Pluripotent Hemopoietic Stem Cells in Murine Postmortem Bone Marrow

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ABSTRACT

Although 12-hours postmortem murine bone marrow cells exhibit extensive degeneration, these cells when infused into irradiated mice produce erythrocytic, granulocytic, megakaryocytic and mixed colonies in their spleen. These observations clearly demonstrate the presence of pluripotent hemopoietic stem cells and their proliferative capability in 12-hours postmortem, murine bone marrow. These observations suggest that cadaveric bone marrow transplantation may be possible in patients with hematologic disorders.

Introduction

In our previous report, evidence was presented that proliferative function persists in human and murine cadaveric bone marrow. Utilizing methylcellulose clonal cell culture assays, erythropoietic burst and granulopoietic colony formation from bone marrow cells, collected as late as 19-hours postmortem was observed. Postmortem persistence of hemopoietic stem cells in mice as assayed by spleen colony technique paralleled that of the committed precursors assayed in clonal cultures. In this communication, ultrastructural, histological and cytological evidence are presented for the pluripotent nature of the murine cadaveric spleen colony forming cells.

Materials and Methods

PREPARATION OF DONOR BONE MARROW CELLS

The animals used were male BDF mice weighing 25 to 30 g.* Mice were killed by cervical dislocation and kept at room temperature. Samples of fresh and 12-hour postmortem bone marrow cells suspensions were collected from nine mice by flushing the femur with 1 ml of α-medium by means of a #23 gauge needle attached to a syringe. Single cell suspensions were separated into samples containing $2 \times 10^5$ cells per ml of
α-medium for 0-hr postmortem group and $8 \times 10^5$ cells per ml of α-medium for 12-hours postmortem group, respectively.

**In Vivo Assay for Murine Hemopoietic Precursors**

Murine hemopoietic stem cells were assayed using the in vivo spleen colony technique developed by Till and McCulloch. On day 1, 40 mice were irradiated with 900 rads (250 KVP GE maxitron units at 30 mA and a quarter-copper filtration).† On day 2, the irradiated mice were infused via their tail veins with either 12-hours postmortem murine marrow cells (24 mice) or fresh murine marrow cells (11 mice). The remaining five irradiated mice were injected with α-medium only for the control purpose. On day 10, the mice were sacrificed. Their spleens were removed and dissected. Numerous imprints of the spleens were stained with Wright's stain. Spleens were then fixed in Bouin's solution and serial sections of the entire spleen were stained with hematoxylin and eosin. Using the imprints and H&E stained serial histologic sections of spleens, the number of spleen colonies were counted and classified as erythrocytic, granulocytic, megakaryocytic and mixed according to their principal hemopoietic cell lines. For ultrastructural study, individual colonies were removed from the spleens under a dissecting microscope. These tissues were prepared for transmission electron microscopy by fixation in 3 percent glutaraldehyde and processed routinely.

**Results**

In figure 1 is illustrated the low magnification light microscopic picture of hemopoietic colonies in a mouse spleen who received whole body irradiation followed by an infusion of 12-hours postmortem bone marrow cells. The spleen of the non-irradiated mice, which were infused with α-medium alone, shows no spleen colonies (figure 2). Irradiated mice who do not receive marrow transfusions died within five days to a month, and autopsy of these mice revealed no identifiable hemopoietic colonies in their spleens. Conversely, most of the irradiated mice who received either fresh or 12-hours postmortem marrow transfusion have survived more than six months. Compared to fresh marrow cells, the 12-hours postmortem marrow cells were approximately 25 percent as efficient in pro-

† Courtesy of the Division of Radiotherapy, Medical University of South Carolina.

**Figure 1.** A section of spleen from an irradiated mouse infused with 12-hours postmortem bone marrow cells showing microscopic hemopoietic colonies (H&E stain × 9).

**Figure 2.** A section of spleen from a non-irradiated mouse injected with α-medium only showing absence of colonies (H&E stain × 9).
FIGURE 3. An imprint of the mixed colony showing a megakaryocyte (m) and numerous granulocytic cells (Wright's stain × 700).

FIGURE 4. An imprint of the granulocytic colony showing numerous immature granulocytic cells (Wright's stain × 700).

FIGURE 5. An imprint of the erythrocytic colonies showing nucleated erythrocytic cells (Wright's stain × 700).

These spleen colonies consist of differentiated hematopoietic cell lines such as erythrocytic, granulocytic and megakaryocytic or mixed cell lines. Imprints of the spleen colonies demonstrate mixed (granulocytic and erythrocytic or granulocytic and megakaryocytic), granulocytic and erythrocytic colonies as shown in figures 3, 4 and 5. The erythrocytic cells in the erythrocytic colonies may be misinterpreted as lymphocytes in routine hematoxylin and eosin sections.

<table>
<thead>
<tr>
<th>Colonies (Percent)</th>
<th>Fresh Marrow Cells</th>
<th>12-Hour Postmortem Marrow Cells</th>
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</thead>
<tbody>
<tr>
<td>Erythrocytic</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>Granulocytic</td>
<td>26</td>
<td>12</td>
</tr>
<tr>
<td>Mixed</td>
<td>33</td>
<td>39</td>
</tr>
<tr>
<td>Megakaryocytic</td>
<td>1</td>
<td>2</td>
</tr>
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Figure 6. An erythrocytic colony of spleen derived from infusion of 12-hour postmortem murine bone marrow cells showing erythrocytic cells with characteristic chromatin pattern (H&E stain × 1,200).

Figure 7. Electron micrograph of early and late erythroblasts in a spleen colony (× 3,000).
However, ultrastructural examination of erythrocytic colonies and higher magnification of Wright's stain on imprint of erythrocytic colonies demonstrated the presence of characteristic erythrocytic cells in various stages of development. These cells are demonstrated in figures 6 and 7. The percentage of various types of colonies in the postmortem and fresh marrow cells are presented in table I. In both samples, about 33 percent of the colonies could be classified as mixed colonies. These data agree with the previous work reported by Curry and Trentin and indicate that postmortem bone marrow cells possess pluripotent hemopoietic stem cells.

Discussion

Recently, allogenic bone marrow transplantation has emerged as a viable therapeutic approach in various hematologic disorders. One major limitation is that the successful transplantation is limited mostly to transplantation between histocompatible siblings. In order to examine the possibility that cadaveric human bone marrow cells could be utilized for bone marrow transplantation between unrelated individuals, the proliferative function of human and murine cadaveric bone marrow cells was examined by us. Significant postmortem survival of hemopoietic functions were observed. However, while the postmortem survival of marrow function has been demonstrated by us, we have not proven the pluripotent nature of the cadaveric spleen colony forming cells. Ultrastructural and cytological analysis of the spleen colonies described in this paper confirms the postmortem persistence of pluripotent hemopoietic stem cells. Establishment of bone marrow banks to store cadaveric human bone marrow cells may broaden the indications for bone marrow transplantation and provide methods to procure larger amounts of histocompatible bone marrow cells in the future.

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References