Studies on the Relationship between Leukemic Fibroblast Colony Forming Cell and Hemopoietic Cells

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Summary: 36 patients with leukemia, including AML, CML and ALL were studied. Fibroblast colony forming cells (CFU-F) in patients with different types of leukemia were less than in normal adults (P<0.05-0.001). There was no correlation observed between CFU-F and CFU-Mix/L-CFU in normal adults and leukemic patients (P>0.05). It was found that CFU-F is a single clone not originating from hemopoietic cells. After fetal muscular conditioned media were added, we observed that myelogenous leukemic cells showed the ability to adhere to the bottom of the dish, indicating the existence of quantitative and qualitative defects of CFU-F in leukemia. The mechanism and clinical significance of this phenomenon are discussed.

Key words: leukemia, CFU-F, CFU-Mix, L-CFU

In 1985, we have studied fibroblast colony forming cells (CFU-F) derived from normal adults and patients with acute nonlymphocytic leukemia. The study covered conditions of colony growth, changes in CFU-F and suspended cells within 4—5 weeks, and effects of fibroblast on granulocyte-macrophage progenitor cells (CFU-GM)[1]. The purpose of the present work is to investigate the relationship between CFU-F and hemopoietic stem cells, and to detect the changes in CFU-F of leukemic patients.

MATERIALS AND METHODS

36 patients (24 males, 12 females, aged 14—62 years) were studied, 18 with acute myelogenous leukemia (AML; 2 with M1, 6 with M2, 1 with M3, 7 with M5 and 2 with M4), 12 with chronic myelogenous leukemia (CML), and 4 with acute lymphoblastic leukemia (ALL; 3 with L1, 1 with L2). Based on the results of scanning electron microscopy, one undifferentiated leukemia and one lymphoid-myeloid leukemia were classified as ALL group. All of them were untreated.

12 normal adults (2 males and 10 females), aged 16—45, served as controls.

Method

1—2 ml of bone marrow were drawn by using heparin as anticoagulation agent. Interface mononuclear cells were collected after Ficoll-Hypaque gradient (density 1.077) centrifugation of bone marrow at 1500 g for 30 min, and then they were washed 3 times in the media.

Both CFU-Mix and CFU-F were cultured at the same time.

CFU-F assay

RPMI 1640 6.5 ml, calf serum 2 ml, fetal muscular conditioned solution 1 ml (method as described in our previous report[2]), and cells 0.5 ml (final concentration 1×10⁶/ml) were put into culture dish. The cells were cultured in humidified air with 5% CO₂ at 37°C with a
2 cm² slide dipped in. On the 14th day the culture media were discarded. The slide was stained and cytomorphologically observed for CFU-F. The bottom of the dish was dried in air and stained for calculating the colony numbers. Aggregates containing more than 100 cells were counted as colony (CFU-F). The result was scored as 1 × 10^7.

**CFU-Mix assay**

Mononuclear cells were suspended in media containing RPMI 1640, 15% calf serum, 15% horse serum, 2.5% PHA-thymocytes conditioned solution, 2.5% fetal muscular conditioned media, erythropoietin 0.2U/microwell, 2-mercaptoethanol 5 × 10⁻⁶ mol, 0.4% L-glutamine. The cells were planted in Linbro 96 microwells culture plate from 4 × 10⁴ to 2.5 × 10⁵ concentration, 0.2 ml/microwell. They were cultured at 37°C in 5% CO₂ and 100% humidity. The colonies were scored on the 14th day of culture according to probability formulae and calculated as 1 × 10⁶.

**RESULTS**

**Colonies and correlation coefficient (table 1)**

There was significant difference between CFU-F of patients and that of normal adults (P<0.05—0.001). However, no marked difference was found among AML, CML and ALL (P<0.05). There was no difference between AML and CML and between the subtypes of AML (P>0.05).

Correlation analysis was carried out for CFU-F with CFU-Mix/L-CFU, and no difference was found therefrom (P>0.05).

Light microscopy, scanning electron microscopy and transmission electron microscopy revealed that leukemic progenitor cells (L-CFU), after being cultured by CFU-Mix culture, accounted for 90% and CFU-Mix for 10% in leukemia. In normal adults, granulocyte (G) - erythrocyte (E) - megakaryocyte (Meg) - macrophage (Mφ) accounted for 12%, G Meg for 28%, G-lymphocyte for 10%, GE for 23% and GMφ for 27%.

**Cytomorphological examination**

In CFU-F of normal adults the fibroblasts were arranged in radiating direction. They were connected with each other forming nets (fig.1). Cellular plasma was abundant. Nuclei turned pink by staining with Giemsa-Wright. There were 3—4 nuclear nucleoli. Large amounts of granulocytes and macrophages were located in CFU-F, and occasionally CFU-GM could also be found.

The ability of myelogenous leukemia to form CFU-F was greater than that of lymphocytic leukemia, CFU-F of M₁ subtype were pure, and their cytomorphology was the same as normal (fig.2,3). There were many granulocytes and macrophages in CFU-F of M₂, M₃ and CML. It was noticed that myelogenous leukemic cells could adhere to the bottom of the dish. They had elongated pseudopodia like fibroblasts (fig.4). Fibroblasts were scatteredly distributed under oil microscope. Lymphoblasts did not adhere to the bottom.

**Table 1. CFU-F, L-CFU and their correlation in leukemia (X ±SE)**

<table>
<thead>
<tr>
<th>Group</th>
<th>CFU-F (X±SE)</th>
<th>CFU-Mix</th>
<th>L-CFU (X±SE)</th>
<th>P*</th>
<th>P**</th>
<th>r***</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>35.94±8.56</td>
<td>&lt;0.05</td>
<td>92.00±29.61</td>
<td>&gt;0.05</td>
<td>-0.15</td>
<td></td>
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<tr>
<td>CML</td>
<td>23.17±6.84</td>
<td>&lt;0.005</td>
<td>174.83±51.57</td>
<td>&lt;0.01</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>ALL</td>
<td>3.83±1.47</td>
<td>&lt;0.001</td>
<td>122.67±62.86</td>
<td>&lt;0.05</td>
<td>-0.04</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>58.00±7.49</td>
<td></td>
<td>34.92±11.10</td>
<td>&lt;0.01</td>
<td>-0.28</td>
<td></td>
</tr>
</tbody>
</table>

* Comparison between CFU-F of leukemia and that of normal.
** Comparison between L-CFU of various groups of leukemia and CFU-Mix of normals,
*** Correlation coefficients of CFU-F with L-CFU/CFU-Mix.