Autografts of Bone Marrow in Dogs After Lethal Total-Body Radiation

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Apen and Baum have shown that infusions of autologous marrow induce recovery in dogs after exposure of the entire body to 400 or 600 r of 250 kv x-rays.1 The purpose of this communication is to report that recovery in dogs can be induced promptly by autologous marrow infusion after exposure to x-ray or gamma radiation in dosages up to 1500 r. Both bone marrow and lymphoid tissue are restored. The restoration of the latter tissue is of an order not yet obtained with homologous marrow in this species after similar amounts of irradiation.2

Material and Methods

Eight purebred beagle dogs were used as experimental animals, 2 male and 6 female. The dogs were between 5 and 18 months of age and weighed from 6 to 8 Kg. They were caged separately or in pairs in a general animal room and were exposed, because of a transient population of sick dogs, to the same seasonal canine disorders as the group of dogs grafted with homologous marrow previously reported from this laboratory.2 Prior to radiation all dogs were dewormed and actively immunized against distemper and hepatitis by attenuated vaccine.*

Dogs were kept fasting on the day of irradiation and were fed only ground beef on the evening before in order to insure a minimum of osseous and other calcium-containing material in the gut at the time of irradiation. All animals were given daily intramuscular injections of 400,000 U. of procaine penicillin and 0.5 Gm. streptomycin beginning on the day of irradiation and continuing for at least 7 days thereafter. Other antibiotics, parenteral fluid therapy, fresh blood transfusions and dog hyperimmune serum† were used as required.

Baseline determinations of hemoglobin concentration, hematocrit, white blood cell count, differential count, reticulocyte count and platelet count were performed on all animals prior to irradiation and were repeated on the sixth or seventh day after irradiation and thereafter at biweekly intervals until full hematologic recovery had occurred.

Serum samples for filter paper electrophoresis were drawn on four of the eight dogs prior to irradiation and at two weeks following irradiation. Samples were drawn on all dogs on the sixth or seventh day after irradiation and thereafter at biweekly intervals until full hematologic recovery had occurred.

Irradiation was given by two methods:

Method I.—Dogs were anesthetized with intravenous pentobarbital sodium (25 mg./Kg.) and placed under a standard 250 kvp x-ray therapy machine. Target distance was 100 cm. to midline of the animal. Dose rate was 5 r/min. in air at theoretical midline with filters 0.25 mm. Sn, 0.4 mm. Cu and 1.0 mm. Al, HVL 2.2 mm. Cu, 250 kvp and 10 ma. Animals

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Fromm Laboratories, Grafton, Wis. and Pitman-Moore Co., Indianapolis, Ind.

Genrously supplied by Dr. H. L. Taylor, Immunochemistry Department, Pitman-Moore Co., Indianapolis, Ind.
were turned after each 100 r, so as to expose both sides to the same amount of radiation. Four dogs were irradiated by this method. Dogs 65 and 54 received 600 r as a single dose. Dog 82 received 800 r as a single dose, and dog 83 received 800 r in divided doses of 400 r each at a 24 hour interval. Method 2.—Dogs in a plywood cage with no metal parts were placed between two Co60 sources. No anesthesia was used. Target distances were 2.0 and 2.3 meters. Three 1/4 inch lead plates were used as filters for each source. Dose rate from each was 0.9 r/min. in air at midline at subject; total air dose, 1.8 r/min.

Four dogs were irradiated by this method. Dog 95 received 1000 r as a continuous dose; dog 94, 1200 r; dog 128, 1300 r; and dog 111, 1500 r.

Autologous bone marrow was obtained immediately prior to irradiation. Under anesthesia an incision was made over the lateral cortex of the femur. A rectangular window was cut in the cortex approximately 2.5 by 0.5 cm. The exposed marrow was aspirated through a cannula into a suction flask. The aspirated marrow was processed immediately through screened syringes as described by Thomas, and stored, still suspended in heparinized TC-199, at 4 C. until ready for use. Total nucleated cell counts were made immediately after processing. Marrow yield in this series ranged from 1.4 to 3.7 billion nucleated cells.

The marrow suspensions were warmed and given back to the dogs by the intravenous route as soon as irradiation was completed. Popliteal or iliac lymph node biopsies were performed on dogs 54, 65, 94, 95 and 111 approximately 10, 20, 30, 60 and 90 days after irradiation.

Results

All dogs survived irradiation for 39 days or more. This was in contrast to control dogs in this laboratory which showed a 100 per cent 15 day mortality after an air dose of 600 r total-body irradiation.

Clinical Course

In this series no correlation could be made between an animal’s clinical course and the dose of radiation administered. Dogs 65 and 111 received 600 r and 1500 r, respectively, and made the most benign recoveries. Similarly there was no apparent correlation of clinical behavior with the quantity of marrow injected. A summary of radiation doses, marrow doses and length of follow-up at time of present writing is presented in table 1.

The immediate clinical response to irradiation was mild. Dogs 2 and 3 received 800 r of 250 kvp x-ray and had mild anorexia for 24 to 48 hours after irradiation. There was no instance of the gastrointestinal syndrome described by Conard et al. Rectal temperature customarily remained in the normal range until the sixth and seventh day after irradiation. It then was invariably elevated a degree or more for a variable period. Weight began to fall at this time, and all except dog 65 developed respiratory infections, usually of a mixed bacterial type that required antibiotic therapy for two to four weeks. All except dogs 65 and 111 received parenteral fluid therapy during this period. Dogs 82, 83 and 94 received one or more injections of hyperimmune dog serum and dog gamma globulin while infection was clinically manifest. Dog 95 received fresh blood transfusions from an unrelated donor because of petechial hemorrhages in the gums. By the twentieth day after irradiation all dogs had improved clinically. Temperatures returned to normal levels; the dogs appeared well and active and began to gain weight. Dogs 65, 83, 95, 111 and 128 have continued to appear well since that time.
Table 1

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Age (mo.)</th>
<th>Radiation dose (r)</th>
<th>Marrow dose (in billions)</th>
<th>Result</th>
<th>Survival to date</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>65</td>
<td>18</td>
<td>600 r 250 kv</td>
<td>1.9</td>
<td>Survival</td>
<td>10 months</td>
<td>None</td>
</tr>
<tr>
<td>54</td>
<td>5</td>
<td>600 r 250 kv</td>
<td>1.4</td>
<td>Survival</td>
<td>7 months</td>
<td>None</td>
</tr>
<tr>
<td>82</td>
<td>7</td>
<td>800 r 250 kv</td>
<td>3.2</td>
<td>Died</td>
<td>30th day</td>
<td>Pancreatic insufficiency and convulsions</td>
</tr>
<tr>
<td>83</td>
<td>7</td>
<td>800 r 250 kv*</td>
<td>2.5</td>
<td>Survival</td>
<td>8 months</td>
<td>None</td>
</tr>
<tr>
<td>96</td>
<td>8</td>
<td>1000 r Co(^{60})</td>
<td>3.7</td>
<td>Survival</td>
<td>7 months</td>
<td>None</td>
</tr>
<tr>
<td>94</td>
<td>8</td>
<td>1200 r Co(^{60})</td>
<td>3.4</td>
<td>Died</td>
<td>54th day</td>
<td>Intestinal obstruction</td>
</tr>
<tr>
<td>111</td>
<td>6</td>
<td>1500 r Co(^{60})</td>
<td>1.9</td>
<td>Survival</td>
<td>6 months</td>
<td>None</td>
</tr>
<tr>
<td>128</td>
<td>9</td>
<td>1500 r Co(^{60})</td>
<td>2.4</td>
<td>Survival</td>
<td>5 months</td>
<td>None</td>
</tr>
</tbody>
</table>

*Given in divided doses of 400 r at 24 hr. interval.

Dog 82 developed an upper respiratory infection on the twenty-ninth day post-irradiation, followed by bacterial pneumonitis and death from convulsion on the thirty-ninth day.

Dog 94 developed intestinal obstruction on the eighty-third day from an adhesion formed at a previous laparotomy and died on the eighty-fourth day.

Six weeks after irradiation dog 54 developed a syndrome of weight loss, voracious appetite and copious foul-smelling stools containing grossly undigested food. A tentative diagnosis of pancreatic insufficiency was made, and the animal was placed on oral pancreatic enzyme. *Gastrointestinal function returned to normal, and the dog regained weight. After three weeks, treatment was discontinued and the syndrome recurred. Therapy was resumed and is being continued at the present time. The other five surviving dogs have given no evidence of this or any other illness.

The clinical behavior of this group of dogs is strikingly different from that of a group of similarly irradiated dogs receiving homologous marrow, reported previously from this laboratory. *Whereas 5 of the 8 dogs in the autologous series have remained entirely well, dogs in the homologous series have lost weight, been intermittently febrile, and have usually died of viral, bacterial or parasitic disease before 100 days postirradiation.

**Hematology**

Figure 1a–c shows the range of hematologic parameters in the postirradiation period. White blood counts fell rapidly after radiation, reaching a nadir at 6 or 7 days and then rose gradually, beginning at about the tenth day. Normal levels were usually attained between the twentieth and twenty-fifth days. During the early part of the recovery period, differential counts showed a preponderance of polymorphonuclear leukocytes with many young forms and the frequent appearance of nucleated red blood cells. Lymphocytes usually did not appear in significant numbers until nearly the thirtieth day. Differentials returned to normal between 30 and 40 days.

Platelet counts in this series diminished at about the same rate as the white blood count, reaching their lowest levels between 7 and 10 days. Recovery

*Panretic Granules, Parke, Davis and Company, Detroit, Mich.
was more gradual, however; normal levels were reached about the thirty-fifth day.

Reticulocyte counts fell from normal levels to zero by the seventh day, and an elevation was usually not apparent until the fourteenth or fifteenth day. Counts remained elevated, usually, until about the thirtieth day, a time roughly corresponding to the return of hemoglobin values to normal.

Dog 82, that expired on the thirty-ninth day, had demonstrated the expected return of all hematologic parameters to normal except for an es-
Marrow Autografts After Lethal Radiation

Fig. 1(c).—Serial reticulocyte counts and hemoglobin determinations on the same 6 dogs.

Fig. 2.—Serial total lymphocyte counts in the peripheral blood of 3 dogs (54, 65, 95) of the present autologous series compared with similar counts on 3 dogs, which survived for 30 days or longer, in the previously reported homologous series.

Sessential absence of lymphocytes from the differential white blood count. A similar failure of lymphocyte counts to return to normal was observed in the series of homologous marrow grafted dogs. Figure 2 compares the range of total lymphocyte counts in the postirradiation period of dogs 54, 65 and 95 of this series with similar counts made in homologous dogs surviving for more than 30 days.

Histologic Studies of Lymph Nodes

The lymph nodes excised from the dogs that received infusions of their own marrow were compared histologically with the nodes removed at postmortem
Fig. 3.—(a) Lymph node from a dog 9 days after supralethal irradiation and homologous marrow. The supporting framework is shown. There are isolated lymphocytes but neither lymph follicles nor aggregates of lymphocytes. (24×) (b) Higher magnification of the lymph node shown in (a). (130×) (c) Lymph node taken from dog 95, 10 days after supralethal irradiation and autologous marrow. Cortical portions of the node contain many immature and mature lymphocytes grouped in foci suggestive of a follicular pattern. (24×) (d) Higher magnification of (c) showing two foci of lymphocytes. (130×)

from previously reported dogs that received similar amounts of radiation and homologous marrow. Comparisons were made between nodes removed at similar time intervals following irradiation. If there was histologic evidence of local infection, the node was excluded from the comparative series.

In the homologous series the nodes from dog 2 showed no lymph follicles and no aggregates of lymphocytes 9 days after irradiation (fig. 3 a,b). Isolated lymphocytes were present. Sinusoids contained many red cells, and the cytoplasm of the lining reticuloendothelial cells was filled with phagocytized red
Figure 4.—(a) Lymph node removed from a dog 29 days after supralethal irradiation and homologous marrow. There are scattered lymphocytes in the trabeculae but no lymphoid follicles. (24×) (b) Higher magnification of the lymph node shown in (a). (130×) (c) Lymph node from dog 95, 20 days after supralethal irradiation and autologous marrow. There is marked restitution of lymphoid tissue with some germinal centers of normal appearance. (24×) (d) Higher magnification of (c) showing a germinal center. (130×)

cells. In the autologous series a lymph node removed at 10 days from dog 95 showed similar changes in the sinusoids plus large numbers of plasma cells in the connective tissue septa (fig. 3 c,d). The cortical portions of the node, however, contained many immature and mature lymphocytes which were grouped in foci suggestive of a follicular pattern.

In the homologous series the nodes of dog 21 at 29 days showed only a few scattered lymphocytes and no lymphoid follicles (fig. 4 a,b). In the autologous series a node from dog 95 at 20 days showed a marked restitution
MANNICK, LOCHTE, JR., ASHLEY, THOMAS AND FERREBEE

Fig. 5.—(a) Lymph node from a dog 105 days after supralethal irradiation and homologous marrow. There are numerous collections of mature lymphocytes which have the size and shape of follicles but do not have germinal centers. (24×) (b) Higher magnification of the lymph node shown in (a). (130×) (c) Lymph node from dog 54, 84 days after supralethal irradiation and autologous marrow. There are numerous well defined follicles containing normal appearing germinal centers. (24×) (d) Higher magnification of the lymph node shown in (c). (130×)

of lymphoid tissue with some germinal centers of normal appearance (fig. 4 c,d). A similar amount of hemosiderin was seen in the phagocytic cells of nodes from both dogs.

The nodes of one exceptional dog in the homologous series showed nearly complete restoration to normal at the time of the animal's death, 57 days after irradiation. A node from dog 65, in the present autologous series, at 63 days following irradiation showed a comparable degree of lymphoid recovery.

The longest survivor among the dogs in the homologous group lived for 105 days. Nodes removed from this animal at autopsy (fig. 5 a,b) showed
numerous collections of mature lymphocytes that had the size and shape of lymphoid follicles but did not have germinal centers. The sinusoids contained increased numbers of phagocytic cells laden with cellular debris and hemosiderin. In contrast a node removed from dog 54 in the autologous group at 84 days (fig. 5 c,d) showed essentially normal histologic appearance with numerous, well defined follicles containing germinal centers of normal appearance.

The nodes from the two groups of dogs reported above may not be precisely comparable since the nodes from the dogs receiving autologous marrow were obtained by biopsy, whereas those from the dogs receiving homologous marrow were removed at autopsy. However, the histologic evidence demonstrates a rapid recovery of lymphoid tissue after supralethal irradiation in dogs that received infusions of autologous marrow. In the series of dogs that received homologous marrow, restoration of lymph node structure was greatly delayed despite the fact that the animals in the homologous group showed clinical and hematologic evidence of repopulation of bone marrow.

Electrophoretic Pattern

The serum electrophoretic patterns showed a slight to moderate diminution of the gamma globulin fraction by the fifteenth day after irradiation that persisted through the thirtieth day. Patterns similar to preirradiation controls were usually obtained by the sixtieth day. Dog 65 has continued to show an unexplained increase in the beta-1 globulin. One dog grafted with homologous marrow showed essentially no gamma globulin at 90 days.

Discussion

The results of the present experiment confirm in the dog the generally accepted thesis that a graft of autologous marrow produces a less stormy and more certain recovery from supralethal irradiation than does a similar graft of homologous marrow. The superiority of autologous marrow appears related to the fact that autologous grafts repopulate lymphoid tissue as well as bone marrow and promote recovery of normal immunologic activity as well as marrow function. The dogs with autologous grafts survived exposure to endemic disease. The dogs given homologous marrow did not. The dogs treated with autologous marrow showed a return of normal quantities of circulating lymphocytes. The dogs treated with homologous marrow showed a return to normal of all hematologic measurements other than the lymphocyte count. The lymph nodes of the dogs in the autologous series showed a rapid return of normal histologic appearance; the nodes in the homologous series failed to show comparable recovery. The gamma globulin patterns on serum electrophoresis returned to normal after irradiation in the autologous series; in the homologous series the gamma globulins remained diminished.

In discussing the results of homologous marrow transplants in dogs in a previous report from this laboratory, it was suggested that the failure to restore lymphoid structures and resistance to disease might be the result of (A), too much x-ray or (B), a “foreign marrow reaction” or (C), that in the dog adult marrow might be a poor source of cells of the type needed to re-
MANNICK, LOCHTE, JR., ASHLEY, THOMAS AND FERREBEE

populate lymph nodes and splenic follicles. The normal restoration of lymphoid tissue observed in the present series of autologous marrow transplants disposes of possibilities (A) and (C) and emphasizes "foreign marrow reaction" as the cause of the abnormalities observed in the lymphoid tissue of the dogs receiving homologous marrow.

It is possible that the lymph node recovery observed in the autologous series represented regrowth of cells that were not destroyed at the time of irradiation. This possibility seems unlikely. Equal recoveries were observed irrespective of radiation dose in the range 800 to 1500 r. It appears more likely that recovery was due to the repopulation of lymph nodes by cells infused with the marrow. This restoration excludes the possibility that failure after homologous marrow might be caused by a direct radiation effect upon the structural framework of the node. In mice a higher dose of marrow cells is required for successful transplants of homologous marrow than is required for autologous transplants. A similar dose effect may be involved in the repopulation of dog lymph nodes. However, a large body of evidence suggests that the deficiencies observed in the nodes are the consequences of a "foreign marrow reaction". Immunologically effective lymphoid cells present in the donor's marrow "home" to the lymphoid structures of the host and there react with host tissue antigens. This reaction of the graft against the host impairs recovery of normal population and structure.

In the observations presented the implications for the treatment of radiation injury in man are obvious. Transplants of autologous marrow have been shown to be effective treatment of supralethal radiation exposures in the dog. Transplants of isologous marrow have been found to be effective treatment for exposures of 800 to 1100 r in man. Satisfactory methods for the collection and preservation of human bone marrow are available. Consideration might therefore be given the establishment of autologous marrow banks for personnel potentially exposed to radiation and for patients about to receive consequential radiation or chemotherapy.

SUMMARY

1. Four beagles were exposed to supralethal total-body irradiation from a 250 kv x-ray therapy machine. Four were exposed to similar irradiation from a Cobalt-60 teletherapy unit. Dosages administered ranged from 600 to 1500 r, calculated as air dose at theoretical midbody line.

2. After irradiation each dog was given an intravenous infusion of 1.4 to 3.7 billion cells of his own bone marrow. This sample of autologous marrow was aspirated through a surgical window in the femur immediately prior to the animal's irradiation and was stored in plasma and tissue culture fluid at 4 C. while awaiting use.

3. All dogs survived the acute radiation injury. There were two late deaths, one from intercurrent infection and one from small bowel obstruction.

4. The six surviving dogs are in apparent good health 5 months to 10 months after irradiation. Control dogs in this laboratory have a 100 per cent 15 day mortality after air dose of 600 r total-body irradiation.

5. A comparison with a previous series of dogs grafted with homologous marrow...
marrow shows that autologous marrow induces a recovery after lethal irradiation that is superior to the recovery induced by homologous marrow.

6. The superior results observed after autologous marrow appear attributable to a rapid postirradiation recovery of lymphoid tissue as well as bone marrow. Circulating lymphocytes reappear, lymph nodes regain normal histologic appearance, and the serum gamma globulin pattern returns to normal. These latter events do not occur promptly after infusions of homologous marrow.

SUMMARIO IN INTERLINGUA

1. Quatro braccos anglese del raccia beagle esseva exponite a doses supraletal de irradiation del corpore total ab un machina de roentgenotherapia de 250 kv. Quatro alteres esseva exponite a doses supraletal de irradiation del corpore total ab un machina de teletherapia a cobalt-60. Le dosages administrate variava inter 600 e 1500 r, calculate ccomo dose in aere al linea centricorporee theoretic.

2. Post le irradiation le canes recipeva individualmente infusiones de 1,4 a 3,7 milliardos cellulas de lor proprie medulla ossee. Iste quantitate de medulla autologe habeva essite aspirate via un fenestra chirurgic in le femore immediatamente ante le irradiation e esseva preservate in liquido cultural de plasma e tissu a 4 C usque al tempore de su uso.

3. Omne le canes superviveva le acute injuria de irradiation. Occurreva duo mortes tardive, le un in consequentia de infection intercurrente, le altere in consequentia de obstruction del intestino tenue.

4. Le sex supervivente canes se trova in apparentemente bon sanitate 5 a 10 menses post le irradiation. Canes de controlo in iste laboratorio ha tin mortalitate de 100 pro cento 15 dies post un irradiation del corpore total in un dose aeree de 600 r.

5. Le comparation con un previe serie de canes graffate con medulla homologe monstra que medulla autologe induce un restablimento post doses letal de irradiation que es superior al restablimento inducite per medulla homologe.

6. Le resultatos superior observate post le administration de medulla autologe pare esser attribuibile a un rapide restablimento post-irradiational de tissu lymphoide si ben como de medulla ossee. Lymphocytos reappare in le circulation, le nodos lymphatic regania lor normal apparentia histologic, e le globulina gamma del sero retorna a configurationes normal. Iste evenimentos non superveni promptemente post infusiones de medulla homologe.

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