CONCISE REPORT

A Murine Model System for the Study of the Human Leukemia-Associated Inhibitory Activity

By John Marcelletti and Philip Furmanski

Mice inoculated with the RFV strain of Friend virus develop an erythroleukemia that spontaneously regresses. Bone marrow cells from leukemic mice produce a factor that inhibits colony formation in agar by normal CFU-Cs. CFU-Cs from leukemic animals are resistant to the factor. Bone marrow cells from some mice whose leukemia has regressed continue to produce the factor, and CFU-Cs from some regressed mice retain resistance to the factor. These observations duplicate findings reported for patients with acute leukemias and those with leukemia in remission. The RFV system thus serves as an accurate experimental model for the study of hematopoietic cell interactions and the proliferative advantage of neoplastic cells in human leukemias.

PATIENTS with acute leukemia exhibit depressed levels of normal hematopoiesis. This may be responsible for the apparent proliferative advantage of leukemic cells in their host and leukemia-associated effects, such as anemia and immunosuppression.

Recently, Broxmeyer et al. have reported that extracts or media conditioned by bone marrow cells from leukemic patients inhibit colony formation in agar by normal human granulocytic stem cells (CFU-C).1 3 Colony formation by CFU-Cs from leukemic patients was unaffected. An important finding was that CFU-Cs from most patients whose leukemia was in complete clinical remission were also resistant to the extracts. Broxmeyer et al. have proposed that suppression of hematopoiesis and the proliferative advantage of leukemic cells may derive from the factor(s) produced by the leukemic bone marrow cells, which they have termed LIA, or other similar factors and hematopoietic cell interactions.

Further study in human systems of this important phenomenon in clearly required. However, it would also be highly advantageous to have available a parallel model system for studies of LIA that are not possible or practical in leukemic patients. In this article, we show that the phenomenon of leukemic bone marrow inhibition of normal granulocytopenesis is duplicated exactly in the mouse during erythroleukemogenesis induced by the RFV strain of Friend virus and its spontaneous regression.4 5 This system, therefore, serves as an approximate model for the study of LIA and its interaction with elements of the hematopoietic system.

MATERIALS AND METHODS

Leukemia Induction

Virus stocks were prepared from spleens of leukemic mice (20% w/v in phosphate-buffered saline, PBS) as previously described6 and stored in sealed ampules at −70°C. Random-bred Swiss/ICR and inbred N/PLCR mice were injected i.p. with 0.5 ml PBS containing 100 ID50 of virus. Mice were checked biweekly by spleen palpation for leukemia development and regression. Spleen weight determined by palpation is an accurate indicator of leukemic status.7

Preparation of Bone Marrow Conditioned Medium (LIA)

Bone marrow cells from normal, leukemic, and regressed animals were prepared from marrow plugs expressed from femurs using a needle and syringe containing RPMI 1630 medium plus 10% calf serum. The cells were dispersed by pipetting and washed once with PBS. The cells were adjusted to 106 nucleated cells/ml with RPMI 1640 plus 10% calf serum, and 5 ml was plated into a T-25 flask. After 3 days of incubation at 37°C, the resulting supernatants were passed through a 0.22-μm Millipore filter. These cell-free conditioned media were stored at −70°C until used.

Assay of LIA

Resident peritoneal macrophages were obtained from normal mice by peritoneal lavage using RPMI 1640 plus 10% calf serum. The cells were plated onto 35-mm tissue culture dishes at a density of 5 × 105 macrophages per dish. After 1 hr incubation at 37°C in 10% CO2 to allow for macrophage adherence, the plates were washed twice with PBS and overlaid with fresh RPMI 1640 plus 10% calf serum containing 0.5% agar. After 3-5 days of incubation, these macrophage monolayers served as a source of granulocyte-macrophage colony-stimulating factor for the target cell for LIA, CFU-C.

Bone marrow cells, obtained as described above, were adjusted to 105 nucleated cells/ml with RPMI 1630 plus 10% calf serum and 0.5 ml of the suspension aliquoted into individual tubes. To these cell suspensions was added 50 μl of fresh medium or the appropriate dilution of medium conditioned by bone marrow cells from either normal, leukemic, or regressed animals. The cells were placed at 37°C in a shaking water bath for 30 min, diluted to 103 nucleated cells/ml with RPMI 1640 plus 10% fetal calf serum and 0.3% agar.

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One milliliter of the mixture was then plated onto each of three of the macrophage monolayers described above. The cultures were incubated at 37°C in 10% CO₂ for 3–5 days, at which time the number of colonies was scored under a dissecting microscope (25×).

Elimination of Virus From LIA Preparations

Aliquots of 0.5 ml of bone marrow conditioned medium were admixed with 0.1 ml goat anti-Rauscher gp70 (obtained from the Division of Cancer Cause and Prevention, National Cancer Institute) and incubated at room temperature for 20 min and then on ice for an additional 40 min. Where indicated, 0.1 ml of rabbit anti-goat Ig antiserum was then added, and the tubes incubated at room temperature for an additional 30 min. Treated and untreated culture supernatants were then diluted tenfold and subjected to ultracentrifugation for 90 min (44,000 rpm, SW 50.1 rotor). The upper half of the supernatant was then removed, passed through a 0.22-μm Millipore filter, and assayed for LIA activity.

Determination of the Cell-Cycle Specificity of LIA

Bone marrow cells were adjusted to a density of 10⁷ nucleated cells/ml with RPMI 1630 plus 10% calf serum, and 0.5 ml of the suspension aliquoted into each of three tubes. To each tube was added either ³H-thymidine (20 Ci/mmol) to a final concentration of 50 μCi/ml, or an equal concentration of unlabeled thymidine or an equal amount of medium. The cells were incubated at 37°C for 1 hr, washed twice with fresh RPMI 1630 containing 10% calf serum and 100 μg/ml of unlabeled thymidine, and once with medium without added thymidine. The cells were then assayed for susceptibility to LIA.

RESULTS AND DISCUSSION

The essential features of the LIA phenomenon, as described by Broxmeyer et al.¹ and the analogous observations in the mouse system are given below.

1. Extracts or conditioned media from cultures of bone marrow cells from patients with acute leukemia inhibit colony formation by normal CFU-Cs. Similar products from bone marrow cells of normal individuals do not affect CFU-C colony formation.

Mice inoculated with the RFV strain of Friend virus develop an acute erythroleukemia characterized by massive splenomegaly, hepatomegaly, and viremia.⁴,⁵ Media conditioned by unfractionated bone marrow cells from all leukemic mice tested inhibited colony formation by normal CFU-Cs (Fig. 1). Media conditioned by bone marrow cells from normal mice did not inhibit CFU-C colony formation. As observed in the human system, the degree of inhibition varied with different LIA preparations, and generally ranged from 20% to 50%.

2. CFU-C colony formation by cells from leukemic patients is unaffected by LIA preparations.

As shown in Fig. 2, colony formation by bone marrow CFU-Cs from leukemic mice was not inhibited by an LIA preparation that significantly inhibited normal CFU-C colony formation.
3. Bone marrow cells from some leukemia patients in complete clinical remission continue to elaborate LIA, and CFU-C colony formation from most patients in remission remains resistant to LIA.

In about half of the RFV-induced leukemic mice, the disease spontaneously regresses. The spleen and liver return to normal size, architecture, and histology during regression, and in fully regressed mice, little to no infectious virus can be recovered from the tissues and plasma.

About 40% of the regressed mice tested elaborated LIA (Fig. 1). The remainder were the same as the normal uninoculated mice, i.e., no inhibition of CFU-C colony formation was observed with media conditioned by their bone marrow cells. In addition, as observed in the human system, bone marrow CFU-C colony formation by most of the regressed mice remained resistant to LIA (Fig. 2).

4. LIA is active only against CFU-Cs in S-phase.

Triitated-thymidine suicide was used to deplete the target normal mouse bone marrow population of cells in S-phase, and the remaining cells were tested for CFU-Cs and their sensitivity to LIA (Table 1). As reported for the human system, inhibition by LIA of colony formation was not observed in the depleted population, indicating that the LIA-sensitive cells were those in S-phase at the time of exposure to the factor.

5. LIA acts on CFU-Cs, in contrast to the polymorphonuclear leukocyte inhibitory activity, which inhibits CFU-C colony formation by inhibition of the production of colony-stimulating factor (CSF).

To test for LIA, media conditioned by test bone marrow cells are added to target CFU-Cs in agar cultures underlayered with macrophage monolayers. The macrophages provide the CSF for CFU-C colony formation. Inhibition of colony formation by LIA could, therefore, occur by suppression of CSF formation or by direct action on the CFU-C cells. To distinguish between these possibilities, LIA was tested in cultures in which preformed CSF from macrophage monolayers was added directly to the cultures. As observed in the human system, LIA exhibited the same inhibitory effect irrespective of whether CSF was added directly or produced by macrophage monolayers (data not shown).

In addition, LIA in the mouse system resembles that in humans by its very high titer in media conditioned by bone marrow cells from leukemic animals and the observation that inhibition of CFU-C colony formation by LIA is obtained at 3-6 days of incubation but not after 8 days (data not shown).

Since in the mouse system, leukemogenesis is virus-induced and infectious virus particles are probably released during conditioning of the medium by bone marrow cells from leukemic mice (LIA production), it was of interest to determine whether the presence of infectious virus is related to CFU-C colony inhibition. Removal of virus from the conditioned media (LIA) by ultracentrifugation, neutralization of virus with high-titered monospecific anti-gp70 antisera, or precipitation and removal of viral antigen by reaction with goat anti-gp70 serum and (secondary) rabbit anti-goat immunoglobulin and centrifugation did not diminish LIA activity.

We propose that RFV-induced erythroleukemogenesis and its spontaneous regression in the mouse serves as an appropriate model for the study of LIA and perhaps other factors and hematopoietic cell interactions observed in patients with leukemia. Broxmeyer et al. have recently observed that mice infected with Abelson virus express an LIA-like activity and that bone marrow CFU-Cs from infected animals were insensitive to inhibition by LIA. However, regression of Abelson virus-induced leukemias has not been reported, and thus, this potential model might not exhibit some of the essential features of the LIA system. In addition, leukemia recurs in about half of the mice with regressed RFV-induced erythroleukemia. It will, therefore, be possible to evaluate LIA production and sensitivity with respect to prognosis and disease recurrence in our model.

Clearly, continued study of LIA in humans is necessary. But the availability of the accurate, quantified, and manipulable mouse model system we have described here will allow many experiments to be carried out relevant to the origin, activity, and biologic significance of the LIA phenomenon, which could not readily be done in human subjects. In vivo administration of LIA and the influence on the LIA phenomenon of manipulations of the hematopoietic or immune systems might be of particular interest in this regard.

Table 1. Cell-Cycle Specificity of LIA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LIA</th>
<th>Colonies/10^5 Cells</th>
<th>Percent Inhibition of CFU-C Colony Formation by LIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>Medium</td>
<td>–</td>
<td>339 ± 10*</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>273 ± 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thymidine</td>
<td>–</td>
<td>397 ± 17</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>308 ± 17</td>
<td>23%</td>
</tr>
<tr>
<td></td>
<td>^3H-thymidine</td>
<td>–</td>
<td>169 ± 6</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>175 ± 5</td>
<td>(–3%)</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>Medium</td>
<td>–</td>
<td>161 ± 11</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>119 ± 8</td>
<td>26%</td>
</tr>
<tr>
<td></td>
<td>Thymidine</td>
<td>–</td>
<td>157 ± 4</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>106 ± 5</td>
<td>32%</td>
</tr>
<tr>
<td></td>
<td>^3H-thymidine</td>
<td>–</td>
<td>87 ± 1</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>85 ± 2</td>
<td>2%</td>
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*Mean ± SD.
REFERENCES


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