Discrete Clusters of Hematopoietic Cells in the Marrow Cavity of Man After Bone Marrow Transplantation

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Discrete aggregates of hematopoietic cells were observed in the bone marrows of patients with aplastic anemia or acute leukemia 14 days after marrow transplantation. The great majority of such colonies were of a single cell type, and less than 3% contained two or more cell types. Erythroid, myeloid, and undifferentiated hematopoietic colonies were approximately equal in frequency.

In 1961 Till and McCulloch described a technique for the detection of primitive hematopoietic stem cells by the formation of colonies in the spleens of lethally irradiated mice. These stem cells are present at low frequency in mouse bone marrow, spleen, liver, and blood, and have the capacity to form macroscopic discrete colonies of erythroid, granulocytic neutrophil, eosinophilic, megakaryocytic, or undifferentiated hematopoietic cells. In contrast, lymphoid cells grow diffusely in this environment. Microscopic erythroid colonies are detectable by 4 to 5 days within the red pulp of the spleen and appear as surface colonies as early as day 6. Granulocytic colonies are characteristically distributed along the trabeculae and in the subcapsular regions, and are frequently more diffuse than erythroid colonies. Undifferentiated colonies account for 0–16% of the total, depending on the histologic criteria used and the time allowed for colony development. Mixed colonies containing two or more types of hematologic cells constitute 0–47% of all colonies, again depending on histologic criteria and time of analysis.

Hematopoietic cell colonies also develop within the marrow cavities of irradiated mice injected with appropriate cell suspensions. These colonies are more often granulocytic in nature than the corresponding spleen colonies. Observations of hematopoietic bone marrow colonies developing in irradiated dogs have also been described.

The opportunities of observing hematopoietic colony formation in man are limited for obvious reasons. One occasion does arise when patients with aplastic anemia or patients with leukemia treated with lethal whole-body irradiation are transplanted with allogeneic bone marrow. This report describes hematopoietic colonies in man observed during the course of bone marrow transplantation.
MATERIALS AND METHODS

Four patients with severe aplastic anemia, and 10 patients with acute myelogenous leukemia (AML) were studied. All received marrow transplantation from HLA-identical, mixed-lymphocyte-culture-nonreactive siblings. The aplastic anemia patients were prepared for bone marrow transplantation with high doses of cyclophosphamide as previously described. The patients with AML received the UCLA-SCARI regimen, which included 1000 rads whole-body irradiation as previously described. Bone marrow biopsy specimens obtained prior to and at 7-day intervals after intravenous administration of allogeneic marrow were processed by standard techniques and stained with Maximow's stain.

Sections were examined for the presence of discrete aggregates of more than eight cells. Such aggregates will be referred to as colonies without implying that they are clonal in origin. These were cataloged on the basis of morphology into erythroid, eosinophilic, megakaryocytic, myeloid (neutrophils or monocytes), or undifferentiated colonies. In occasional patients clusters of more than eight plasma cells were also identified. Neutrophilic granulocytes and monocytes were classified together as "myeloid" colonies because of the difficulty in distinguishing between monocytes and myelocytes in histologic sections. At least 100 cell aggregates were counted in each patient.

RESULTS

In the four aplastic patients, no discrete hematopoietic colonies were observed in marrow biopsy specimens prior to transplantation. By the seventh day, rare clusters of 3-6 nucleated cells were found widely scattered in the marrow space. By day 14 individual discrete colonies were identified against the background of stroma and empty fat spaces in all patients. These clusters usually consisted of groups of 8 to more than 200 cells. By day 21 these colonies were usually confluent. Consequently, marrow specimens obtained 14 days after transplantation were used for subsequent analyses.

Examples of erythroid, myeloid, undifferentiated, and mixed megakaryocytic-erythroid colonies are shown in Fig. 1A-1D, respectively. As illustrated in Fig. 1C, mitotic figures were frequent in these colonies. As shown in Table 1, approximately equal proportions of pure erythroid, myeloid, and undifferentiated colonies were observed. The great majority of colonies were of a single cell type, and less than 3% of colonies were mixed. Mixed colonies usually consisted of two cell types, and less than 0.25% of all colonies contained three cell types. Erythroid and undifferentiated colonies were usually compact, whereas myeloid and eosinophil colonies were generally diffuse and spread along a stromal network. Similar colony morphology has been observed in the mouse spleen. Eosinophil colonies were surprisingly common (4.9%-22.1%) and were observed in patients without evidence of graft-versus-host disease. Plasma cell colonies were rare (less than 0.25% of all the colonies).

Table 1. Differential Analysis of Marrow Hematopoietic Colonies in Aplastic Anemia Patients 14 Days After Bone Marrow Transplantation

<table>
<thead>
<tr>
<th>Patients</th>
<th>Erythroid</th>
<th>Myeloid</th>
<th>Undifferentiated</th>
<th>Eosinophil</th>
<th>Megakaryocyte</th>
<th>Mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26.6</td>
<td>27.5</td>
<td>22.0</td>
<td>17.4</td>
<td>4.6</td>
<td>1.8</td>
</tr>
<tr>
<td>2</td>
<td>37.3</td>
<td>38.2</td>
<td>14.7</td>
<td>4.9</td>
<td>2.9</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>13.7</td>
<td>34.7</td>
<td>24.2</td>
<td>22.1</td>
<td>2.1</td>
<td>3.3</td>
</tr>
<tr>
<td>4</td>
<td>29.6</td>
<td>23.5</td>
<td>33.7</td>
<td>12.2</td>
<td>ND*</td>
<td>1.0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>26.8 ± 9.8</td>
<td>31.0 ± 6.7</td>
<td>23.7 ± 7.8</td>
<td>14.2 ± 7.4</td>
<td>2.4 ± 1.9</td>
<td>1.8 ± 1.1</td>
</tr>
</tbody>
</table>

*ND: not done.
Fig. 1. (A) Erythroid colony in the bone marrow of a patient with aplastic anemia 14 days after bone marrow transplantation. (B) Myeloid colony in the bone marrow of a patient with aplastic anemia 14 days after bone marrow transplantation. (C) Colony of undifferentiated hematopoietic cells in the bone marrow of a patient with aplastic anemia 14 days after marrow transplantation. (D) Mixed megakaryocytic-erythroid colony in the marrow of a patient with aplastic anemia 14 days after marrow transplantation.
Bone marrow specimens from transplanted leukemic patients were more difficult to interpret because mature red cells and/or fibrous tissue often filled the medullary cavity between days 7 and 21. Specimens of only 4 of the 10 patients were considered suitable for analysis. In these, the percentages of different colony forms were as follows: erythroid 28% ± 15%, myeloid 24% ± 12%, eosinophil 6% ± 4%, undifferentiated hematopoietic cells 34% ± 20%, megakaryocytes 3.3% ± 2.6%, and mixed colonies 1.7% ± 0.8%. This pattern was similar to that observed in the aplastic anemia patients.

DISCUSSION

Discrete clustering of hematopoietic cells was routinely observed in marrow specimens of aplastic anemia patients and frequently seen in leukemic patients 14 days after bone marrow transplantation. For convenience, these clusters have been referred to as cell colonies. The homogeneous cell population and frequent mitotic figures suggested that these colonies arose from the local proliferation of precursor cells rather than the aggregation of circulating cells. Furthermore, at the time of colony formation, isolated single nucleated hematopoietic cells were exceedingly rare in the marrow cavity and generally nonexistent in the blood. It was impossible, however, with current technology to prove or disprove the clonal origin of such cell colonies.

The great majority of human hematopoietic colonies at 14 days were of a single cell type rather than mixed. In the irradiated mouse spleen, most colonies appear to arise from pluripotent stem cells. At 6-9 days post-marrow infusion, 6%-16% of mouse colonies are of mixed morphology, and by days 10-12 between 23% and 47% are mixed. The observed differences between mouse and man are two: a smaller percentage of mixed colonies and a greater percentage of eosinophil colonies in man. These differences might relate to the existence of disease in the human subjects or to the time course of expression of committed versus pluripotent stem cells in different species.

REFERENCES

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