BRIEF NOTE

Marrow Repopulating Ability of Peripheral Blood Cells Compared to Thoracic Duct Cells

By R. STORR, R. B. EPSTEIN AND E. D. THOMAS*

STUDIES IN MICE,1 guinea pigs,2 and dogs3 have shown hemopoietic stem cells to be present in the circulating blood. Infusion of 10 to 20 × 10⁹ autologous blood leukocytes sufficed to repopulate bone marrow in lethally irradiated dogs.3 The morphologic characterization of the stem cell is difficult.4 In mice5 and dogs6 evidence has been obtained that hemopoietic stem cells may be morphologically similar to small lymphocytes. It has been suggested that small lymphocytes enter the peripheral blood via lymphatic vessels and then seed the bone marrow where they differentiate into hemopoietic precursor cells.7 Attempts in mice and rats to test this hypothesis have given contradictory results. Delorme8 observed that 9 out of 21 lethally irradiated rats showed prompt restoration of bone marrow function after infusion of syngeneic thoracic duct cells. Gesner and Gowans,9 however, reported that syngeneic thoracic duct cells did not prolong the life of lethally irradiated mice nor did they repopulate the bone marrow with hemopoietic precursor cells. Furthermore, no evidence was obtained to suggest that lymph nodes in mice contain cells capable of restoring bone marrow function.10,11 The present study was undertaken to test for the presence of hemopoietic stem cells in the thoracic duct lymph of the dog. The ability of autologous thoracic duct lymphocytes to repopulate the bone marrow in lethally irradiated dogs was compared with that of autologous peripheral blood leukocytes.

MATERIALS AND METHODS

Ten mongrel or beagle dogs weighing 7 to 15 Kg. were used. All were dewormed and immunized against hepatitis and distemper. The dogs were divided into two groups. Group I comprised 5 dogs that received 1,200 r of whole body irradiation followed by...

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infusions of stored autologous thoracic duct lymphocytes. Group II consisted of 5 dogs given 1,200 r. and infusions of stored autologous peripheral blood leukocytes.

The thoracic duct was cannulated indirectly by inserting a teflon-silastic cannula into the left external jugular vein after tying the vessel distally. Subsequently the subclavian, innominate, and other branching veins were tied off leaving the thoracic duct as the only vessel entering the cannulated part of the external jugular vein. Lymph was collected from the unanesthetized dogs during 48 hours following cannulation. The lymph was collected in iced, sterile 150 ml. Fenwal plastic bags containing 2 mg. of heparin (Connaught Laboratories, Toronto). The lymph was collected at 6 hour intervals and then centrifuged for 10 minutes at 140 x g. Five to 10 ml. of cellular sediment was obtained and resuspended in an equal volume of a mixture of 7 parts TC 199 (Difco), 2 parts of dimethyl-sulfoxide and 1 part of autologous serum.3 The plastic bags containing the cell suspension were placed between copper plates, frozen to −25 C. at a rate of 1 C. per minute,12 and then stored at −80 C. The cannula was removed at the termination of the lymph collection.

Buffy coat cells from peripheral blood were harvested on 2 consecutive days as previously described.13 After 2 to 6 hours storage on ice the cell suspension was recentrifuged for 20 minutes at 240 x g. to reduce the total fluid volume. The buffy coat was then resuspended in the dimethyl-sulfoxide mixture and frozen to −80 C. as described above.

Two to three weeks after cell collection the dogs were given 1,200 r. of whole body irradiation from dual 60Co sources at a dose rate of 9.2 r. per minute and with a source-target distance of 180 cm. The radiation dose was monitored by a Victoreen “r” meter and lithium fluoride radioluminescence dosimetry. Within 2 hours of irradiation each dog received its own stored cells by intravenous infusion. These cells were rapidly thawed in a water bath at 37 C. and injected within 10 minutes. Cell clumping may occur when the interval between thawing and injection exceeds 10 minutes.3 No attempt was made to remove dimethyl-sulfoxide prior to infusion since experience with dogs has shown it to be nontoxic.14 Differentiation of the injected cells was done by phase-contrast microscopy.

The irradiated dogs received supportive therapy with fluid and electrolytes during periods of vomiting and diarrhea. Ampicillin, 250 mg. twice daily, was administered from the third day after irradiation. White cell counts, platelet counts and hematocrits were done at least three times weekly. Complete autopsies with histology were performed on all dogs that died.

**RESULTS**

Table 1 summarizes the results. The 5 dogs in Group I received a total of 6.9 to 22.1 x 10⁹ thoracic duct cells. Most infused cells were lymphocytes, 78 to 92 per cent of them being small lymphocytes and the remainder being medium sized and large lymphocytes. All five dogs died 6 to 11 days after irradiation from hemorrhage or infection. Their survival and course was indistinguishable from that of dogs given 1,200 r. and supportive therapy.15 The white cell counts fell below 1000/mm³ by the fifth day and then continued to decline (Fig. 1). None of the dogs showed a rise in white cell count prior to death. The platelet counts remained above 100,000/mm³ until the eighth day and then declined rapidly to low values. The bone marrow at autopsy showed absence of hemopoietic precursor cells. The cells present were stroma cells, reticulum cells, lymphocytes and plasma cells. The lymph nodes and lymphatic follicles in the spleen in all 5 dogs were considerably more cellular than is usually observed in radiation controls.15 There were signs of
Table 1.—Summary of data on dogs given 1,200 r of whole body irradiation. Group I received infusions of autologous thoracic duct cells. Group II received infusions of autologous peripheral blood leukocytes.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dog No.</th>
<th>Number of infused cells x 10⁶</th>
<th>Survival (days)</th>
<th>Rise in WBC</th>
<th>Bone Marrow Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>6.9 (L) 6.4 (G) 0.5 (N)</td>
<td>9</td>
<td>No</td>
<td>No hemopoietic precursor cells present</td>
</tr>
<tr>
<td>I</td>
<td>2</td>
<td>20.9 (L) 20.9 (G) -- (N)</td>
<td>6</td>
<td>No</td>
<td>No hemopoietic precursor cells present</td>
</tr>
<tr>
<td>I</td>
<td>3</td>
<td>22.1 (L) 22.1 (G) -- (N)</td>
<td>11</td>
<td>No</td>
<td>No hemopoietic precursor cells present</td>
</tr>
<tr>
<td>I</td>
<td>4</td>
<td>15.1 (L) 15.1 (G) -- (N)</td>
<td>8</td>
<td>No</td>
<td>No hemopoietic precursor cells present</td>
</tr>
<tr>
<td>I</td>
<td>5</td>
<td>16.2 (L) 16.1 (G) 0.1 (N)</td>
<td>10</td>
<td>No</td>
<td>No hemopoietic precursor cells present</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>74.5 (L) 19.4 (G) 50.3 (N)</td>
<td>11</td>
<td>Yes</td>
<td>Minimal cellularity, erythroid and myeloid precursor cells and occasional megakaryocytes present</td>
</tr>
<tr>
<td>II</td>
<td>7</td>
<td>21.4 (L) 4.6 (G) 16.4 (N)</td>
<td>12</td>
<td>Yes</td>
<td>Minimal cellularity, erythroid and myeloid precursor cells and occasional megakaryocytes present</td>
</tr>
<tr>
<td>II</td>
<td>8</td>
<td>41.9 (L) 8.9 (G) 31.3 (N)</td>
<td>16</td>
<td>Yes</td>
<td>Moderate cellularity, erythroid and myeloid precursor cells and occasional megakaryocytes present</td>
</tr>
<tr>
<td>II</td>
<td>9</td>
<td>22.7 (L) 4.0 (G) 17.5 (N)</td>
<td>&gt;100</td>
<td>Yes</td>
<td>Normal</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>27.2 (L) 5.5 (G) 20.3 (N)</td>
<td>&gt;100</td>
<td>Yes</td>
<td>Normal</td>
</tr>
</tbody>
</table>
MARROW REPOPULATING ABILITY

Fig. 1.—White blood cell counts of dogs given 1,200 r. of whole body irradiation followed by thoracic duct cells or by buffy coat cells.

active regeneration with large immature cells, plasma cells, active germinal centers and numerous mitotic figures.

The 5 dogs in Group II received 21.4 to 74.5 × 10⁶ blood leukocytes (Table 1). 4.0 to 19.4 × 10⁶ of the infused cells were lymphocytes, 0.4 to 4.9 × 10⁶ monocytes and 16.4 to 50.3 × 10⁶ granulocytes. Three of the 5 dogs died from infection, 11, 12 and 16 days after irradiation. The white cell counts in these dogs declined to below 1000 per mm.³ and then started to rise indicating beginning hemopoietic recovery (Fig. 1). The bone marrow at autopsy showed myeloid and erythroid cells at all stages of maturation. The 2 remaining dogs in this group are alive beyond 100 days after irradiation. Their white cell and platelet counts slowly returned to normal.

DISCUSSION

Infusion of about 7 to 22 × 10⁶ autologous thoracic duct cells did not produce clinical or histologic signs of bone marrow repopulation in lethally irradiated dogs. This is in agreement with the findings of Gesner and Gowans in mice and suggests that hemopoietic stem cells are not present in the thoracic duct lymph of the dog in an appreciable number. Repopulation of the bone marrow was observed after infusion of about 21 to 74 × 10⁶ autologous peripheral blood leukocytes. Cavins et al. reported that even infusion of 10 × 10⁶ autologous blood leukocytes may suffice to give rise to hemopoiesis after lethal irradiation. These numbers become even more meaningful when it is considered that the granulocytes are nondividing cells
that do not participate in the repopulation of the hemopoietic tissues. Thus, the hemopoietic stem cells in the peripheral blood of the dog must be present among the $4.0 \times 10^9$ lymphocytes or $0.4 \times 10^9$ monocytes. It must be assumed that they enter the circulating blood by routes other than the thoracic duct.

**Summary**

Ten dogs were exposed to 1200 r. of whole body irradiation at a dose rate of 9.2 r./min. Five of these dogs were then given infusions of $21 \times 10^9$ to $74 \times 10^9$ autologous peripheral blood cells which had been previously stored at $-80\,^\circ\mathrm{C}$. $4.0 \times 19.4 \times 10^9$ of these cells were lymphocytes, $0.4 \times 4.9 \times 10^9$ were monocytes and $16.4 \times 50.3 \times 10^9$ were granulocytes. All five dogs showed clinical or histologic evidence of bone marrow repopulation. The remaining five dogs were given $7 \times 22 \times 10^9$ autologous thoracic duct lymphocytes. In none of these dogs was marrow repopulation observed.

It was concluded that hemopoietic stem cells are not present in the thoracic duct lymph of the dog in any appreciable number.

**SUMMARIO IN INTERLINGUA**

Dece canes esseva exponite a 1200 r de irradiation del corpore integre a un dosage de $9.2\ r$ per minuta. Subsequentemente 5 de iste canes recipeva infusiones de inter $21 \times 74 \times 10^9$ autologe cellulas de sanguine peripheric le quales habeva previemente essite preservate a $-80\,^\circ\mathrm{C}$. Inter $4.0 \times 19.4 \times 10^9$ de iste cellulas esseva lymphocytos, inter $0.4 \times 4.9 \times 10^9$ monocytos, e inter $16.4 \times 50.3 \times 10^9$ granulocytos. Omne iste canes manifestava evidentia clinic o histologic de repopulation del medulla ossee. Le remanente cinque canes recipeva inter $7 \times 22 \times 10^9$ autologe lymphocytos de ducto thoracic. Repopulation del medulla non esseva observate in ulle de iste canes.

Es concludite que hematopoietic cellulas primordial non es presente in le lympha del ducto thoracic de canes, o al minus non in numeros substantial.

**REFERENCES**


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