BRIEF REPORT

Transplantation of Allogeneic Canine Bone Marrow Stored at -80 C. in Dimethyl Sulfoxide

By R. STORB, R. B. EPSTEIN, R. F. LE BLOND, R. H. RUDOLPH, and E. D. THOMAS

PROTECTION against otherwise lethal doses of ionizing radiation by infusion of isogeneic bone marrow stored at low temperatures has been demonstrated in rodents,1 dogs,2,3 and primates.4 In rodents, a few attempts have been made to transplant stored allogeneic marrow.5-7 This study was undertaken to evaluate the feasibility of obtaining grafts with allogeneic canine marrow preserved in dimethyl sulfoxide at -80 C.

MATERIALS AND METHODS

Twenty dogs, 6 to 12 months of age, were dewormed and immunized against distemper and hepatitis. They were isolated for at least 3 weeks prior to use and observed carefully to insure good health. All were typed for canine red cell antigens A and C. Histocompatibility typing with four canine lymphocyte cytotoxic antisera designated as anti-A, anti-B, anti-C and anti-D was carried out as previously described.8

The donor dogs, weighing 7 to 18 kg., were sacrificed with pentobarbital sodium. Bone marrow was removed under aseptic conditions from all major bones and suspended in TC 199 tissue culture medium (Difco Laboratory, Detroit, Michigan) containing 10 per cent autologous serum and 6.6 mg. of heparin (Connaught Laboratories, Toronto, Canada). The marrow cell suspension was then passed through stainless steel screens of 300 and 200 microns.9 Thirty to 50 ml. of the cell suspension was placed in each of 2 to 4 600 ml. plastic blood administration bags (Fenwal Laboratory, Morton Grove, Illinois). An equal volume of a mixture of 70 per cent TC 199, 20 per cent dimethyl sulfoxide, and 10 per cent autologous serum was added to the bags. The bags were then placed between copper plates to secure a uniform thin layer and frozen to -25 C. at a rate of 1 C. per minute. They were stored at -80 C. for 7 to 40 days.10

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Table 1.—Dogs Given 1200 r. and Infusion of Allogeneic Bone Marrow Cells Stored at −80° C.

<table>
<thead>
<tr>
<th>Dog No.*</th>
<th>Relationship</th>
<th>Red Cell Type</th>
<th>Lymphocyte Type</th>
<th>No. of Bone Marrow Cells X 10^6</th>
<th>Marrow Storage Time in Days</th>
<th>Marrow Take</th>
<th>Marrow Rejection</th>
<th>Survival in Days</th>
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<tr>
<td>506-D</td>
<td>Unrelated</td>
<td>A−C+</td>
<td>ABC</td>
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<td>AB</td>
<td>B</td>
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<td>Yes</td>
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<td>A+C+</td>
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<td>B</td>
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<td>AB</td>
<td></td>
<td></td>
<td>Yes</td>
<td>No</td>
<td>&gt;100</td>
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</table>

* D = bone marrow donor, R = bone marrow recipient.
† Dashes indicate negative reaction with all four antisera.
Fig. 1.—White blood cell changes in 10 dogs treated with 1200 r. and infusion of stored allogeneic bone marrow.

elapsed time from death of the donor dogs to freezing of marrow was approximately 2 hours.

The recipient dogs, weighing 7 to 12 kg., were given 1200 r. (midline air dose) of whole body radiation delivered by 2 opposing ⁶⁰Co sources at a dose rate of 9.2 r. per minute with a source-target distance of 158 cm. The radiation dose was monitored by a Victoreen "r" meter and by lithium fluoride radioluminescence dosimetry.

Of the 10 donor-recipient pairs, 4 were litter mate pairs matched by histocompatibility testing, and 6 were unrelated dog pairs in which donor and recipients were of different breeds. Three of the 6 unrelated dog pairs were matched by histocompatibility testing while 3 pairs were mismatched. The aliquots of marrow were administered intravenously over a period of 4 to 18 hours after radiation. Each aliquot of marrow was thawed rapidly in a 37 C. water bath and infused within 15 minutes of thawing. All recipient dogs received immunosuppressive therapy with methotrexate, 0.5 mg./kg. intravenously, on days 1, 3 and 6. Sodium ampicillin, 250 mg., was given twice daily during the period of leukopenia (white counts less than 1,000/mm³). Parenteral fluids and electrolytes were administered twice daily for the first 10 days following radiation. White cell counts, platelet counts, and hematocrits were performed at least 3 times per week. Bone marrow engraftment was indicated by a rise in white cell counts to over 1,000/mm³ subsequent to the post-irradiation decline, and by marrow histology. Cytogenetic analysis of bone marrow cells was carried out in 1 dog (518) 70 days following radiation. This male dog received bone marrow from a female donor. Only cells with a chromosome count of 78 were evaluated. All dogs that died underwent gross and histologic post mortem examinations.

**Results**

All 10 recipient dogs showed initial marrow engraftment as indicated by a rise in white cell counts to over 1,000/mm³ after the post-irradiation nadir
ALLOGENEIC CANINE BONE MARROW

(Table 1 and Fig. 1). The 3 unrelated dogs that were mismatched with their donors by histocompatibility testing subsequently rejected the foreign marrow during the second week after radiation. Rejection was manifested by declining white cell and platelet counts, and by severe marrow hypoplasia at autopsy. The 7 dogs that matched their donors by histocompatibility testing showed sustained hemopoietic recovery. Four of the 7 are alive and well with normal peripheral blood cell counts more than 100 days after transplantation. Three of the 7 died. One (520) died on day 14 with severe enteritis. The white cell count in this dog rose to 4,200/mm³ prior to death, and the bone marrow at autopsy was cellular with hemopoietic precursor cells at all stages of maturation. Dogs 533 and 541 died on days 42 and 58 with pneumonia. These dogs showed normal hematocrits, platelet counts and granulocyte counts, and the autopsy bone marrow showed normal cellularity. The lymphocyte counts in these dogs ranged from 3,200 to 3,500/mm³ before irradiation.

Bone marrow for cytogenetic analysis was obtained on one of the long-term survivors (518) 70 days after transplantation. This male dog received bone marrow from a female donor. All 20 cells analyzed were of female karyotype. Dogs 517, 518 and 541 had red cell types different from their donors. When tested 74, 68 and 36 days respectively after transplantation, their red cells were of donor type.

DISCUSSION

Since the report of Barnes and Loutit in 1955, numerous investigations have been carried out in a variety of animals concerning technics of freezing and storing bone marrow. The efficacy of these storage technics has been established by infusing isogeneic bone marrow into lethally irradiated recipients. In contrast, only a limited number of studies have been carried out in rodents testing the efficacy of stored allogeneic marrow. Schwarzenberg et al. grafted stored DBA/2 x C57BL/6 marrow into CBA x C57BR mice and found that 70 per cent of the animals survived longer than 60 days. Kurnick et al. were able to obtain grafts with stored bone marrow in non-isogeneic CF₁ mice. Porter and Murray demonstrated the presence of female drumsticks in male rabbits that received stored female marrow following lethal radiation. The present study demonstrates that sustained hemopoietic engraftment can be achieved in lethally irradiated dogs using allogeneic bone marrow stored at -80 C. in dimethyl sulfoxide. The data suggest that success or failure of grafts with stored allogeneic marrow depend upon the histocompatibility relationship between donor and recipient dogs. Successful grafts were obtained only when donor and recipient were matched by histocompatibility testing. The predictive value of histocompatibility testing for marrow transplantation has been shown previously in litter mates and unrelated dogs using grafts of fresh allogeneic hemopoietic cells.

It appears that marked differences exist between animal species with respect to the effectiveness of the various marrow storage technics. This fact com-
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Complicates the extrapolation of animal data to man, and so far only suggestive evidence is available that storage methods effective in animals are adequate for human bone marrow. Nevertheless, cautious optimism seems to be justified regarding collection and storage of human cadaver bone marrow for future use in allogeneic transplantation. Rapid advances in human histocompatibility typing may permit selection of the stored marrow most compatible with the intended recipient.

Summary

Prompt initial bone marrow engraftment was observed in 10 lethally irradiated dogs receiving infusions of $9.8 \times 10^8$ to $30.0 \times 10^8$ allogeneic marrow cells stored at $-80\,\text{C}$ in dimethyl sulfoxide. The 3 recipients of bone marrow from unrelated donors, mismatched by canine histocompatibility testing, subsequently rejected their grafts and died within 16 days with marrow hypoplasia. The 3 dogs with matched unrelated donors and the 4 with matched litter mate donors all showed sustained marrow engraftment. Evidence of marrow repopulation by allogeneic cells was obtained by cytogenetic studies in one and by change to donor red cell type in 3 instances.

Summary in Interlingua

Un prompte ingraffamento de medulla ossee esseva observate in 10 letalmente irradiate canes que recipeva infusiones de inter $9,8 \times 10^8$ e $30,0 \times 10^8$ allogenic cellulas medullari preservate a $-80\,\text{C}$ in sulfoxydo dimethylic. Le 3 animalis in le serie que recipeva medulla ossee ab nonrelationate donatores, mal appareate per tests del histocompatibilitate canin, rejiceva subsequentemente lor graffos e moriva intra 16 dies con hypoplasia medullari. Le 3 canes con appareate sed non relationate donatores e le 4 con appareate donatores confraterne manifestava sin exception sustenite ingraffamento medullari. Evidentia de repopulation de cellulas allogenic esseva obtenite per studios cytogenetic in un caso e per le costatation del transition al typo erythrocytic del donator in tres casos.

Acknowledgment

We are grateful to Doctor J. W. Ferrebee, Cooperstown, New York, who provided some of the dogs used in these studies.

References

8. Epstein, R. B., Storb, R., Ragde, H.,
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