Responsiveness of Human Granulocytic Leukemic Cells to Colony-stimulating Factor

By D. Metcalf, M. A. S. Moore, J. W. Sheridan, and G. Spitzer

The proliferative response of normal and leukemic human granulocytic cells to stimulation by varying concentrations of the normal regulator, colony-stimulating factor (CSF), was determined by cluster counts in agar cultures of 150 blood or marrow specimens stimulated by monkey lung conditioned medium. Acute myeloid leukemic cells were slightly more responsive than normal at low concentrations of conditioned medium, but chronic myeloid leukemic cells were slightly less responsive at all concentrations. Marrow cells from acute leukemic patients in remission exhibited a normal pattern of responsiveness. Average plasma CSF levels in leukemic patients were two to three times higher than the concentration of CSF in cultures maximally stimulated by monkey lung conditioned medium. The observed responsiveness of leukemic cells to stimulation by CSF-containing material is further evidence in support of the conclusion that most myeloid leukemias in man are conditioned, rather than autonomous, neoplasms.

Granulocytic progenitor cells from patients with chronic myeloid leukemia (CML) or acute myeloid leukemia (AML) can proliferate in agar cultures to form granulocytic colonies or clusters if stimulated by underlayers containing normal white cells or media conditioned by such cells. Karyotypic and other evidence indicates that in cultures from CML or untreated AML patients the proliferating cells are representative members of the leukemic populations, whereas in cultures from AML patients in full remission, the proliferating cells appear to be normal granulopoietic cells. Evidence has been obtained to indicate that the active component in the underlayers which stimulates the proliferation of normal or leukemic granulopoietic cells is the glycoprotein, colony-stimulating factor (CSF). Although some colony or cluster formation can occur in unstimulated cultures, this is due to the activity of CSF-producing cells in the cultured suspension, and if these are first removed by differential centrifugation in bovine serum albumin, neither leukemic nor normal human colony- or cluster-forming cells can proliferate in the absence of an added exogenous source of CSF. “Spontaneous” colony or cluster formation in unstimulated cultures of unfrac-tionated marrow or blood cells is markedly concentration-dependent and is usually absent when low cell concentrations are cultures.
Serum and urine CSF levels are elevated at some stage of the disease in all AML patients and are often elevated in CML. However, a proper assessment of the potential importance of these elevated CSF levels as a proliferative stimulus for leukemic cells in vivo requires quantitative studies on the proliferative responsiveness of leukemic cells to various concentrations of CSF compared with normal granulocytic cells.

In the present experiments, dose–response curves have been established for the responsiveness of leukemic and normal human granulocytic cells to stimulation by various concentrations of medium containing colony-stimulating factor.

**MATERIALS AND METHODS**

**Monkey Lung Conditioned Medium**

The technique used to prepare monkey lung conditioned medium was similar to that described previously for the preparation of mouse lung conditioned medium. Fresh lung tissue from Rhesus monkeys was cut into 100-mg pieces and pairs of pieces placed in 5 ml volumes of serum-free Eagle’s medium in capped plastic tubes. These were incubated at 37°C for 72 hr in an atmosphere of 10% CO₂ in air. The medium was harvested, heated to 56°C for 30 min, centrifuged at 12,000 g for 15 min, then dialyzed against three changes of distilled water at 4°C for 3 days. The initial dialysis water contained 0.02% sodium azide and subsequent dialysis, water penicillin (2 × 10⁵ U/liter) and streptomycin (200 mg/liter). The dialyzed medium was again centrifuged, then Millipore filtered and stored at -20°C.

**Human Marrow and Blood Cultures**

Portions of bone marrow and blood specimens collected for diagnostic or follow-up clinical assessment were kindly made available by the Hematology Departments of the following Melbourne hospitals: Royal Melbourne Hospital, Alfred Hospital, St. Vincent’s Hospital, Prince Henry’s Hospital, the Austin Hospital. The diagnosis and morphologic classification of acute leukemia were performed by the collaborating hematology departments and were subjected to independent review in this Unit. Morphologic subdivisions were according to the criteria of Hayhoe. The cell suspensions in preservative-free heparin were centrifuged at 500 g for 3-5 min, and the buffy coats washed three times in Eisen’s salt solution. Cultures were prepared in 35-mm plastic Petri dishes using modified Eagle’s medium in 0.3% agar. The composition of this medium and the general technique used have been described elsewhere. One-milliliter underlayers were prepared containing 0.1 ml of serial twofold dilutions in water of the monkey lung conditioned medium in modified Eagle’s medium in 0.5% agar. In each assay, three replicate cultures were prepared for each of the seven dilutions of conditioned medium used, together with an eighth set (unstimulated) containing 0.1 ml of saline.

The human cells to be cultured were placed in 1-ml overlayers in modified Eagle’s medium in 0.3% agar and allowed to gel. Cultures contained 25,000 nucleated cells to minimize endogenous CSF production, except in two instances when 10,000 cells were cultured. Cultures were incubated for 4 days at 37°C in a fully humidified atmosphere of 10% CO₂ in air. Each marrow or blood specimen was also cultured in cell doses of 10,000, 50,000, 100,000 cells per ml on underlayers containing 10⁶ human peripheral blood cells to determine the incidence at day 7 of cluster-forming and colony-forming cells per 10⁶ cells.

**Scoring of Cultures**

Cultures were scored at × 40 magnification using an Olympus dissecting microscope with indirect lighting. The size achieved by colonies is dependent on CSF concentration, and thus the lower size limit for colonies needs to be reduced progressively when scoring colonies stimulated by progressively lower concentrations of CSF. To avoid this subjective element in scoring, all aggregates of three or more cells were scored (total cluster count) in every culture dish. Furthermore, because total aggregate numbers decline rapidly in many human cultures after
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day 4 or 5 of incubation, due mainly to dispersion of aggregates, \(^5\) total plate counts were performed in most cases on day 4 of incubation, at which time all aggregates were quite discrete and unequivocal. Total cluster counts for each dilution were expressed as a percentage of the maximum cluster count obtained (usually with undiluted conditioned medium).

**Plasma CSF Assays**

Assays were performed on dialyzed plasma specimens using the technique described previously.\(^6\) While dialysis does not remove all high molecular weight serum CSF inhibitors, the majority of dialyzed plasma specimens give linear dose-response curves with no evidence of high-dose inhibition which permits an assessment of approximate CSF concentrations. Plasmas were assayed in replicate cultures at doses of 0.1 and 0.05 ml, with 75,000 C57BL bone marrow cells and colony counts at day 7, standardized using a correction factor derived from five standard CSF preparations (four human, one monkey) included in each assay run.

**RESULTS**

Assays using monkey lung conditioned medium in single-layer cultures of human marrow cells indicated that the medium was capable of stimulating the development of typical colonies and clusters, although the mean number of colonies varied from 30\% to 50\% of the number stimulated by underlayers containing \(10^6\) human peripheral blood nucleated cells.

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**Fig. 1.** Total clusters per \(10^5\) cultured cells from various types of patients comparing day-7 cluster counts in underlayer-stimulated cultures (left hand points) with day-4 cluster counts in conditioned medium-stimulated cultures (right-hand points).
<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Disease</th>
<th>Cells Cultured</th>
<th>Treatment</th>
<th>Marrow Cellularity</th>
<th>Marrow Differential</th>
<th>Calculated No. Clusters per 10^6 Cells</th>
<th>Lung CM (1:1)</th>
<th>Plasma CSF*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acute lymphoid leukemia Remission</td>
<td>BM</td>
<td>Daunorubicin, cytosine arabinoside, vincristine</td>
<td>–</td>
<td>B1 11, Pro 5, Myel 10, Meta 6, P 26, E 4, Ly 12, Ery 26</td>
<td>0 416 17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Iron-deficiency anemia</td>
<td>BM</td>
<td>None</td>
<td>–</td>
<td>Bl 1, Pro 3, Myel 7, Meta 8, P 38, E 6, Ly 6, PC 1, Ery 30</td>
<td>0 464 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Fever, unknown origin</td>
<td>BM</td>
<td>None</td>
<td>N</td>
<td>Bl 1, Pro 4, Myel 5, Meta 2, P 17, E 2, Mono 1, Ly 3, Ery 65</td>
<td>0 660</td>
<td></td>
<td></td>
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<tr>
<td>4</td>
<td>Gastrointestinal bleeding</td>
<td>BM</td>
<td>None</td>
<td>N</td>
<td>Bl 1, Pro 4, Myel 3, Meta 4, P 30, E 4, Ly 8, Ery 46</td>
<td>0 452 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Lymphoma</td>
<td>BM</td>
<td>Pretreatment</td>
<td>N</td>
<td>Bl 0, Pro 2, Myel 8, Meta 12, P 26, Ly 16, Ery 36</td>
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<tr>
<td>6</td>
<td>Multiple myeloma</td>
<td>BM</td>
<td>Pretreatment</td>
<td>N</td>
<td>Bl 0, Pro 2, Myel 9, Meta 6, P 39, E 2, PC 23, Ery 18</td>
<td>92 1022</td>
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<td></td>
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<tr>
<td>7</td>
<td>Thrombocytopenic purpura</td>
<td>BM</td>
<td>None</td>
<td>N</td>
<td>Bl 2, Pro 6, Myel 4, Meta 12, P 22, E 2, Ly 12, PC 4, Ery 36</td>
<td>0 480 16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Lymphosarcoma</td>
<td>BM</td>
<td>Cytosine arabinoside</td>
<td>+</td>
<td>Bl 0, Pro 0, Myel 1, Meta 1, P 1, Ly 97</td>
<td>0 164 36</td>
<td></td>
<td></td>
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<tr>
<td>9</td>
<td>Lymphosarcoma</td>
<td>BM</td>
<td>Pretreatment</td>
<td>N</td>
<td>Bl 2, Pro 2, Myel 19, Meta 12, P 24, E 6, Ly 8, PC 1, Ery 26</td>
<td>0 1376 6</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Diagnosis</td>
<td>Source(s)</td>
<td>Responsiveness to CSF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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</tr>
<tr>
<td>10</td>
<td>Acute lymphoid leukemia remission</td>
<td>BM Daunorubicin cytosine arabinoside, vincristine</td>
<td>Bl 3, Pro 4, Myel 8, Meta 6, P 43, E 8, Ly 14, Ery 14</td>
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<td>82</td>
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<td>11</td>
<td>Rheumatoid arthritis</td>
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<td></td>
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<td>12</td>
<td>Lymphosarcoma</td>
<td>BM Pretreatment</td>
<td>Bl 0, Pro 1, Myel 7, Meta 5, P 15, Mono 1, Ly 44, Ery 27</td>
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<td>600</td>
<td>5</td>
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<td></td>
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<td>13</td>
<td>Sideroblastic anemia</td>
<td>BM None</td>
<td>Bl 0, Pro 5, Myel 13, Meta 10, P 22, Ly 4, Ery 46</td>
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<td>116</td>
<td>—</td>
<td></td>
<td></td>
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<tr>
<td>14</td>
<td>Iron-deficiency anemia</td>
<td>BM None</td>
<td>Bl 2, Pro 2, Myel 11, Meta 12, P 39, E 3, Mono 1, Ly 7, PC 1, Ery 22</td>
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<td>332</td>
<td>3</td>
<td></td>
<td></td>
</tr>
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<td>15</td>
<td>Liver disease</td>
<td>BM None</td>
<td>Bl 1, Pro 2, Myel 8, Meta 9, P 20, E 1, Ly 8, PC 1, Ery 47</td>
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<td>5120</td>
<td>11</td>
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<td></td>
</tr>
<tr>
<td>16</td>
<td>Iron-deficiency anemia</td>
<td>BM None</td>
<td>Bl 2, Pro 2, Myel 8, Meta 11, P 20, E 1, Ly 29, Ery 26</td>
<td>0</td>
<td>108</td>
<td>2</td>
<td></td>
<td></td>
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<tr>
<td>17</td>
<td>Megaloblastic anemia</td>
<td>BM Pretreatment</td>
<td>Bl 2, Pro 2, Myel 7, Meta 7, P 32, E 1, Ly 14, PC 2, Ery 33</td>
<td>0</td>
<td>428</td>
<td>2</td>
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</tr>
</tbody>
</table>

Abbreviations used: Bl, blasts; Pro, promyelocytes; Myel, myelocytes; Meta, metamyelocytes; P, polymorphonuclears; E, eosinophils; Ly, lymphocytes (normal and abnormal); PC, plasma cells; Ery, nucleated erythroid cells; Mono, monocytes; BM, bone marrow; PB, peripheral blood.

Marrow cellularity: —, slightly reduced; N, normocellular; +, slightly increased; ++, increased.

*Colony numbers stimulated by 0.1 ml plasma.
In an initial pilot study, 57 marrow or blood specimens were cultured with undiluted monkey lung conditioned medium or human peripheral blood underlayers. Comparison of total aggregates at day 4 using human peripheral blood underlayers (Fig. 1) confirmed previous observations that total aggregate counts fall sharply between day 4 and day 7 in cultures of most human marrows. With the exception of cells from one case of AML, and one case of CML which responded very poorly to stimulation by monkey lung conditioned medium, there was a general parallelism between the growth obtained in the two types of cultures, and monkey lung conditioned medium did not appear to selectively stimulate or fail to stimulate cells from various types of patients.

Based on the results of this pilot study, it was decided to use monkey lung conditioned medium as the stimulus for cultures, since optical conditions were better than in cultures using white cell underlayers. Detailed analysis was made of cluster formation in cultures of cells from nonleukemic and leukemic patients using varying concentrations of lung conditioned medium. Data from marrow cultures of 17 patients with a variety of hematologic disorders, exclusive of myeloid leukemia, were used to establish the “normal” dose–response curve of human cells to monkey lung conditioned media. The clinical details of this group of patients are listed in Table 1, together with the absolute D4 cluster counts in unstimulated cultures and cultures stimulated by the highest concent-

![Fig. 2. Dose-response curves of cluster formation stimulated by increasing concentrations of monkey lung conditioned medium. Shaded area depicts mean values for normal marrow cells ± 1 SD. Points are mean values for each group, vertical bars are standard deviations of mean values. Number of patients indicated in parentheses. CML, chronic myeloid leukemia; SubAML, subacute myeloid leukemia.](image-url)
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tration of lung conditioned medium used. A sigmoid dose–response curve was obtained which, together with the standard deviations of the mean values, is shown as the shaded area in Figs. 2 and 3. In addition to a progressive increase in total aggregate numbers, there was also a progressive size increase in aggregates with increasing concentrations of monkey lung conditioned medium. It should be noted that, because of the low cell concentrations used, no aggregates developed in unstimulated cultures of 15 of the 17 marrows analyzed. Although maximum aggregate numbers varied widely between different marrows, this had no significant influence on the shape of the dose–response curves.

Cells cultured from the bone marrow or blood of six patients with CML showed a dose–response curve of similar shape to the normal curve, but the mean values were at, or just below, the lower limits of the normal range (Fig. 2). The clinical and hematologic details of these patients are listed in Table 2. As in the case of the control cultures, the shape of the dose–response curves was not influenced by variations in the maximum number of clusters developing in cultures from individual patients.

![Graph showing dose-response curves](image)

**Fig. 3.** Dose–response curves of cluster formation stimulated by increasing concentrations of monkey lung conditioned medium. Shaded area depicts mean values for normal marrow cells ± 1 SD. Points are mean values for each group, vertical bars are standard deviations of mean values. Number of patients indicated in parentheses. AML, acute myeloid leukemia, including two patients with acute myelomonocytic leukemia.
<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Disease</th>
<th>Cells Cultured</th>
<th>Treatment</th>
<th>Marrow Cellularity or WBC</th>
<th>Blood or Marrow Differential</th>
<th>Calculated No. Clusters per 10^9 cells</th>
</tr>
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<tr>
<td>18</td>
<td>CML</td>
<td>PB</td>
<td>Pretreatment</td>
<td>130,000</td>
<td>BL 6, Pro 16, Myel 10, Meta 20, P 34, E 2, B 5, Mono 0, Ery 5, Ly 2</td>
<td>8  2740  15</td>
</tr>
<tr>
<td>19</td>
<td>CML</td>
<td>PB</td>
<td>6-MP methotrexate 2 wk before</td>
<td>137,000</td>
<td>BL 9, Pro 2, Myel 28, Meta 11, P 25, E 2, B 15, Mono 1, Ery 0, Ly 6</td>
<td>0  68  37</td>
</tr>
<tr>
<td>20</td>
<td>CML</td>
<td>PB</td>
<td>Melphelan</td>
<td>25,800</td>
<td>Pro 3, Myel 5, Meta 4, P 74, E 2, B 7, Ly 5</td>
<td>0  832  3</td>
</tr>
<tr>
<td>21</td>
<td>CML</td>
<td>PB</td>
<td>Pretreatment</td>
<td>300,000</td>
<td>BL 3, Pro 6, Myel 11, Meta 16, P 52, E 1, B 7, Mono 2, Ly 2</td>
<td>0  2320  26</td>
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<tr>
<td>22</td>
<td>CML</td>
<td>BM</td>
<td>Pretreatment</td>
<td>+ +</td>
<td>BL 4, Pro 5, Myel 18, Meta 8, P 61, E 4, B 0, Mono 0, Ly 0</td>
<td>0  14000  -</td>
</tr>
<tr>
<td>23</td>
<td>CML</td>
<td>PB</td>
<td>Pretreatment</td>
<td>300,000</td>
<td>BL 3, Pro 7, Myel 19, Meta 19, P 49, E 1, B 2, Mono 0, Ly 0</td>
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<td>24</td>
<td>AMML</td>
<td>BM</td>
<td>Pretreatment</td>
<td>N</td>
<td>BL 18, Pro 0, Myel 12, Meta 4, P 10, Mono 56</td>
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<tr>
<td>25</td>
<td>AML</td>
<td>BM</td>
<td>Cytosine arabinoside Thioguanine</td>
<td>N</td>
<td>BL 12, Pro 2, Myel 19, Meta 20, P 29, Mono 1, Ly 3, Ery 14</td>
<td>24  700  23</td>
</tr>
<tr>
<td>26</td>
<td>AML</td>
<td>BM</td>
<td>Pretreatment</td>
<td>+</td>
<td>BL 82, Pro 1, Myel 3, Meta 0, P 1, PC 1, Ery 7</td>
<td>88  .1760  6</td>
</tr>
<tr>
<td>27</td>
<td>Smouldering AML</td>
<td>BM</td>
<td>Pretreatment</td>
<td>N</td>
<td>BL 18, Pro 12, Myel 10, Meta 4, P 8, Ly 1, PC 1, Ery 46</td>
<td>0  168  46</td>
</tr>
<tr>
<td>28</td>
<td>AMML</td>
<td>BM</td>
<td>Pretreatment</td>
<td>+</td>
<td>BL 16, Pro 1, Myel 4, Meta 5, P 10, Mono 35, Ly 4, PC 3, Ery 22</td>
<td>4  760  1</td>
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AML/AMML Pretreatment or Relapse
<table>
<thead>
<tr>
<th>Case</th>
<th>Diagnosis</th>
<th>Stage</th>
<th>Treatment</th>
<th>Colony Numbers</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>AML</td>
<td>BM</td>
<td>Pretreatment</td>
<td>+  Bl 55, Pro 0, Myel 4, Meta 0, P 2, E 3, Mono 6, Ly 18, Ery 12</td>
<td>0 144 21</td>
</tr>
<tr>
<td>30</td>
<td>Smouldering AML</td>
<td>BM</td>
<td>Pretreatment</td>
<td>+  Bl 10, Pro 10, Myel 8, Meta 2, P 56, E4, Ery 10</td>
<td>0 36 8</td>
</tr>
<tr>
<td>31</td>
<td>AML</td>
<td>BM</td>
<td>Pretreatment</td>
<td>+  Bl 14, Pro 10, Myel 44, Meta 10, P 18, Ery 4</td>
<td>8 3360 8</td>
</tr>
<tr>
<td>32</td>
<td>AML</td>
<td>BM</td>
<td>Cytosine arabinoside, methotrexate, vincristine 20 days before</td>
<td>N  Bl 0, Pro 1, Myel 27, Meta 27, P 25, E 4, Ery 16</td>
<td>0 150 1</td>
</tr>
<tr>
<td>33</td>
<td>AML</td>
<td>BM</td>
<td>Cytosine arabinoside, thio guanine 10 days before</td>
<td>N  Bl 7, Pro 5, Myel 2, Meta 3, P 13, Mono 1, Ly 12, Ery 57</td>
<td>4 300 22</td>
</tr>
<tr>
<td>34</td>
<td>AML</td>
<td>BM</td>
<td>Cytosine arabinoside, vincristine, prednisone 21 days before</td>
<td>N  Bl 6, Pro 3, Myel 10, Meta 19, P 24, Mono 2, Ly 5, PC 1, Ery 36</td>
<td>0 464 24</td>
</tr>
<tr>
<td>35</td>
<td>AMML</td>
<td>BM</td>
<td>Cytosine arabinoside, thio guanine 18 days before</td>
<td>N  Bl 13, Pro 23, Myel 6, Meta 3, P 10, Ly 24, Ery 27</td>
<td>4 1088 19</td>
</tr>
<tr>
<td>36</td>
<td>AML</td>
<td>BM</td>
<td>6-MP methotrexate continuous</td>
<td>N  Bl 2, Pro 5, Myel 5, Meta 18, P 36, Ly 8, Ery 26</td>
<td>0 556</td>
</tr>
<tr>
<td>37</td>
<td>AML</td>
<td>BM</td>
<td>VAMP 6 days previously</td>
<td>N  Bl 5, Pro 3, Myel 8, Meta 4, P 18, Ly 6, Ery 56</td>
<td>4 348 14</td>
</tr>
</tbody>
</table>

For abbreviations see footnotes Table 1. AML, acute myeloid leukemia; AMML, acute myelomonocytic leukemia; CML, chronic myeloid leukemia.

*Colony numbers stimulated by 0.1 ml plasma.
Cells from three patients gave highly abnormal dose–response curves (Fig. 2). One of these patients was diagnosed as subacute myeloid leukemia, and the other two were Philadelphia-negative myeloproliferative disorders, one with a myelomonocytic dyscrasia, the other with splenomegaly, mature eosinophilia, and neutrophilia with low neutrophil alkaline phosphatase scores. The cells from these three patients were highly responsive to stimulation by monkey lung conditioned medium, and exhibited some cluster formation in the unstimulated cultures.

Cells growing in agar from the marrow or blood of eight patients with untreated AML or AML in relapse (including two patients with acute myelomonocytic leukemia) also exhibited responsiveness to stimulation by increasing concentrations of conditioned medium, but the dose–response curve was of abnormal shape (Fig. 3). Although, as in the case with control and CML cultures, only 25,000 cells were cultured per dish, a significant degree of cluster formation was observed with cells from three of the eight cases in unstimulated cultures. AML cells were more responsive than normal cells at low concentrations of conditioned medium, but the over-all dose–response curve was flatter than those obtained with either normal or CML cells. In contrast, bone marrow cells from six patients with AML in full clinical and hematologic remission gave dose–response curves which were wholly within the normal range (Fig. 3).

Bone marrow or blood cells from six patients with myeloproliferative disorders (two with aplastic anemia, one with myelofibrosis, two with megakaryocytic myelosis, and one with polycythemia vera) gave dose–response curves which were wholly within the normal range. As with the leukemic patients, neither the level of marrow cellularity nor the absolute numbers of clusters per culture significantly altered the shape of the dose–response curves.

The above observations on the different responsiveness patterns of normal and leukemic cells were confirmed in a further series of 59 cultures from patients with leukemia or other disorders using two other batches of monkey lung conditioned medium and in cultures of a smaller group of 22 patients using human spleen conditioned medium as the stimulus.

In all, 150 marrow and blood specimens were cultured and analyzed using one or other form of conditioned medium. Table 3 presents a summary of the over-all results obtained with cells from the leukemic patients surveyed, which comprised 32 patients with AML or AMML, 12 with CML, and four in the acute transformation phase of CML. Cells from 15 of these patients failed to grow in agar, and nothing can be said regarding the responsiveness of these cells to lung conditioned medium. Of the cells from the 11 AML/AMML pa-

<table>
<thead>
<tr>
<th>Disease</th>
<th>Number of Patients Studied</th>
<th>Autonomous Growth</th>
<th>Growth Responsive to Conditioned Medium</th>
<th>No Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML/AMML</td>
<td>32</td>
<td>0</td>
<td>21</td>
<td>11</td>
</tr>
<tr>
<td>CML</td>
<td>12</td>
<td>0</td>
<td>9</td>
<td>3</td>
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<td>CML acute transformation</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>
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tients that failed to grow when stimulated by lung conditioned medium, one
grew well and seven formed small numbers of clusters when stimulated by
human white cell underlayers. Three cell suspensions failed to grow in any type
of culture, but two of these were peripheral blood specimens from treated
patients, whose bone marrow cells did proliferate in agar. All four of the CML
and acute transformation cell suspensions which failed to proliferate when
stimulated by lung conditioned medium were from heavily treated aplastic pa-
tients, but the cell suspensions did form low numbers of clusters when stimu-
lated by human white cell underlayers. In six cases the number of clusters which
formed was too low to permit construction of complete dose–response curves
(maximum number of clusters less than 25 per culture dish), but in every case
the maximally stimulated cultures contained higher numbers of clusters than
dishes with lower concentrations, and most of the latter had no clusters. From
each of the remaining 26 patients, cells were cultured and analysed on one to
four occasions. In every instance the leukemic cells exhibited responsiveness to
stimulation by one or other type of conditioned medium, and no example of an
autonomous leukemic population was encountered in which cell growth was
not stimulated by added conditioned medium.

To determine the relative concentrations of CSF in the conditioned media
used in these experiments, compared with CSF levels in the plasma of patients
with CML and AML, assays for colony-stimulating activity were performed on
dialyzed plasma specimens from the patients listed in Tables 1 and 2 and com-
pared with the activity of monkey lung conditioned medium. All assays were
performed using 75,000 C57BL bone marrow cells as target cells.10

The mean number of colonies stimulated by 0.1 ml of undiluted monkey lung
conditioned medium (the maximum concentration used in the dose–response
curves) was 62 ± 10. As is shown in Tables 1 and 2, the mean number of
colonies stimulated by 0.1 ml of dialyzed plasma was 11 ± 10 for the control
patients and 16 ± 12 for the myeloid leukemic patients.

Since the human plasmas were assayed at a tenfold dilution (0.1 ml plasma
in a 1-ml culture), extrapolation of this data to the in vivo situation indicates
that in normal and leukemic humans, average plasma CSF concentrations are
two to three times higher than the concentrations of monkey lung CSF ob-
served to cause maximum stimulation of cluster formation by normal and
leukemic cells in vitro.

DISCUSSION

The present study has confirmed that growth in agar by granulocytic cells
from patients with CML or AML is dependent on stimulation by material con-
taining colony-stimulating factor, CSF. No example was encountered in which
the growth of leukemic cells in vitro in unstimulated cultures was equal to that
following stimulation by CSF-containing conditioned medium. No conclusions
can be reached regarding the CSF responsiveness of cells from the minority
group of myeloid leukemic patients whose cells failed to grow in agar. Similarly,
although karyotypic and other data suggest that the cells proliferating in
agar are genuinely representative of the leukemic population,5,6 some caution
needs to be used in interpreting data from those patients where the plating efficiency of the leukemic cells was very low.

Although both AML and CML cells responded progressively to increasing concentrations of conditioned medium, the dose–response curves for both were abnormal and significantly different from one another. CML cells were slightly less responsive than normal, whereas AML cells were more responsive than normal at low concentrations of conditioned medium.

The normal responsiveness of marrow cells cultured from AML patients in remission reinforces earlier karyotypic, density gradient, and cell cycle data, indicating that, in remission, clones of normal granulopoietic cells can reappear and displace preexisting leukemic populations.

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RESPONSIVENESS TO CSF

these cells to CSF-containing material supports previous evidence, indicating that most myeloid leukemias in man are conditioned, rather than autonomous, neoplasms. If this conclusion is valid, the elevated levels of CSF commonly observed in these patients should represent a significant factor influencing the progressive proliferation of the leukemic populations.

ACKNOWLEDGMENT

The authors are indebted to Mrs. J. Thompson and Mrs. J. Yeats for technical assistance throughout this work. We wish to thank the following individuals for their assistance in providing clinical material: Dr. T. Hurley and Dr. D. Cowling, Royal Melbourne Hospital, Professor D. Penington and Dr. B. Rush, St. Vincent's Hospital, Professor B. Firkin and Dr. M. Whiteside, Alfred Hospital, Dr. D. Forster, Prince Henry's Hospital, and Dr. P. Castaldi, Austin Hospital.

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Responsiveness of Human Granulocytic Leukemic Cells to Colony-stimulating Factor

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