Marrow Engraftment by Allogeneic Leukocytes in Lethally Irradiated Dogs

By R. Storb, R. B. Epstein, H. Ragde, J. Bryant and E. D. Thomas

A major problem of allogeneic bone marrow grafting in outbred animals and in man has been the procurement of a quantity of marrow from a single living donor sufficient for rapid restoration of marrow function. Recent studies in mice, guinea pigs, and dogs indicate that cells with marrow repopulation potential circulate in the peripheral blood. These cells could be used instead of marrow or as a supplement to marrow from living donors for transplantation purposes. This possibility was explored in dogs given 1200 r of whole body irradiation. When these animals were cross-circulated with normal partners, consistent marrow grafts could be demonstrated after a week of daily cross circulation. The technic of cross circulation, however, did not allow the quantitation of the types of cells transferred. In addition, severe secondary disease was observed following cross circulation, possibly due to immunization of the donor by cells from the recipient during cross circulation.

In the present study, a technic of leukapheresis was used to achieve allogeneic marrow engraftment in the dog. This technic made possible determination of the quantity and type of cells transferred and did not expose the donor to recipient antigens.

Materials and Methods

Ten unrelated donor-recipient pairs of dogs were matched for canine red cell antigen A. The weight of the donor animals ranged from 17 to 35 kilograms, and the weight of the recipients ranged from 7 to 16 kilograms. All animals were dewormed and immunized against hepatitis and distemper.

Silastic-teflon arteriovenous shunts were placed in the donor dog as previously described. Blood, 400 to 800 ml., was withdrawn from the arterial side of the shunt into sterile 50-ml. glass centrifuge tubes containing 2 mg. of heparin. After centrifugation at 260 g for 20 minutes, the buffy coat layer was drawn into sterile 50-ml. plastic syringes and stored at 4 C. for a maximum of 3 hours. White blood cell and differential counts were obtained prior to infusion into the recipient. The red cells and plasma were returned to the donor through the venous side of the shunt while another 400 to 800 ml. of whole blood...
were obtained from the arterial cannula. Two to 8 such volumes of blood were processed each day, with the time of the buffy coat separation averaging about 30 minutes.

The recipients received 1200 r of whole-body irradiation at a dose rate of 9.2 r per minute from dual Co-60 sources with a source-target distance of 180 centimeters. The radiation dose was monitored by a Victoreen "r" meter as well as by lithium fluoride radioluminescence dosimetry. Following the irradiation, the recipients were given daily infusions of leukocytes for 8 consecutive days. An arbitrary schedule was selected so that each received a total of approximately 100 x 10^6 white blood cells from his donor. Approximately 20 x 10^6 cells were given on the day of irradiation and on the day following. Subsequent injections consisted of approximately 10 x 10^6 white blood cells daily for 6 days.

Two groups of recipients were studied. Group I was composed of 5 dogs that received no immunosuppressive treatment following irradiation. Group II included 5 recipients that received immunosuppressive therapy with methotrexate, 0.25 mg/Kg. of body weight subcutaneously on days 1, 3, 5, and 8. All irradiated animals were given ampicillin, 250 mg. twice daily intravenously, and supported with fluids and electrolytes as needed.

White blood cell count, platelet count, and hematocrit were obtained daily from the irradiated dogs prior to leukocyte infusion. The onset of allogeneic bone marrow function was indicated by an increase in white blood cell counts after termination of leukocyte infusions and by bone marrow histology. Cytogenetic analysis of a peripheral blood and bone marrow sample taken on one dog (214) 20 days following irradiation was carried out as previously described. This male animal received leukocytes from a female donor. Complete autopsies with histologic studies were performed on all recipients.

**RESULTS**

By the use of teflon-silastic arteriovenous shunts, large quantities of blood could be collected or reinjected within minutes. The volume of blood processed per donor during the 8-day period of leukapheresis was 9200 ± 1500 ml., and the number of leukocytes obtained from 1000 ml. of blood was 11.6 ± 3.8 x 10^6. The efficiency of leukapheresis was determined by calculating the percentage of white cells recovered from each 400 ml. of blood exchanged in one donor (192) over the 8-day period. This was found to be 75 ± 15 per cent.

Hematocrits on the buffy coat samples ranged between 20 and 30 per cent. The volume containing 10 x 10^6 white cells was 70 to 120 ml. A similar volume of blood was removed from the recipient prior to infusion. The daily number of platelets infused with the buffy coat was determined for 2 dogs (229 and 231) and varied from 30 to 550 x 10^6.

All donor dogs tolerated leukapheresis well. In most dogs clotting of the arteriovenous shunts occurred after 5 or 6 days, and new cannulae were placed in the contralateral vessels. All donors developed a moderately severe anemia and a slight thrombocytopenia during the collection period. The white blood cell count remained within normal ranges. When leukapheresis was discontinued, hematologic recovery was rapid and complete.

Table 1 summarizes the results obtained in the two groups of recipient dogs studied. The total number of leukocytes infused and the constituent cell types are shown for each dog. All dogs showed histologic evidence of bone marrow repopulation at autopsy. The dogs in Group I survived between 6 and 9 days with a mean of 7.8 days and demonstrated no rise in peripheral white blood cell counts. In contrast, the dogs of Group II survived between 8 and 22 days, with a mean of 16.8 days. In four of these animals a
<table>
<thead>
<tr>
<th>Group</th>
<th>Dog No.</th>
<th>Number of infused leukocytes x 10⁶</th>
<th>Survival in days</th>
<th>Rise in white blood cell count</th>
<th>Bone marrow histology</th>
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<tr>
<td></td>
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<td>Total</td>
<td>Bands</td>
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<td>197</td>
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<tr>
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<td>88.3</td>
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<tr>
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<td>41.8</td>
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rise in white blood cell count occurred prior to death. Figure 1 illustrates the white blood cell changes in the dogs of Group II. In both groups platelet counts were maintained in the range of 50,000 to 200,000 per cu. mm. during the buffy coat infusion but thereafter fell to low levels.

The results of the cytogenetic analysis of peripheral blood and bone marrow obtained on dog 214 20 days after irradiation are shown in Table 2. This male recipient received buffy coat cells from a female donor. All cells analysed showed the XX chromosomes characteristic of the female karyotype.

Diarrhea and wasting were observed in all irradiated animals. Histologically, these dogs showed necrosis of the surface epithelium of the ileum with mononuclear infiltration of mucosa and submucosa. The mucosa of the colon showed varying degrees of hemorrhage and necrosis. Liver cell necrosis and icterus of varying degree was apparent in 8 animals. Evidence of a terminal pneumonia was found in 7 dogs. The lymph nodes showed absence of the follicular structure, and the degree of cellularity varied from poor to near normal.
DISCUSSION

The results extend those obtained with cross circulation, indicating that marrow function in lethally irradiated dogs can be restored by allogeneic hemopoietic progenitor cells derived from the peripheral blood of a living donor. Within 7 days of irradiation and infusion of allogeneic leukocytes, the bone marrow showed histologic evidence of early restoration. Within 10 days newly formed leukocytes and platelets appeared in the peripheral blood. In one male recipient dog, bone marrow and peripheral blood cells were shown to be of the female donor karyotype when analyzed by cytogenetic technic.

The rate of hemopoietic recovery in dogs given infusions of allogeneic leukocytes was slower than that observed in dogs after cross circulation or marrow infusion. It appears that $100 \times 10^9$ homologous leukocytes are near the minimal number for prompt marrow engraftment. In previous studies in the dog it was shown that $20 \times 10^9$ stored autologous leukocytes produced a slow marrow recovery, in contrast to a rapid recovery after $1-4 \times 10^9$ autologous marrow cells.

The present study provides information about the quantities of the different cell types that were infused, but it provides no information about the morphologic characteristics of the cells with marrow repopulating potential. Presumably, these cells were present in the approximately $20 \times 10^9$ lymphocytes or $10 \times 10^9$ monocytes that were infused. Suggestive evidence has been obtained in mice and dogs indicating that the stem cells are similar to small lymphocytes. In the rat, however, the stem cell may resemble the monocyte. Resolution of this problem must await improved methods of separating the different cell types.

Dogs with allogeneic marrow grafts achieved by cross circulation died early, presumably of severe "secondary disease" since administration of methotrexate delayed death. The cause of the death of these animals was complex and included the complications of marrow hypoplasia as well as the secondary syndromes. It appeared that this severe secondary disease might be due to exposure of the recipient to donor cells immunized by contact with cellular antigens of the recipient during cross circulation. In the mouse preimmunization of the marrow donor with recipient tissue has been shown to increase the severity of secondary disease. In the present study marrow engraftment was achieved by peripheral blood cells without immunization of the donor. The resulting secondary disease was comparable to that seen after cross circulation in that it appeared quickly, was severe, and was only partially ameliorated by methotrexate. The peripheral blood appears to be a rich source of immunologically competent cells, since the secondary disease in these dogs was more severe than that usually observed after infusion of marrow or marrow and spleen cells. Similarly, in the mouse peripheral blood was shown to produce a killing effect in the marrow grafting experiments.

It has been shown in mice, guinea pigs, and dogs that stem cells are present in the circulating blood. However, the presence of these cells in man has been questioned. If, indeed, such cells are present in man, large num-
bers could be collected and stored, thus increasing the likelihood of a prompt marrow transplant from an individual donor. Clinical application of marrow grafting by circulating stem cells must await control of secondary disease through better matching of donor and recipient and improved immunosuppressive therapy.

**SUMMARY**

Infusion of white blood cells separated from peripheral blood produced allogeneic bone marrow engraftment in lethally irradiated dogs. Approximately $100 \times 10^6$ leukocytes obtained from a single donor over an 8-day period were adequate to establish marrow repopulation. Marrow engraftment was indicated by rising blood count, marrow histology, and, in one instance, cytogenetic studies. Marrow grafts were associated with a severe secondary syndrome. Survival was prolonged with methotrexate.

**SUMMARIO IN INTERLINGUA**

Le infusion de leucocytos, separate ab sanguine peripheric, produceva ingraffamento de allogeneic medulla ossee in letalmente irradiate canes. Approximativamente $100 \times 10^6$ leucocytos, obtenite ab le mesme donator in le curso de un periodo de 8 dies, esseva sufficiente pro initiar le repopulation medullari. Le ingraffamento del medulla esseva evidentiate per augmentos in le numerations sanguinee, studio histologic del medulla, e—in un caso—examine cytogenetic. Graffos medullari esseva associate con un sever syndrone secundari. Methotrexato serviva a prolongar le superviventia.

**REFERENCES**

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