Autologous Bone Marrow Transplantation Studies in Dogs Irradiated by $^{90}$-DTPA Urine-Recycling Technic

By H. S. Winchell, Myron Pollycove, W. D. Loughman and J. H. Lawrence

Relatively selective beta irradiation of the lymphatic system in the dog has been achieved with internally administered Yttrium$^{90}$ chelated with diethylene triamine penta-acetic acid ($^{90}$-DTPA). Intravenous injection of $^{90}$-DTPA followed by continuous intravenous infusion of the animal's own urine containing the excreted $^{90}$-DTPA maintains a constant quantity of radioactivity in the body for a specified period.

Lymphopenia, without concomitant depression of other circulating formed elements such as granulocytes, reticulocytes or platelets, was produced in dogs given sublethal irradiation with $^{90}$-DTPA. Dogs lethally irradiated in the same way showed virtual absence of circulating lymphocytes and a less marked depression of circulating granulocytes, reticulocytes, and platelets. Further studies of bone marrow antografts, in dogs lethally irradiated with $^{90}$-DTPA, were made to determine whether or not the animal's death was due primarily to bone marrow destruction.

Materials and Methods

The irradiation procedure employed was the same as previously described. All animals were purebred male beagles aged between 6 months and 2 years. They were given daily injections of penicillin and streptomycin (600,000 units penicillin; 0.75 Gm. streptomycin) during the 4 weeks following irradiation. Tissues were fixed in Bouin's solution, embedded in paraffin or celloidin, and stained with hematoxylin and eosin, Pollak's trichrome, and methylene blue. Duplicated counts of platelets were made by phase-contrast microscopy, and those of WBC and PCV by routine technics. Blood creatinine values were determined by method of Folin and Wu.

Just prior to irradiation of each dog, bone marrow was obtained by multiple aspirations using Bierman needles. Siliconized syringes containing small amounts of preservative-free heparin (from Connaught Laboratories, Toronto, Canada) were used. The bone marrow was placed in heparinized Hank's solution in a 300 ml. Fenwall plastic transfer pack (100-110 ml. Hank's solution plus 50 mg. heparin) and stored at 4-6 °C. Counts of nucleated cells were made from the final bone-marrow mixture without attempting to homogenate bone marrow spicules, and the counts were corrected for maximal inclusion of circulating white blood cells. Particulate matter was not removed other than by passing the bone marrow through a commercial blood-administration filter at the time of marrow administration. In some transplantation experiments, autologous bone marrow was infused intravenously into non-anesthetized dogs; in others it was injected, 18-24 hours after the termination of urine recycling, through a cannula in the femoral artery with the animals under sodium pentobarbital anesthesia. Blood was drawn 2 or 3 times a week for hematologic studies.

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Submitted May 3, 1963; accepted for publication Sept. 7, 1963.

Blood, Vol. 23, No. 1 (January), 1964
Experimental Results

1. The clinical course and pathologic results in dogs irradiated with Y\(^{90}\)-DTPA and given autologous bone marrow, and which survived for more than 1 month, are summarized in table 1a. In figure 1 are given body weight and hematologic changes in these animals.

The initial clinical course of those animals given lethal radiation plus autologous bone marrow was benign compared with that of animals given comparable radiation exposures but without bone marrow treatment.\(^1\) This difference became apparent during the first few days after administration of bone marrow and before significant growth of cells from the graft might be expected to influence the animal's clinical course. These clinical findings are supported by the relative stability of body weight following radiation of dogs in this series (fig. 1) compared with the weight loss in dogs given lethal irradiation but no bone marrow autografts.\(^1\) This clinical behavior suggests some beneficial effect from infused autologous bone marrow beyond its proliferation and repopulation of depleted marrow cavities.\(^3\)

Although four dogs in this series (BE, W-4, W-5 and 14-D) showed good clinical recovery following autologous bone marrow infusions, they could not be considered "normal": dog BE succumbed apparently to an illness which similarly exposed dogs in the colony were able to resist; dog W-4 died of gastrointestinal bleeding 6 weeks after irradiation; dog 14-D succumbed to hemorrhagic pneumonia 9 months after irradiation; and W-5 died of heat stroke while other dogs were able to survive the adverse climatic conditions.

Dog Fatty showed normal cellularity in bone marrow, lymph nodes and spleen although the last blood count performed before its death revealed that the absolute lymphocyte count remained below 1000/mm\(^3\).\(^3\) Postmortem histologic studies on W-4 revealed damage to the gastric mucosa and only moderate repopulation of lymphatic structures. Dog W-5 had marked lymphoid hypoplasia at the time of its death 4 months after irradiation. The relation of these findings either to canine diseases or to late radiation effects is not clear.

2. Hematologic findings in all animals included postirradiation lymphopenia that persisted despite administration of autologous bone marrow (fig. 1). Even 6 weeks to 3 months following irradiation, dogs W-4, 14-D, W-5 and W-8 had peripheral lymphocyte counts significantly below those found in Andersen's dogs which survived 300 r from 250-kv x-rays without bone marrow treatment,\(^4\) (plotted in figure 1 as a heavy line).

Alpen and Baum,\(^5\) and Mannick et al,\(^6\) reported that autologous bone marrow is able to restore lymphopoiesis rapidly in the x-irradiated dog. The limited return of circulating lymphocytes in animals irradiated with Y\(^{90}\)-DTPA followed by autologous bone marrow administration, in contrast to the return in x-irradiated dogs treated with autologous marrow, may in part reflect more severe damage of lymphatic tissue with the use of Y\(^{90}\)-DTPA. It is also possible that the amount of Y\(^{90}\) remaining in the animal's body after the procedure may adversely affect the growth of transplanted cells.

In this series the return of circulating granulocytes in Y\(^{90}\)-DTPA irradiated dogs given autologous bone marrow was slower than in x-irradiated dogs.
**Table 1**

*Summary of Dogs Given Autologous Bone Marrow Transplants after Irradiation and Surviving More Than 1 Month*

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Per Pound Body Weight Administered</th>
<th>Nucleated Bone Marrow Cells Administered</th>
<th>Clinical History</th>
<th>Survival Status</th>
<th>Gross Pathology</th>
<th>Histological Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>491*</td>
<td>&gt;8.77</td>
<td>4.09 x 10^6</td>
<td>Renal graft attempted 4th day, technically a failure. Animal healed surgical wound uneventfully and had an unremarkable, normal recovery.</td>
<td>Living 15 mos.</td>
<td></td>
<td></td>
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<tr>
<td>14-D†</td>
<td>9.16</td>
<td>1.64 x 10^6</td>
<td>Normal until 18th day when anorexia, drooling, emesis and weight loss appeared. Began to improve after 30th day. By end of 2nd month was back to normal. Subsequently hair sparse, but normal in all other details until brief terminal coughing, anorexia, and hypoactivity.</td>
<td>Died 10 mos.</td>
<td>Hemorrhagic pneumonia. Petechial hemorrhages in small bowel. Lymph nodes fibrotic.</td>
<td>Alveoli atelectatic and hemorrhagic, edema. Kidneys normal. Bone marrow, lymph nodes, and spleen showed normal cellularity.</td>
</tr>
<tr>
<td>B. E.</td>
<td>11.4</td>
<td>2.28 x 10^6</td>
<td>Appeared normal during first few months after radiation, but a few weeks before demise animal became thin, hypoactive, and anorectic. Blood creatinine taken preterminally was slightly elevated.</td>
<td>Died 4 mos.</td>
<td>Animal emaciated, tissues unremarkable except for thinning of the renal cortex (cortex:medullary ratio of 1:3 or 1:4).</td>
<td>(Postmortem autolysis precluded examination.)</td>
</tr>
<tr>
<td>Animal</td>
<td>Weights</td>
<td>Mortality</td>
<td>Clinical Findings</td>
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<tr>
<td>Fatty?</td>
<td>11.57</td>
<td>Died 33rd day of pulmonary embolism</td>
<td>Lymph nodes fibrotic. Antibiotic suspension seen in small vessels of lungs. Lymph nodes and spleen showed normal cellularity. Bone marrow normally cellular. Kidney grossly normal but area of microabscess seen.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W-4</td>
<td>12.3</td>
<td>Died 43rd day.</td>
<td>Lymph nodes fibrotic. About 300 cc. of blood in stomach with small amounts distally in G-I tract. No definite gastric ulcer seen. Lymph nodes and spleen showed 30 to 40% repopulation, hemosiderin, and much collagenous fiber. Stomach showed necrotic slough of distal mucose—only base of gastric glands being viable. Bone marrow moderately hypoplastic.</td>
<td></td>
<td></td>
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<tr>
<td>W-8</td>
<td>12.9</td>
<td>Living 12 mos.</td>
<td></td>
<td></td>
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<tr>
<td>Blackie</td>
<td>13.9</td>
<td>Living 16 mos.</td>
<td></td>
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<tr>
<td>W-5</td>
<td>14.24</td>
<td>Died 4 mos.</td>
<td>Very thin, with many large, blue-black lymph nodes. Lymph nodes and spleen markedly hypoplastic and completely without germinal follicles; composed chiefly of fibrous tissue with hemosiderin. Many Langhans type giant cells in spleen. Kidneys showed marked loss of tubules with interstitial fibrosis.</td>
<td></td>
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Table 1.—(Continued)

1b.—Summary of Dogs Given Autologous Bone Marrow after Radiation and Surviving Less Than 1 Month

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>mc. Y* Per Pound Body Weight Administered</th>
<th>Nucleated Bone Marrow Cells Administered</th>
<th>Clinical History</th>
<th>Survival Status</th>
<th>Gross Pathology</th>
<th>Histological Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Gt</td>
<td>8.4</td>
<td></td>
<td>Hypoactive, anorexia from first day, emesis and mucoid diarrhea from 2nd day. Began to show improvement (more active, started eating, etc.) on day 7. On 11th day passed tarry stools and had bloody emesis, and died with GI bleeding on 12th day.</td>
<td>Died 12th day.</td>
<td>Some submucosa petechiae in bronchioles and stomach. Marked suberosal hemorrhage around ileocecal and appendiceal areas. Digested blood in stomach and tarry material in lower duodenum—no definite ulcer in GI tract seen. Mesenteric lymph nodes hemorrhagic.</td>
<td>Lymph nodes and spleen showed moderate cellularity with 40 to 50% of normal cellularity. Bone marrow showed early regeneration. Colon showed marked submucosal hemorrhage but mucosa was intact.</td>
</tr>
<tr>
<td>Chain</td>
<td>9.87 4.01 x 10⁶</td>
<td></td>
<td>Remained normal in appearance except for slow weight loss until 17th day, when animal became anorexic. On 19th day was hypoactive, coughing, anorexic, and vomited. Died on 20th day.</td>
<td>Died 20th day.</td>
<td>Diffuse hemorrhagic pneumonia, submucosal petechiae in pyloric end of stomach and in colon. Mesenteric lymph nodes enlarged and markedly hemorrhagic.</td>
<td>Lymph nodes and spleen quite cellular (70 to 100% of normal), germinal follicles present. Hyperemia, edema, and congestion in lungs.</td>
</tr>
<tr>
<td>X-50</td>
<td>19.8 3.00 x 10⁶</td>
<td></td>
<td>Known to have suffered small pulmonary emboli during urine recycling from clotted blood in tubing. Hypoactive after radiation but ate and drank well. Developed large hematomas on 2nd day in area of femoral artery cut-down. Anorexic, dehydrated, and hypoactive on 3rd day, dead on 6th day—markedly dehydrated.</td>
<td>Died 6th day.</td>
<td>————</td>
<td>————</td>
</tr>
</tbody>
</table>

*Spill during urine recycling precludes dosimetry estimations.
†In dogs Fatty, 14-D and 4-Gt, the bone marrow transplant was administered intravenously, 2 hours after cessation of urine recycling.
Fig. 1.—Changes in body weight, and hematologic parameters in irradiated dogs which received autologous bone marrow and survived longer than 1 month.
given autologous bone marrow.\textsuperscript{5,6} Dogs 14-D and Fatty, both given autologous bone marrow intravenously 2 hours after cessation of urine recycling, had the greatest depression and the slowest return of granulocytes of any dogs in this series. Indeed, the rate of peripheral granulocyte recovery is not as rapid as in the LD\textsubscript{60,30} x-irradiated survivors among Andersen's dogs.\textsuperscript{4} It appears therefore that the capacity of autologous bone marrow to save the lives of these two lethally irradiated dogs was not well correlated with its ability to raise peripheral granulocyte levels.

The remaining dogs in this series, each of which received an intra-arterial autologous marrow infusion 18-24 hours after the cessation of urine recycling, had peripheral granulocyte levels above 1000 cells per mm.\textsuperscript{3} of blood. There was a gradual decrease in platelet counts, reaching the lowest point at about the 20th day. No profound thrombocytopenias and only a few platelet values below 100,000/mm.\textsuperscript{3} were seen, a result anticipated from a platelet-sparing effect of the Y\textsuperscript{90}-DTPA technic. The alterations in the reticulocyte count reflect the pattern of changes in the circulating granulocyte counts.

The clinical course and pathologic results in the three dogs treated with autologous bone marrow and given supralethal irradiation, and which survived less than 1 month, are summarized in table 1b. Figure 2 shows body weights and hematologic changes in these animals. The first animal in this series, 4-G, received the marrow autograft 2 hours after the termination of urine recycling. On the 12th day it died as the result of a hemorrhagic diathesis with 40-50 per cent cellularity of lymph nodes and spleen. The bone marrow showed early regeneration in lymphopoietic and hematopoietic structure despite which this animal continued to have severe depression of lymphocytes, granulocytes, and reticulocytes in its peripheral blood until it died. In this dog the autografted marrow cells apparently were responsible for the cellularity in the lymph nodes and bone marrow, because none of the dogs in the control series of untreated animals given lethal amounts of Y\textsuperscript{90}-DTPA\textsuperscript{1} and which died about the 12th day showed histologically any regeneration of lymphatic and hematopoietic structures.

Dog X-50 died on the 6th day of pulmonary emboli from clotted blood in the intravenous recycling unit tubing during the recycling procedure, and from the loss of several hundred ml. of blood in the area of the femoral arterial cannulation following the bone marrow infusion.

Dog Chain, which received the highest radiation dose in the series treated with autologous bone marrow, remained normal in appearance until the 17th day, but died on the 20th day of hemorrhagic pneumonia, at which time the lymph nodes and spleen showed almost normal cellularity. Its circulating blood did not reflect this return of lymphopoiesis, and preterminally it had a lymphocyte count of less than 100 cells/mm.\textsuperscript{3} of blood.

**Discussion**

The results obtained from both series of dogs established that administration of bone marrow autografts can prevent the death of dogs given lethal irradiation with Y\textsuperscript{90}-DTPA. The autologous marrow apparently promotes
the return of circulating granulocytes, reticulocytes, and platelets, but not of lymphocytes. Three dogs were given autologous bone marrow intravenously 2 hours after cessation of recycling, at a time when significant amounts of isotope remained in the body. Two of these dogs survived, but the pattern of their peripheral blood elements suggested autogenous return. The third animal (4-G) showed recovery of cellularity in lymphatic and hematopoietic structures, although peripheral granulocytes, reticulocytes and lymphocytes were markedly depressed, and the dog died on the 12th day.

It thus appears that autologous bone marrow administered intravenously 2 hours after cessation of urine recycling is able to repopulate lymphatic and hematopoietic structures, although evidence of returning hematopoiesis is delayed to a greater extent than when the marrow is injected later than 24 hours after the radiation procedure.

CONCLUSIONS

1. Autologous bone marrow infusions are able to save the lives of dogs lethally irradiated with Y\(^{90}\)-DTPA.

2. In certain cases the autologous marrow was able to save the life of the
Y\textsuperscript{90}-DTPA-irradiated dogs without any marked early elevation in circulating formed blood elements.

3. In contrast to results obtained using external irradiation technics, animals treated with Y\textsuperscript{90}-DTPA and autologous marrow did not show early return of circulating lymphocytes.

**Summario in Interlingua**

1. Infusiones de autologe medulla ossee es capace a salvar le vita de canes irradiate letalmente con Yttrium-90 in chelation con acido diethyleno-triamino-penta-acetic (Y\textsuperscript{90}-ADTP).

2. In certe casos le autologe medulla esseva capace a salvar le vita de canes irradiate per Y\textsuperscript{90}-ADTP sin le precoce occurrenzia de marcate elevationes de formate elementos sanguinee in le circulation.

3. Per contrasto con resultatos obtenite per le uso de technicas de irradiation externe, animales tractate con Y\textsuperscript{90}-ADTP e medulla autologe non monstrawa un precoce retorno de circulante lymphocytos.

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