The Recovery of Lethally Irradiated Dogs Given Infusions of Autologous Leukocytes Preserved at -80 C.

By JOHN A. CAVINS, STUART C. SCHEER, E. DONNALL THOMAS and JOSEPH W. FERREBEE

LETHALLY irradiated dogs usually recover promptly if given several billion fresh, autologous, marrow cells.\textsuperscript{1,2} Autologous marrow, stored at low temperature in glycerol or dimethyl-sulfoxide, induces a similar recovery.\textsuperscript{3,4} Recent studies of lethally irradiated mice have shown that infusions of fresh, isogenic leukocytes, if given in sufficient number, are followed by survival and return of marrow function.\textsuperscript{5-9} Atkins, Goodman, Perry and Kerby\textsuperscript{10-12} have shown that glycerol exerts a protective effect on blood leukocytes stored at low temperatures. This communication reports the survival of dogs given x-irradiation in a dosage of 1200 r and infusions of autologous leukocytes that had been stored at -80 C. in 10 per cent dimethyl-sulfoxide.*

MATERIALS AND METHODS

Nine mongrel dogs were used in this study. The dogs were 6 to 18 months of age and weighed 15 to 30 pounds. Seventy-five to 100 ml. of blood were withdrawn 2 to 4 times weekly with heparin as anticoagulant. Intramuscular iron-dextran\textsuperscript{f} (50 mg.) was given twice weekly to maintain hemoglobin levels.

One volume of blood was added to two volumes of a 1.5 per cent solution of polyvinylpyrrolidone (Plasdone C)\textsuperscript{\|} in normal saline in a 150 ml. Fenwal plastic bag,\textsuperscript{\|} mixed thoroughly, the bag suspended vertically, and the red cells allowed to sediment for 1 hour. The supernatant fluid containing white cells, platelets and a few red cells was removed with a Fenwal plasma extractor\textsuperscript{\|} and centrifuged for 20 minutes at 670 g. A cellular sediment of approximately 10 ml. was obtained. To this was added an equal volume of a 20 per cent solution of dimethyl-sulfoxide, and the cells were resuspended by gentle mixing in the bag. The bags were placed between copper plates and frozen slowly to -80 C.\textsuperscript{4} The solution of 20 per cent DMSO was made by adding two parts concentrated dimethyl-sulfoxide to one part serum and seven parts of tissue culture medium.\textsuperscript{38}

Storage periods of individual samples ranged from 3 days to 6 weeks. Total collections from individual donors ranged from 2 to 20 billion cells. One to two weeks after storage of his final leukocyte sample, each dog was irradiated with 1200 r from dual Co\textsuperscript{60} sources at a dose rate of 4-5 r per minute. The LD\textsubscript{100} dose of this radiation in this laboratory

*We are grateful to the Crown Zellerbach Co., Camas, Wash., for providing the dimethyl-sulfoxide used in these studies.

\textsuperscript{f}Lakeside Lab., Milwaukee, Wis.
\textsuperscript{\|}Antara Chemicals, General Aniline & Film Corp., New York, N. Y.
\textsuperscript{\|}Fenwal Laboratories, Morton Grove, Ill.
\textsuperscript{38}TC-199, Difco Laboratories, Detroit, Mich.

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Table 1.—Summary of Experiments in Storing Leukocytes in Dimethyl-Sulfoxide

<table>
<thead>
<tr>
<th>Dog No</th>
<th>Irradiation Dose (r) x 10⁶</th>
<th>White Blood Cells Infused x 10⁶</th>
<th>ml. Infused</th>
<th>No. of Infusions</th>
<th>Hematopoietic Recovery</th>
<th>Survival Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>1086</td>
<td>1200</td>
<td>2.1</td>
<td>71</td>
<td>1</td>
<td>no</td>
<td>14 days</td>
</tr>
<tr>
<td>860</td>
<td>1200</td>
<td>6.0</td>
<td>166</td>
<td>1</td>
<td>partial</td>
<td>25 days</td>
</tr>
<tr>
<td>836</td>
<td>1200</td>
<td>3.3</td>
<td>112</td>
<td>1</td>
<td>no</td>
<td>14 days</td>
</tr>
<tr>
<td>1109</td>
<td>1200</td>
<td>4.1</td>
<td>64</td>
<td>1</td>
<td>no</td>
<td>14 days</td>
</tr>
<tr>
<td>835</td>
<td>1200</td>
<td>9.0</td>
<td>197</td>
<td>1</td>
<td>complete living and well</td>
<td></td>
</tr>
<tr>
<td>718</td>
<td>1200</td>
<td>20.0</td>
<td>502</td>
<td>1</td>
<td>complete living and well</td>
<td></td>
</tr>
<tr>
<td>716</td>
<td>1347</td>
<td>18.6</td>
<td>428</td>
<td>2</td>
<td>partial</td>
<td>18 days</td>
</tr>
<tr>
<td>939</td>
<td>1347</td>
<td>19.0</td>
<td>451</td>
<td>2</td>
<td>partial</td>
<td>21 days</td>
</tr>
<tr>
<td>941</td>
<td>1200</td>
<td>19.0</td>
<td>430</td>
<td>1</td>
<td>complete living and well</td>
<td></td>
</tr>
</tbody>
</table>

is approximately 600 r. Care before and after radiation was given the dogs as previously described.¹³

Experience with dog bone marrow has shown it to be unnecessary to remove dimethyl-sulfoxide prior to infusion.⁵ In the experiments described here the leukocyte samples were administered intravenously to the original donor immediately after rapid thawing in a 37 C. water bath. A problem of cell clumping was noted if the interval between thawing and infusion was greater than 10 minutes.

**Results**

Table 1 summarizes the results. The dose of leukocytes given the first five dogs ranged from 2.12 to 9.0 x 10⁶ cells in volumes up to 200 ml. Three of these dogs survived 14 days, but showed no evidence of bone marrow regeneration. One dog (860) that received a dose of 6.04 x 10⁶ cells survived 28 days and demonstrated early marrow recovery with a rising white count. Dog (835) received 9 x 10⁶ cells and survived. Four dogs were given leukocyte doses of approximately 20 x 10⁶ cells each in volumes up to 400-500 ml. Two were given their cells in a single infusion 18 hours after radiation exposure (718 and 941). Two were given their cells in two approximately equal infusions 18 and 42 hours following the radiation exposure (716 and 939). Both of the former have survived, whereas both of the latter showed beginning marrow regeneration but died 3 weeks post-radiation of infection or hemorrhage.

Figure 1 shows the white cell responses of those dogs that showed evidence of regeneration of marrow. Figure 2 compares this response with the responses seen in dogs recovering from lethal radiation after infusions of bone marrow or after supportive treatment only.²,⁵,¹⁴ Dog (681) given autologous marrow showed prompt recovery of the white count after a nadir on the 7th post-radiation day; dog (835) given autologous leukocytes showed a slower, but progressive return with a white count of 1000 on the 15th post-radiation day after a nadir on the 7th day. The previously reported dog (271) given only supportive treatment with almost daily transfusions of irradiated allogeneic blood, fluids, and antibiotics had a white count of 750 cells 55 days after radiation and a very slow recovery thereafter.¹⁴ The platelet counts of the dogs given frozen, autologous leukocytes showed a very slow rise after a
Fig. 1.—Peripheral white blood cell counts of dogs exposed to 1200 r of Co\(^{60}\) radiation and given an infusion of a sample of autologous white cells that had been stored at \(-80\) C. in 10 per cent dimethyl-sulfoxide.

nadir at approximately the 15th day post-irradiation. Several plateaus were observed at levels of 1000, 10,000, and 50,000 platelets, each plateau lasting several days to 2 weeks. The over-all hematopoietic recovery was delayed 2 to 3 weeks as compared to dogs receiving fresh or frozen bone marrow.

DISCUSSION

These studies show that lethally irradiated dogs recover following administration of samples of autologous leukocytes that have been stored at \(-80\) C. in 10 per cent dimethyl-sulfoxide. Three dogs given 9, 20, and 20 billion cells respectively survived with complete but delayed hematopoietic recovery. The plateaus seen in their rising platelet counts are mentioned without explanation other than a possible relationship to the number of platelet precursors or stem cells infused. The two dogs given 20 billion cells in divided dosage died of infection, and hemorrhage from platelet insufficiency. Since rate of restoration of platelet counts appeared borderline in all animals, the effect of split dosage on recovery rates can hardly be evaluated. Three dogs that received small numbers of cells showed a beginning return of marrow function but also died of hemorrhage at 3 to 4 weeks post-radiation. The number of cells given thus appears a critical factor with no apparent differences in pattern of recovery being observed with doses between 9 and 20 \(x\) \(10^9\) cells. Dogs given less than 9 \(x\) \(10^9\) cells showed evidence of marrow regeneration, but at a rate insufficient to permit survival.

The radio-protective effect of chronic marrow stimulation by multiple bleedings has not been evaluated in the lethal range of radiation in dogs.\(^{15}\) The recoveries reported here after 1200 roentgens appear beyond what might be anticipated on this basis. The observations suggest that there are cells in
the peripheral blood of dogs that are capable of inducing recovery of marrow function in dogs that have been exposed to lethal radiation. As in the mouse, the percentage of the necessary primitive cells in the peripheral blood appears less than in the marrow. The number of peripheral blood cells needed to induce prompt recovery in the dog and in the mouse seems of the order of 10 times the number of marrow cells required.\textsuperscript{2,3}

**Summary**

Nine normal mongrel dogs were exposed to 1200 r whole-body irradiation at 4 to 5 r per minute. They were then given intravenous infusions of 2.12 to 20 x 10\textsuperscript{9} autologous leukocytes that had been previously stored at \(-80\) C. in 10 per cent dimethyl-sulfoxide.

Three dogs survived with delayed but complete hematopoietic recovery. Three showed beginning marrow regeneration but died within 3-4 weeks of irradiation. Three given less than 6 x 10\textsuperscript{9} cells died within 21 days. The number of leukocytes infused was critical since there was no survivor among the dogs receiving less than 9 x 10\textsuperscript{8} cells.

It is concluded that peripheral blood contains primitive cells capable of repopulating marrow spaces and restoring marrow function.

**Summario in Interlingua**

Novem normal canes hybrida esseva exponite a 1200 r de irradiation del corpore total, in un concentration de 4 a 5 r per minuta. Postea le canes recipeva infusiones intravenose de 2,12 a 20 x 10\textsuperscript{9} autologe leucocytos que habeva previemente esite preservate a un temperatura de minus 80 C in dimethyl-sulfoxydo de 10 pro cento.
Tres del canes superviveva con tardive sed complete restablimento hematopoietico. Tres alteres manifestava un comenciante regeneration medullari sed moriva intra 3 a 4 septimanas post le irradiation. Le remanente tres, tractate con minus que 6 x 10⁶ cellulas moriva intra 21 dies. Le numero del infusionate leucocytos esseva critic; inter le canes supervivente, nullo habeva recipite minus que 9 x 10⁹ cellulas.

Es concludite que sanguine peripheric continue cellulas primitive que pos-sede le capacitate de repopular spatios de medulla e de restaurar le function medullari.

REFERENCES

RECOVERY OF LETHALLY IRRADIATED DOGS

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