CONCISE REPORT

Host Origin of the Human Hematopoietic Microenvironment Following Allogeneic Bone Marrow Transplantation


The origin of marrow stromal cells post allogeneic bone marrow transplantation (BMT) was studied. Two groups of patients receiving HLA-identical marrow grafts from sex mismatched siblings were included in the study: the first group (eight patients) received conventional marrow grafts and the second group (ten patients) received stromal cell and T cell depleted grafts. All patients showed hematopoietic engraftment with donor cells. Marrow aspirates obtained from these patients were used to establish stromal layers in long-term marrow cultures (LTMC) for 4 to 6 weeks. In both groups, karyotype analysis of nonhemato-

poietic cultured stromal cells showed host origin even as late as day 760 posttransplantation. Immunofluorescence methods using monoclonal antibodies against components of fibroblasts, macrophages, and endothelial cells, showed that the composition of stromal layers was similar to those obtained from normal controls. Our data indicate that marrow stromal progenitors capable of proliferation are nontransplantable and do not originate from a hematopoietic-stromal common progenitor.

From the Memorial Sloan-Kettering Cancer Center Laboratories of Developmental Hematopoiesis, Cancer Genetics & Cytogenetics, and Bone Marrow Transplantation Service, New York.

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Address reprint request to Joseph Laver, MD, Bone Marrow Transplantation Service, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

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F Materials and Methods

Patients. Eighteen patients who received allogeneic marrow grafts from sex mismatched HLA identical siblings were studied. After informed consent was obtained, patients were prepared for transplantation with hyperfractionated total body irradiation and cyclophosphamide (except one patient with severe aplastic anemia who received total lymphoid irradiation and cyclophosphamide). Eight patients received conventional grafts and ten received marrow depleted of stromal progenitors and T cells. The separation method, involving soybean agglutination and E-rosetting (SBA), was originally used to eliminate T cells and was subsequently found to deplete stromal progenitors as well.

Long-term marrow cultures. Bone marrow aspirates post-BMT were obtained at 14 to 760 days in the conventional group and 14 to 563 days in the SBA separated group. Long-term cultures of human marrow (LTMC) were established according to the method of Gardner and Kaplan with some modifications. Briefly, 20 x 10^6 marrow buffy coat cells in 10 mL of LTMC medium were inoculated into T-25 tissue culture flasks. Cultures were then gassed with 5% CO2 in air and incubated at 33°C. The LTMC medium consisted of supplemented McCoy’s 5A medium containing 12.5% horse serum, 12.5% FCS and 10^-4 mol/L hydrocortisone sodium succinate (Calbiochem-Behring, La Jolla, CA). At weekly intervals, half the supernatant medium and suspension cells were removed and replaced with fresh LTMC medium. The development of marrow stromal layers was assessed every seven days under a phase contrast inverted microscope and the percentage of the flask surface covered by the stromal interlocking network was visually estimated and recorded.

To evaluate the composition of the adherent stromal cells in culture we used immunofluorescence methods using antibodies to components of fibroblasts (collagen type III and fibronectin), endothelial cells (factor VIII related antigen), and macrophages (monocyte surface molecules recognized by 63D3 and 61D3 mouse monoclonal antibodies), as previously described.

Cytogenetic studies. To assess the origin of hematopoietic and stromal cells, chromosomal analysis of fresh bone marrow cells and trypsinized LTMC stromal cells was performed using quinacrine dihydrochloride staining (Q-banding). The binomial distribution was used to calculate the probability to identify donor stromal cells derived from LTMC.
Table 1. Karyotype Analysis of Marrow Hematopoietic and Stromal Cells Following Unseparated Grafts

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age (yr)</th>
<th>Diagnosis</th>
<th>Sex</th>
<th>Host</th>
<th>Donor</th>
<th>Day Post-BMT</th>
<th>Hematopoietic</th>
<th>Stromal</th>
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<td>1</td>
<td>7</td>
<td>ALL</td>
<td>M</td>
<td>F</td>
<td></td>
<td>33</td>
<td>46,XX(30/30)</td>
<td>46,XY(30/30)</td>
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<tr>
<td>2</td>
<td>7</td>
<td>AML</td>
<td>M</td>
<td>F</td>
<td></td>
<td>760</td>
<td>46,XX(30/30)</td>
<td>46,XY(34/34)</td>
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<tr>
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<td>8</td>
<td>AML</td>
<td>F</td>
<td>M</td>
<td></td>
<td>150</td>
<td>46,XX(18/18)</td>
<td>46,XY(30/30)</td>
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<td>F</td>
<td></td>
<td>30</td>
<td>46,XY(30/30)</td>
<td>46,XX(16/16)</td>
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<tr>
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<td>8</td>
<td>AA</td>
<td>M</td>
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<td>60</td>
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<td>M</td>
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<td></td>
<td>640</td>
<td>46,XX(30/30)</td>
<td>46,XY(32/32)</td>
</tr>
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</table>

Abbreviations: AML, acute myelocytic leukemia; AA, aplastic anemia.

In parentheses appears the number of cells with given karyotype as fraction of total number of cells studied.

RESULTS

The eighteen patients included in this study showed engraftment with donor hematopoietic cells. Hematopoietic cells remained of donor origin throughout the study period. In contrast, chromosomal analysis of cultured stromal cells in 4 to 6-week-old LTMC showed host origin in recipients of either unmodified (group 1) or stromal cell depleted marrow grafts (group 2) (Tables 1 and 2). In both groups the composition of stromal layers in LTMC derived from marrows obtained over day 35 of the BMT was similar to that seen in normal controls and included fibroblasts, lipid-containing cells, macrophages, and 5% to 10% endothelial cells (Table 3). In group 1, 311 stromal metaphases were studied. All, including 158 over day 100 post-BMT, showed host origin. In group 2, 367 metaphases including 273 over day 100 post-BMT were studied showing host stromal origin. It is unlikely that the presence of donor stroma was missed as the probability to do so is extremely low. For instance, if the frequency of donor stromal cells was 5%, the probability that none would be identified in a random sample of over 300 metaphases is <0.1%. Furthermore, in our laboratories we have been able to identify as low as 3% mosaicism in marrow specimens obtained after marrow transplantation.

DISCUSSION

The data presented show the host origin of cultured marrow stromal cells obtained from patients successfully transplanted with allogeneic grafts. These findings suggest that chemoradiotherapy used for myeloablation does not fully ablate marrow stromal progenitors. Achievement of successful hematopoietic engraftment in these patients indicates that the host microenvironment plays an important role in the posttransplant period: In the early stages it provides favorable conditions for hematopoietic engraftment, and later it reconstitutes a functional microenvironment capable of sustaining hematopoiesis. Since the reconstitution of the marrow microenvironment requires proliferation of stromal...
progenitors it is important to determine the origin of stromal cells capable of undergoing mitosis.

Our results showing host origin of proliferating marrow microenvironmental cells following BMT suggest that stromal progenitors are nontransplantable. The apparent discrepancy between our results and those reported by Keating et al² can be explained by the differences in the techniques used to assess cultured stromal cells and the type of cells analyzed by these methods. We used chromosomal analysis, which determines the origin of stromal cells capable of undergoing mitosis, whereas Keating by determining the presence of the Y-body chromosome analyzed nondividing cells. Identifying donor derived macrophages that are abundantly present in stromal layers with the Y-body method could lead to the incorrect conclusion that stromal cells post BMT are of donor origin. In contrast, chromosomal analysis assesses only cells undergoing mitosis and excludes differentiated nondividing cells such as macrophages. Using karyotype analysis, other investigators have also shown that certain stromal elements, such as marrow fibroblasts and reticulofibroblastoid cells, are of host origin following allogeneic unmodified marrow grafts.³ Analogous discrepancy exists regarding the origin of stromal cells in patients with clonal hematopoietic disorders such as chronic myelocytic leukemia. This, once again, can be explained by the different cell populations used for study (dividing vs nondividing cells). Chromosomal analysis has demonstrated that stromal cells do not originate in the malignant clone⁴ whereas G6PD studies⁵ of nondividing cells in patients heterogeneous for types A and B of the enzyme, have suggested that stromal cells arise from the malignant hematopoietic clone.

In addition to the nontransplantability of marrow stromal cells, our data are against the notion of a common hematopoietic-stromal progenitor. Marrow transplantation with SBA separated grafts provides a unique model to answer whether stromal and hematopoietic cells share a common progenitor. Following BMT, a microenvironment of donor origin could develop through two possible mechanisms: (1) engraftment with a common stromal-hematopoietic progenitor giving rise to both marrow compartments, or (2) engraftment with a transplantable stromal progenitor that gives rise only to stromal cells and not to hematopoietic cells. Conventional marrow grafts containing stromal and hematopoietic progenitors cannot discriminate between these two mechanisms, whereas marrow grafts depleted of stromal progenitors by eliminating the second possibility can be used to test the first mechanism. Our results with stromal depleted marrow grafts showing engraftment with donor hematopoietic cells without the emergence of donor stromal cells, rule out the hypothesis that stromal and hematopoietic cells share a common progenitor.

REFERENCES

Host origin of the human hematopoietic microenvironment following allogeneic bone marrow transplantation

J Laver, SC Jhanwar, RJ O'Reilly and H Castro-Malaspina