Increase in Circulating Stem Cells in Patients With Myelofibrosis

By Paul A. Chervenick

An increased number of granulocytic and mononuclear cell colonies was grown in vitro from blood of patients with myelofibrosis and ranged between 162–4370 colonies/ml of blood. The number of colonies grown from normal individuals and ranged between 40–120/ml blood. There was no correlation between the number of colonies and number of potentially proliferating granulocytic cells (myeloblasts, promyelocytes, myelocytes) plated. In six patients colony size was similar to that of colonies grown from normal individuals and larger than colonies grown from patients with leukemia. Morphologically, colonies consisted of either eosinophils, neutrophils, monocytes, or macrophages. These data indicate that an increased number of stem cells capable of giving rise to in vitro colonies of granulocytes and mononuclear cells circulate in patients with myelofibrosis. The growth pattern of these colonies differed from colonies grown from leukemia cells suggesting that these may not be closely related diseases.

Colonies of granulocytes and mononuclear cells can be grown in vitro from the blood and bone marrow of animals, and man in the presence of a colony-stimulating factor (CSF). In man, colonies can be grown from normal individuals as well as from patients with various diseases and consist of either neutrophils, eosinophils, monocytes, or macrophages. Colonies are the progeny of a single cell and because of the extensive replication required to produce a colony it is assumed that colony-forming cells represent one class of hematopoietic stem cells. The finding of an increased number of colony-forming cells (stem cells) in blood of patients with myelofibrosis forms the basis of this report.

MATERIALS AND METHODS

Blood anticoagulated with heparin was obtained from seven patients with typical features of myelofibrosis. Six were considered idiopathic and one (E.C.) was diagnosed as polycythemia vera 16 yr earlier. After sedimentation at room temperature blood leukocytes were cultured in a soft gel system using methylcellulose as described previously. Briefly, nucleated blood cells were suspended in 1.6% methylcellulose containing 15% fetal calf serum (FCS) and McCoys 5A tissue culture medium. Initially $5 \times 10^5$ nucleated cells were plated, but because of the large number of colonies which grew, the number plated was reduced to $2 \times 10^5$ plate. One milliliter of the methylcellulose–FCS–cell mixture was plated into 10 × 35 mm tissue culture plates and incubated at 37°C in 7.5% CO₂. Stimulation of colony growth was by a feeder layer of peripheral leukocytes.

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At the same time and in separate plates, stimulation was also achieved with conditioned medium (C.M.) prepared from human leukocytes. After 16–18 days of incubation the number of colonies containing greater than 50 cells was counted. For morphological identification, individual colonies were removed with a Pasteur pipette, smeared between glass coverslips, and stained with Wright's stain.

RESULTS

The clinical and laboratory findings of the patients studied are listed in Tables 1 and 2. All patients had hepatosplenomegaly, “tear drop” red cell changes and marrow fibrosis as seen in biopsy specimens. In six, the fibrosis was extensive and diffuse and in one (B. G.) the fibrosis was focal. None of the patients had chromosome abnormalities and the leukocyte alkaline phosphatase was normal or slightly elevated in four patients and borderline low in one (L.W.). Three patients were receiving androgens while four patients were not receiving any therapy at the time of study.

The number of colonies grown from the blood of patients with myelofibrosis is seen in Table 3. Colonies grew from all patients studied and ranged between 162–4370 colonies/ml of blood. Blood from four normal individuals cultured during the same period grew between 40–120 colonies/ml. In six patients colony size ranged between 50 and 1500 cells and was similar to that observed in normal individuals. In Patient L.W. colonies were smaller with a mean of 250 cells (range 50–500). Morphologically colonies were of various cell types and consisted of either eosinophils, neutrophils, monocytes, or macrophages similar to that observed in normal individuals.

The relationship between the number of colonies and number of potentially proliferating granulocytic cells plated is seen in Table 3. No good correlation was observed between the number of colonies and number of myeloblasts, promyelocytes, or myelocytes. Patient B.G., whose blood smear contained 4.0% potentially proliferating cells (0.5% blasts), gave rise to the largest number of colonies. L.W., whose blood contained 25% proliferating cells (12.5% blasts), gave rise to the second largest number of colonies. E.C., whose blood smear did not contain any recognizable blasts or promyelocytes and 3% myelocytes, grew the third largest number of colonies, while patients E.D. and W.F., whose blood contained many more potentially proliferating cells, grew considerably fewer colonies.

DISCUSSION

The results of this study indicate that increased numbers of cells capable of giving rise to colonies of granulocytes and mononuclear cells in vitro circulate in patients with myelofibrosis. This was true of patients whose disease was considered idiopathic as well as in the one patient who had long standing polycythemia vera prior to the development of myelofibrosis.

The number of colonies grown from the blood of these patients was 4–40 times greater than observed in normal individuals cultured during the same time period. In six patients colony size was similar to that observed in normal individuals and in one (L.W.), colonies were considerably smaller (50–500 cells; mean 250) than observed in the other patients. Whether this represents
### Table 1. Clinical Features of Patients With Myelofibrosis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hepatomegaly</th>
<th>Splenomegaly</th>
<th>Hematocrit (%)</th>
<th>WBC/cu mm</th>
<th>Platelets/cu mm</th>
<th>&quot;Tear drop&quot; red cells</th>
<th>LAP*</th>
<th>Marrow fibrosis</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.G.; 54 M</td>
<td>++</td>
<td>++++</td>
<td>31</td>
<td>7,800</td>
<td>52,000</td>
<td>++</td>
<td>74</td>
<td>++</td>
<td>None</td>
</tr>
<tr>
<td>L.W.; 20 F</td>
<td>++</td>
<td>++++</td>
<td>32</td>
<td>32,000</td>
<td>222,000</td>
<td>++</td>
<td>38</td>
<td>++++</td>
<td>Fluoxymesterone</td>
</tr>
<tr>
<td>E.C.; 81 F</td>
<td>++</td>
<td>++++</td>
<td>40</td>
<td>6,800</td>
<td>97,000</td>
<td>++</td>
<td>--</td>
<td>++++</td>
<td>Deposterosterone</td>
</tr>
<tr>
<td>J.P.; 54 M</td>
<td>++</td>
<td>++++</td>
<td>19</td>
<td>4,200</td>
<td>72,000</td>
<td>++</td>
<td>--</td>
<td>++++</td>
<td>Fluoxymesterone</td>
</tr>
<tr>
<td>E.D.; 47 F</td>
<td>++</td>
<td>++++</td>
<td>24</td>
<td>4,300</td>
<td>329,000</td>
<td>++</td>
<td>134</td>
<td>++++</td>
<td>None</td>
</tr>
<tr>
<td>W.F.; 61 M</td>
<td>++</td>
<td>++++</td>
<td>33</td>
<td>4,600</td>
<td>107,000</td>
<td>++</td>
<td>124</td>
<td>++++</td>
<td>None</td>
</tr>
<tr>
<td>M.B.; 60 M</td>
<td>++</td>
<td>++++</td>
<td>23</td>
<td>1,400</td>
<td>49,000</td>
<td>++</td>
<td>100</td>
<td>++++</td>
<td>None</td>
</tr>
</tbody>
</table>

*Leukocyte alkaline phosphatase (normal 40–90).
†As determined in biopsy specimen (1–4+).

### Table 2. Total and Differential* Blood Leukocyte Count in Patients With Myelofibrosis

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>B.G.</td>
<td>7,800</td>
<td>4.0</td>
<td>3.0</td>
<td>25.0</td>
<td>43.0</td>
<td>--</td>
<td>6.0</td>
<td>17.0</td>
<td>2.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L.W.</td>
<td>32,000</td>
<td>25.0</td>
<td>15.0</td>
<td>21.0</td>
<td>23.0</td>
<td>3.0</td>
<td>2.0</td>
<td>1.0</td>
<td>9.0</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E.C.</td>
<td>6,600</td>
<td>3.0</td>
<td>4.0</td>
<td>46.0</td>
<td>28.0</td>
<td>1.0</td>
<td>--</td>
<td>1.0</td>
<td>15.0</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J.P.</td>
<td>4,200</td>
<td>4.0</td>
<td>2.0</td>
<td>4.0</td>
<td>61.0</td>
<td>--</td>
<td>4.0</td>
<td>8.0</td>
<td>16.0</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E.D.</td>
<td>4,300</td>
<td>13.0</td>
<td>12.0</td>
<td>28.0</td>
<td>13.0</td>
<td>--</td>
<td>1.0</td>
<td>4.0</td>
<td>26.0</td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W.F.</td>
<td>4,600</td>
<td>13.0</td>
<td>8.0</td>
<td>26.0</td>
<td>22.0</td>
<td>2.0</td>
<td>2.0</td>
<td>5.0</td>
<td>21.0</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.B.</td>
<td>1,400</td>
<td>4.0</td>
<td>--</td>
<td>6.0</td>
<td>65.0</td>
<td>--</td>
<td>--</td>
<td>5.0</td>
<td>16.0</td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Based on 500 cell differential count.
Table 3. In Vitro Colonies from Blood of Patients With Myelofibrosis

<table>
<thead>
<tr>
<th>Patient</th>
<th>WBC/cc mm</th>
<th>myelocytes ($\times 10^4$)*</th>
<th>Blasts, promyelocytes, Myeloblasts ($\times 10^3$)*</th>
<th>Colonies*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.G.</td>
<td>7,800</td>
<td>39</td>
<td>312</td>
<td>4,370</td>
</tr>
<tr>
<td>L.W.</td>
<td>32,000</td>
<td>4,000</td>
<td>8,000</td>
<td>2,816</td>
</tr>
<tr>
<td>E.C.</td>
<td>6,600</td>
<td>0</td>
<td>198</td>
<td>2,244</td>
</tr>
<tr>
<td>J.P.</td>
<td>4,200</td>
<td>126</td>
<td>168</td>
<td>756</td>
</tr>
<tr>
<td>E.D.</td>
<td>4,300</td>
<td>86</td>
<td>559</td>
<td>730</td>
</tr>
<tr>
<td>W.F.</td>
<td>4,600</td>
<td>45</td>
<td>585</td>
<td>495</td>
</tr>
<tr>
<td>M.B.</td>
<td>1,400</td>
<td>28</td>
<td>58</td>
<td>162</td>
</tr>
</tbody>
</table>

*Number per milliliter blood.

a true difference in growth pattern or some other phenomenon will require further study. The differences in this patient were the high WBC and the early onset of extensive fibrosis.

Increased numbers of colonies can also be grown from the blood of patients with acute myeloblastic leukemia (AML)\(^6,13,14,16\) and chronic myelocytic leukemia (CML).\(^11\) While an increased number of colonies can be grown in these diseases, colonies from patients with AML and many with CML are smaller than are observed in normal individuals. It is possible that patient L.W.'s disease is more closely related to the myeloid leukemias than the other patients in the present study.

There was no good correlation between the number of colonies and number of potentially proliferating granulocytic cells plated or to any other recognizable blood leukocyte. The largest number of colonies grew from B.G., whose blood contained considerably less potentially proliferating cells than a number of other patients in the study. L.W., whose blood contained 100 times as many blasts and 30 times as many potentially proliferating cells as B.G., grew approximately one-half as many colonies. Patient E.C., who had no blasts or promyelocytes in the blood smear, gave rise to the third largest number of colonies. These findings suggest that only a fraction of the granulocytic cells in the proliferating compartment are capable of giving rise to in vitro colonies or that a cell other than that recognized as belonging to the myeloid series is responsible for production of colonies. It seems quite likely that as the marrow becomes fibrotic, immature cells of many types, those recognized as belonging to the myeloid series as well as even more immature cells, are released from the marrow. Circulation of increased numbers of such cells could result in an increased number lodging in the spleen and liver and could account for the extra-medullary hematopoiesis seen in this disease. Another possible explanation is that the liver and spleen are embryonic hematopoietic organs and are again triggered into this role.

A relationship between the degree of fibrosis and number of circulating stem cells could not be obtained since all but one patient had extensive and diffuse fibrosis at the time of study. However, it is of some interest that patient B.G., who gave rise to the largest number of colonies, had a cellular marrow and focal fibrosis.

The relationship between myelofibrosis, polycythemia vera, and the myeloid
leukemias is not entirely clear. While these diseases have been grouped by
some as the “myeloproliferative disorders” others have questioned the use-
fulness of such a grouping in dealing with these disorders. Features such as
the difference in distribution of the Philadelphia chromosome and leukocyte
alkaline phosphatase indicate that these may be different diseases. Blood
leukocytes from patients with Polycythemia vera studied thus far did not
give rise to an increased number of colonies prior to the development of
myelofibrosis. While a relationship exists between Polycythemia vera and
myeofibrosis, the findings in the present study suggest that these may not
be closely related to the myeloid leukemias.

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