What Radiation Dose for DLA-Identical Canine Marrow Grafts?

By Rainer Storb, Robert F. Raff, Frederick R. Appelbaum, Friedrich W. Schuening, Brenda M. Sandmaier, Theodore C. Graham, and E. Donnall Thomas

In view of reported attempts at marrow grafting after nuclear accidents with a broad range of radiation exposures, the present study explored the total-body irradiation (TBI) conditions needed for engraftment in a canine model by using marrow from DLA-identical littermates. Previous studies have shown that such grafts are consistently successful when recipients are exposed to 920 cGy of TBI delivered at a rate of 7 cGy/min from opposing dual cobalt sources. The present TBI doses were all in the lethal range. Five dogs were administered 450 cGy; seven dogs, 600 cGy; five dogs, 700 cGy; and five dogs, 800 cGy of TBI administered at 7 cGy/min. They received a median of 3.3 x 10^8 marrow cells/kg intravenously after completion of radiation. Results showed transient allogeneic marrow engraftment in all dogs administered the lowest dose of TBI studied (450 cGy). Importantly, transient grafts permitted four of five dogs to live long enough for autologous marrow recovery to occur. At increasing radiation doses, 600, 700, and 800 cGy, the risk of graft failure lessened, with 3 of 7, 2 of 5, and 1 of 5 dogs, respectively, showing graft rejection. Fewer dogs survived with autologous marrow recovery, and more showed sustained allogeneic engraftment (4 of 7, 3 of 5, and 4 of 5 dogs, respectively). We conclude that DLA-identical littermate marrow grafts are beneficial in the setting of otherwise lethal radiation exposures, with most dogs either experiencing sustained allogeneic engraftment or surviving with autologous marrow recovery due to the extended support provided by a transient allogeneic graft.

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Marrow grafts from histocompatible canine littermates are consistently successful when recipients are conditioned with 920 cGy of total-body irradiation (TBI) delivered at a rate of 7 cGy/min from two opposing 60Co sources.1 Previous1 and recent, as yet unpublished, studies from this laboratory have shown that exposure to 400 to 450 cGy of TBI delivered at 7 cGy/min without subsequent marrow infusion is uniformly lethal (none of 21 dogs survived), and most dogs (nine of 16) died following 300 cGy of TBI. When conditioned with 200 cGy all dogs survived with hematologic recovery (seven of seven). Grafts of histoincompatible unrelated marrow following 400 to 600 R (300 to 450 cGy) of TBI were uniformly unsuccessful (15 of 15 dogs), and there was no improvement in survival or evidence of harm.2 Dogs given autologous marrow grafts, as a rule, survived TBI exposures from 400 to as high as 1,400 cGy delivered at 7 cGy/min.2,4

In view of reported attempts at marrow transplants after nuclear accidents with a broad range of radiation exposures, the present study explored the radiation conditions needed for engraftment of marrow from littermates genetically identical for major histocompatibility complex antigens (DLA) in a canine model. The TBI exposures explored were 450, 600, 700, and 800 cGy. We found that genotypically DLA-identical marrow grafts are useful after these lower lethal TBI exposures, since they either engraft transiently, thereby allowing time for autologous marrow recovery, or they engraft permanently, with resulting improvement in survival. There was no evidence that DLA-identical grafts were harmful as described in other species.5,7

MATERIALS AND METHODS

Litters of beagles, harriers, and mongrels were either bred at the Fred Hutchinson Cancer Research Center or purchased from commercial kennels in the states of Washington, Oregon, and Michigan. The dogs weighed from 6.7 to 15.9 (median, 9.7) kg and were 6/5 to 30 (median 8) months in age. They were observed for disease for 2 months before marrow transplantation. All were immunized for hepatitis, leptospirosis, distemper, and parvovirus. Research was carried out according to the principles enunciated in the Guide for Laboratory Animal Facilities and Care, prepared by the National Academy of Sciences, National Research Council. The research protocol was given approval by the Institutional Animal Care and Use Committee of the Fred Hutchinson Cancer Research Center.

Twenty-two littermate donor-recipient pairs were chosen on the basis of identity for the serologically detectable canine histocompatibility antigens DLA-A and -B and mutual nonreactivity of their peripheral blood mononuclear cells in mixed leukocyte culture.6,9 Histocompatibility typing included family studies. Donor-recipient pairs were of the opposite sex in all but two cases. Marrow for transplantation was obtained by needle aspirations from femora and humeri, as described.10 Recipients were administered a single dose of TBI at a rate of 7 cGy/min from two opposing 60Co sources as described in detail previously.12 They were administered marrow, 0.6 to 4.0 (median 3.3) x 10^8 cells/kg intravenously, within four hours of TBI. Dogs were not administered postgrafting immunosuppression. The postgrafting care has been described.16 In addition, all dogs were administered oral antibiotics (polymyxin B sulfate and neomycin sulfate) three times daily beginning on day -5 through the day at which the granulocyte count reached 500/μL postgrafting. The day of TBI and marrow transplantation was designated as “day 0.”

Four groups of recipients were studied. Five dogs in group 1 were administered 450 cGy, 7 dogs in group 2 received 600 cGy, 5 dogs in group 3 received 700 cGy, and 5 dogs in group 4 received 800 cGy of TBI.

Marrow engraftment was assessed by rises in granulocyte and...
platelet counts following the postirradiation decline, histologic features of the marrow from biopsy or autopsy specimens, the documentation of cells with donor karyotype in specimens from peripheral blood and marrow, and the development of graft-v-host disease (GVHD). Graft rejection was defined as the failure of sustained recoveries of granulocyte or platelet counts after the postirradiation nadir, reappearance of cells with host karyotype, extreme marrow hypocellularity at autopsy, and the absence of clinical and histologic features of GVHD.

RESULTS

Tables 1 and 2 summarize the data, and Figs 1 through 3 illustrate the peripheral blood count changes.

All five dogs in group 1, which were administered 450 cGy TBI, showed promptly rising WBC and granulocyte counts following the postirradiation nadir, and four of the five showed moderate rises in platelet counts (Figs 1 through 3). This most likely represents engraftment of marrow from the DLA-identical littermate since dogs administered 450 cGy TBI without marrow infusion failed to show rises in peripheral blood cell counts and die of marrow aplasia within 12 days (Fig 1). Also, dogs administered 450 cGy and histoincompatible unrelated marrow show only a brief rise in WBC counts by day 10 but then go on to die of marrow aplasia within 13 days (Fig 1). Peripheral blood cell counts in the five dogs in group 1 of this study then declined again to very low levels between 13 and 25 days (Figs 1 through 3). Two of the five died of infection (Table 1); one showed beginning hematopoietic recovery in the marrow. Three dogs showed complete hematopoietic recovery and survived (Table 1). Cyto genetic studies were not done in two dogs because they were sex matched with their donors, and in one dog no evaluable karyotype was obtained. In one dog, mixtures of donor and host karyotypes were seen on days 13, 21, and 27, but only host type cells on day 90 (Table 1). One dog showed only cells with host karyotype on days 26 and 138. The hemato logic changes seen in these dogs are consistent with transient allogeneic engraftment followed by autologous recovery in four cases.

All seven dogs in group 2 administered 600 cGy TBI and showed promptly rising granulocyte counts after the postirradiation nadir and, to a lesser extent, rising platelet counts (Figs 2 and 3). In all, cytogenetic studies showed either mixtures of donor and host or only donor cells within the first 3 weeks (Table 1). Two of the five died with sustained grafts matched with their donors, and in one dog no evaluable karyotype was obtained. In one dog, mixtures of donor and host karyotypes were seen on days 13, 21, and 27, but only host type cells on day 90 (Table 1). One dog showed only cells with host karyotype on days 26 and 138. The hemato logic changes seen in these dogs are consistent with transient allogeneic engraftment followed by autologous recovery in four cases.

<table>
<thead>
<tr>
<th>Table 1. Marrow Grafts From DLA-Identical Littermates After Otherwise Lethal Single Doses of TBI Delivered at 7 cGy/min</th>
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<td><strong>Group</strong></td>
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<td>---------------------------------------------------------------</td>
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<tr>
<td>1 (450 cGy)</td>
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<td>C223</td>
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<td>C381</td>
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<td>C431</td>
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<td>2 (600 cGy)</td>
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<td>C430</td>
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<td>C478</td>
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<td>3 (700 cGy)</td>
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<td>C489</td>
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<td>4 (800 cGy)</td>
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*Following the postirradiation nadir.  
†NE: not evaluable, although the remaining marrow is most likely of autologous origin — see dog C551.  
‡Sod Pent, dog killed with sodium pentobarbital injection at the completion of study.  
§On the average, 37 metaphases were analyzed per dog; only spreads with 78 chromosomes were evaluated: H, host origin; D, donor origin; D/H, mixed chimerism, donor cells predominante; H/D, mixed chimerism, host cells predominante; ND, not done; NE, not evaluable.  
Eleven of 24 marrow cells analyzed on day 80 were of the host karyotype.  
Thirteen of 16 cells analyzed on day 47 were of host karyotype; the allogeneic graft seems to undergo late reversal to the host type.
from GVHD-associated complications. Two dogs showed continued allogeneic engraftment, although by days 47 and 80, respectively, increasing numbers of cells of host karyotype were seen, which suggests that dogs were undergoing late graft rejection along with autologous recovery. Three dogs showed declining peripheral blood cell counts after the initial rise; two of these died of infection, and one became a long-term survivor with complete hematologic recovery. Only cells with host karyotype were seen in two of these three dogs after the third week. Thus, early rejection was seen in three dogs, sustained engraftment in two, and persisting mixed chimerism in two, most likely heralding late rejection (Table 2).

Three of five dogs in group 3, which were administered 700 cGy of TBI, showed prompt sustained hematopoietic engraftment (Figs 2 and 3, Tables 1 and 2). Following an initial mixed chimerism, all hematopoietic cells tested had the donor karyotype. These three dogs were in good health and were killed by sodium pentobarbital infusion after completion of the study. Two dogs had transient rises in granulocyte counts but then developed marrow aplasia and died of infection on days 15 and 17.

Four of five dogs in group 4, which were administered 800 cGy of TBI showed evidence of sustained allogeneic engraftment (Figs 2 and 3, Tables 1 and 2). Following initial evidence of mixed chimerism in two of the four, all four had only cells with donor karyotype beyond dog 40. The dogs were in good health and were killed by sodium pentobarbital infusion at the completion of the study. One dog never showed evidence of allogeneic engraftment and died of infection with evidence of early autologous marrow recovery.

For comparison, Table 2 summarizes previously published results in 21 allografted dogs conditioned with 920 cGy TBI at 7 cGy/min.1 Twenty of the 21 had sustained allogeneic engraftment, and one failed to show engraftment.

**DISCUSSION**

The current study examined the radiation doses needed for consistent engraftment of DLA-identical littermate marrow by using marrow cell numbers comparable to those used in human marrow grafting. The study was stimulated by questions regarding the advisability of marrow infusions following otherwise lethal exposures to radiation in nuclear accidents. Previous studies had shown that grafts of histoincompatible unrelated marrow are uniformly unsuccessful following doses of TBI up to 920 cGy, with consistent engraftment possible only after 1,800 cGy.1,2,12

In contrast to the results with unrelated grafts, the current study shows that grafts of genotypically DLA-identical littermate marrow can be useful in extending the survival of dogs exposed to low but lethal TBI. Results suggest that genotypically HLA-identical marrow grafts should be considered a treatment option for human nuclear accident victims exposed to unknown doses of radiation. It must be remembered, however, that current results have been obtained with γ-radiation with fission neutrons thought to have contributed between one quarter to one third of the total exposure.13 Fission neutrons are known to have greater radiobiologic effectiveness than either γ- or x-radiation.14 Nevertheless, within that limitation, the current results have clinical relevance. Transient allogeneic engraftment was seen even after the lowest dose of TBI studied (450 cGy), and...
importantly, transient engraftment permitted a number of dogs to live long enough for autologous hematopoietic recovery to occur. At increasing radiation doses, 600, 700, and 800 cGy, the risk of graft failure lessened, and more dogs survived with allogeneic marrow engraftment. Graft rejections seen at high radiation exposures generally did not lead to autologous recovery, presumably because stem cell damage increases with increasing radiation doses. Nevertheless, it would seem that DLA-identical littermate marrow grafts are beneficial in the setting of otherwise lethal radiation exposures, with most dogs either experiencing allogeneic marrow engraftment or surviving with autologous marrow recovery due to the extended support provided by the transient allogeneic graft.

The rejection of the allograft seen in a number of the dogs in this study is most likely the result of inadequate suppression of host T-cell function through lower exposure of TBI. Previous studies in DLA-identical littersmates conditioned with otherwise lethal doses of the alkylating agent dimethyl-busulfan have shown that inadequate immunosuppression can be improved and “permanent” marrow grafts established through additional treatment with antithymocyte serum.11

Few other studies have investigated the feasibility of marrow grafts from histocompatible littermates following low but otherwise lethal radiation exposures. Bradstock et al12 irradiated recipients with a 6-MeV linear accelerator at 55 cGy/min and found that two of four dogs administered 500 cGy had sustained allogeneic engraftment while two rejected and survived with autologous marrow recovery. At 750 cGy seven of eight dogs showed sustained engraftment, and at 900 cGy all recipients died of gastrointestinal toxicity. Using fission neutron radiation, Monroy et al16 achieved successful engraftment of serologically matched littermate marrow in two of seven dogs administered 255 cGy delivered at 32 cGy/min. These two died of gastrointestinal hemorrhage and infection. Four dogs did not show engraftment but survived with autologous recovery. This compares with one of five survivors among dogs administered 255 cGy TBI and no marrow and four of seven among dogs administered autologous marrow infusion. Their attempts to increase the rate of allogeneic engraftment by raising the neutron dose to 330 cGy or by adding 400 to 600 cGy of γ-radiation failed largely because of early mortality. Vriesendorp et al17 delivered TBI from opposing x-ray machines at a rate of 17 cGy/min. They reported uniform sustained engraftment of DLA-identical littermate marrow after a TBI dose as low as 500 cGy. In their hands, two dogs administered 300 cGy TBI and one of four administered 400 cGy without marrow survived, while five dogs administered 500 cGy and no marrow died within 3 weeks without marrow recovery. All six dogs administered 400 cGy and allogeneic marrow were reported to show autologous marrow recovery. Two of these reports do not provide information of blood genetic marker studies to determine allogeneic engraftment.15,16 although the last study mentions that extensive marker studies were done.17

Differences in results between the current study and the three reports by others may be related in part to differences in the quality of radiation (γ v fission neutron/γ v x-ray) and in dose rates used (7 v 17 v 35 v 55 cGy/min). It has already been established that the toxicity of single-dose TBI increases in direct relationship to the dose rate.4,18 In part, the lack of blood genetic marker studies in some of the reports may have masked late reversals to host type, as suggested in two of the present dogs conditioned with 600 cGy. Also, one study16 relied only on serologic histocompatibility matching and another4 only on mixed leukocyte culture to select donor and recipient pairs, which may raise questions regarding genotypic DLA identity.

Nevertheless, despite some differences in experimental design and results, the data of the present study taken together with those previously reported15-17 suggest that grafts of marrow genotypically matched for the antigens of the major histocompatibility complex follow a known but presumably lethal accidental exposure to radiation may be useful, since they appear to increase survival either by permitting autologous marrow recovery through extended support or through “permanent” allogeneic engraftment.

Studies in mice and monkeys administered lower lethal radiation exposures demonstrated an increase in mortality when they were treated by infusion of autologous hematopoietic cells thought to be due to a graft-v-host reaction.5,7 An increase in mortality was not seen in dogs administered 400 R (300 cGy) followed by grafts of DLA-nonidentical marrow.7 This finding along with the autologous recovery seen by us and others15-17 in recipients of DLA-identical marrow that were administered otherwise lethal doses of radiation would argue against a harmful effect of marrow infusion. The death from GVHD-associated complications seen in two of the dogs in this study that were administered 600 cGy TBI could have been avoided if postgrafting immunosuppression had been used.10 In fact, in human patients administered HLA-identical marrow grafts following presumed lethal TBI, some form of postgrafting immunosuppression would seem mandatory.19,20

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