS AND METHODS

Blood cells were cultured for 48–72 hr. according to technics previously described (11, 15–17). Blood was obtained from seven patients with Ph'1-positive CML and from three subjects with Ph'1-negative CML. In each case the blood contained substantial numbers of immature leukemic cells (myeloblasts, promyelocytes, myelocytes) and lymphocytes. The presence of the latter was necessary, since the division of these cells had to serve as an indicator of the number of metaphases with normal karyotypes observed under various conditions. All the patients with CML had been treated with various chemotherapeutic agents, and their white blood cell counts, when elevated, were not excessively so (8–30,000/cu mm). Whether the patients were Ph'1-positive or Ph'1-negative had been established in the past on the basis of examination of metaphases from bone marrow and in cultured blood cells (16).

RESULTS

A study of the morphology of the cells following culture revealed immature lymphocytic and lymphoblastic-like cells in the smears derived from normal blood (3, 9) and immature granulocytic cells (myeloblasts, promyelocytes, and myelocytes) in the blood of patients with CML grown without phytohemagglutinin. The CML blood cultured with phytohemagglutinin revealed a mixture of immature lymphocytic and immature granulocytic cells. In all smears dividing cells at various stages of mitosis were observed. Possible influence of this abnormal autosome on cell division was investigated.

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identification of metaphases with the Ph¹ chromosome. Cytogenetic evidence indicates that the abnormal Ph¹-autosome is confined to those hemopoietic elements which are of bone marrow origin (granulocytic, megakaryocytic, and possibly the erythroblastic series) and is not present in lymphocytes and in cultured cells of skin and other tissues (16, 19). Metaphases were labeled as Ph'-positive only if the abnormal autosome could be identified with certainty and when it met the morphologic criteria of the Ph¹ chromosome. Metaphases and karyotypes with and without the Ph¹ chromosome are shown in Figures 1, 2. As can be seen, the abnormal Ph¹ can be easily differentiated from the other G-group acrocentrics and from the Y.

Preliminary studies showed that the number of metaphases in CML blood grown in vitro without phytohemagglutinin was higher (twenty per 1000 cells) after 48 hr. of culture than after 72 hr. (ten per 1000 cells); and that in the presence of PHA there was no significant difference. Hence, the results to be presented are based on cells cultured for 48 hr.

The incidence of the Ph¹ chromosome in blood grown for 48 hr. with and without phytohemagglutinin and the percentage of cells in the blood of patients with CML capable of division are shown in Table 1. It is apparent that, in the absence of PHA, a much higher (92 per cent) percentage of metaphases was Ph¹-positive as compared with the incidence of metaphases with the Ph¹-autosome (39 per cent) in PHA-treated cultures.

To test the possibility that the presence of the Ph¹ chromosome increased the mitotic capacity of the leukemic leukocytes to undergo mitosis in the absence of added mitogenic factors, blood cells from three subjects with CML and devoid of the Ph¹ autosomes were cultured with and without PHA. Since the incubated cells did not contain an abnormal "marker" chromosome and were not aneuploid, it cannot be assumed with certainty that the metaphases observed in the absence of PHA are of leukemic origin. Nevertheless, the nearly total absence of metaphases in cultured normal blood incubated without PHA would militate against the metaphases observed in CML bloods as being nonleukemic. In addition, in smears only immature granulocytic cells were observed. The number (range: fifteen to twenty metaphases per 1000 cells) of metaphases observed in the three blood samples did not differ materially whether or not PHA was added to the culture medium.

The experiments cited above indicate that cells present in the blood of patients with CML are capable of division in the absence of PHA regardless of whether the Ph¹ is present, in contrast to cells present in normal blood, which fail to undergo mitosis unless a mitogenic substance is added to the medium.

A series of experiments was performed with the aim of determining whether a mitogenic substance was present in the plasma of subjects with CML or was confined to the cells. Normal and CML leukocytes were incubated with normal or CML serum, in the presence or absence of PHA, and the number of metaphases was determined. CML leukocytes underwent division in both normal and CML serum, with the number of metaphases being higher, in this series of experiments, in the absence of PHA. Interestingly, in several instances a significant number (five to ten per 1000 cells) of metaphases was observed when normal leukocytes were incubated with CML serum and no PHA. Under the same conditions, and with PHA, the number of metaphases was somewhat smaller than in the absence of PHA. Not all (four out of seven) of the CML sera had this mitogenic effect. Whether this is related to previous therapy, stage or complications of the disease, quantity or quality of cells in the blood or marrow, or other factors is at present unknown.

An attempt was made next to determine whether the capacity of CML leukocytes to divide in vitro in the absence of PHA was peculiar to the leukemic state. To this end blood was obtained from several subjects who had

### Table 1

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Number of cells with Ph¹ pos.</th>
<th>Per cent Ph¹ pos.</th>
<th>Number of cells without Ph¹ pos.</th>
<th>Per cent Ph¹ pos.</th>
<th>Per cent cells capable of division</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>With phytohemagglutinin</td>
<td>Per cent Ph¹ pos.</td>
<td>Without phytohemagglutinin</td>
<td>Per cent Ph¹ pos.</td>
<td>Granulocytic cells</td>
</tr>
<tr>
<td>1</td>
<td>f</td>
<td>13</td>
<td>37</td>
<td>26</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>f</td>
<td>15</td>
<td>35</td>
<td>30</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>f</td>
<td>8</td>
<td>22</td>
<td>27</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>f</td>
<td>17</td>
<td>13</td>
<td>57</td>
<td>27</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>m</td>
<td>16</td>
<td>34</td>
<td>32</td>
<td>42</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>m</td>
<td>25</td>
<td>5</td>
<td>83</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>m</td>
<td>9</td>
<td>41</td>
<td>18</td>
<td>44</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>103</td>
<td>187</td>
<td>210</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>
myelocytes and other immature granulocytic cells in the 
blood (two subjects with myeloid metaphasia, one subject 
with a high leukocyte count due to infection complicating 
cancer of the lung). Even though the number of meta-
phases was not as high as that observed with CML cells, 
there was a significant number of metaphases (four to seven 
per 1000 cells) observed in the three blood samples in-
cubated without PHA.

DISCUSSION

Recently published results and the data of the present 
experiments elucidate several parameters of leukocytic 
division in vitro. It would appear that the leukocytes of 
normal blood which are capable of mitosis—are triggered into that process by sub-
stances acting through immune mechanisms (1—3, 5—10, 
12, 13, 18). In the absence of mitogenic substances, for 
all practical purposes no mitotic cells can be seen in nor-
mal blood cells cultured for short periods of time (48—72 
hr.).

In the present study, in six out of seven cases the non-
leukemic leukocyte metaphases outnumber the leukemic 
ones (Ph'-positive cells) in the presence of PHA. It is 
unclear whether this is due to stimulating effects of PHA 
on lymphocytes and/or inhibition of division of the leuk-
emic leukocytes, thus resulting in a higher rate of mitosis 
in the Ph'-negative cells. On the other hand, in the 
absence of PHA, only Ph'-positive cells were seen in mitosis. 
Thus, it appears that PHA is not necessary for leukocytes of 
CML to undergo division. The presence of the Ph' 
chromosome did not seem to endow the leukemic leuko-
cytes with any advantage of in vitro growth as compared 
with leukemic leukocytes devoid of the Ph' autosome. In 
addition, it would appear that blood myelocytes and other 
immature granulocytic cell precursors of nonleukemic origin are capable of division in the absence of PHA. 
These findings indicate: (a) Blood lymphocytes require the 
presence of a mitogenic stimulator in order to undergo 
division in short-term cultures. It is thought that the 
mitotic stimulus is triggered through an immunological 
response. (b) Normal and leukemic granulocytic cells 
(myeloblasts, promyelocytes, myelocytes) are capable of 
division in the absence of exogenous mitogenic substances. 

It is of more than passing importance to note that acute 
leukemic leukocytes did not undergo mitosis when in-
cubated under the same conditions as were the leukocytes of 
normal or CML blood, regardless of whether PHA was 
added to the culture medium (4, 17). Thus, it would ap-
pear that considerable further information has to be 
gathered about the exact parameters controlling division 
of various leukocytes in vitro. The fact that mitosis of 
lymphocytes is initiated by immunological mechanisms 
may be of considerable importance in relation to the 
development of disorders involving these cells or their 
precursors. The fact that serum from CML was capable 
of initiating mitosis of normal lymphocytes may indicate 
that such serum contains mitogenic substances, which 
may have originated from the granulocytic cells, capable 
of inducing division in normal cells.

Even though immunological mechanisms may be 
responsible for initiating and sustaining mitoses in blood 
lymphocytes, it appears that each antigen may provoke 
the cells into division by diverse processes (2). For 
example, it has been shown that phytohemagglutinin and 
tuberculin induce mitosis in normal blood lymphocytes at 
different times and rates, suggesting different loci of action 
of these two substances. These observations indicate that 
the factors controlling and initiating mitosis of leukocytic 
cells may be highly diversified.

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FIG. 1.—Ph'-negative metaphase of cultured blood cell from a 
normal female. PHA was present during the incubation. Arrows 
point to the four small acrocentric chromosomes (groups G21 and 
G22); these autosomes are of normal size, and no Ph' chromosome 
is present.

FIG. 2.—Ph'-positive metaphase of cultured blood cell from 
female patient #4 with CML. PHA was omitted from the incuba-
tion. Long arrow points to the Ph' autosome, the other arrows to 
the three remaining small acrocentrics. Note abbreviated long-
arms of the Ph' autosome.


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