Fractionated Versus Single-Dose Total Body Irradiation at Low and High Dose Rates to Condition Canine Littermates for DLA-Identical Marrow Grafts

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We explored in dogs the immunosuppressive properties of 450 cGy total body irradiation (TBI) delivered from two opposing ^{60}Co sources, as assessed by the criterion of successful engraftment of allogeneic genotypically DLA-identical littermate marrow. Two questions were asked in this study. Firstly, does dose rate affect the immunosuppressive effect of TBI when administered in a single dose? Secondly, does fractionation alter the immunosuppression of TBI when delivered at a very fast dose rate? Dose rates studied included 7 and 70 cGy/min, and fractionation involved four fractions of 112.5 cGy each, with 6-hour minimum interfraction intervals. Six of 7 dogs receiving 450 cGy single-dose TBI at 70 cGy/min showed sustained engraftment of the allogeneic marrow, compared with 1 of 7 dogs receiving single-dose TBI at 7 cGy/min ($P = .01$). Fractionated TBI at 70 cGy/min resulted in sustained allogeneic engraftment in 3 of 10 dogs, a result that was statistically significantly worse than that with single-dose TBI at 70 cGy/min ($P = .03$) and not statistically different ($P = .24$) from that with fractionated TBI delivered at 7 cGy/min (0 of 5 dogs engrafted). A single dose of 450 cGy of TBI delivered at a rate of 70 cGy/min is significantly more immunosuppressive than the same total dose delivered at 7 cGy/min. Fractionated TBI at 70 cGy/min is significantly less immunosuppressive than single-dose TBI at 70 cGy/min and not significantly different from fractionated TBI administered at 7 cGy/min. Results are consistent with the notion that significant DNA repair in lymphoid cells is possible during interfraction intervals at the relatively high dose rate of 70 cGy/min.

TOTAL BODY IRRADIATION (TBI) has been widely used to condition patients with hematologic malignancy for allogeneic marrow transplantation. The goal of TBI is to deliver the greatest marrow toxic and immunosuppressive effects with acceptable toxicity to nonhematopoietic organs. The effects of TBI are governed by at least three variables: total dose, dose rate, and dose fractionation. Numerous studies have attempted to alter the three variables in such a way that greater antileukemic and immunosuppressive effects are obtained without increasing toxicity. It was suggested that, for a given total dose of single or fractionated TBI, the effect on hematopoietic tissues would be the same, whereas sparing of nonhematopoietic tissues would be achieved by dose fractionation, provided that interfraction intervals exceeded 3 hours.[1-12] This suggestion was based on the assumption that rapidly proliferating tissues, such as marrow, have a reduced capacity for repairing sublethal DNA damage during interfraction intervals of TBI, whereas DNA repair is possible in slower growing tissues of nonhematopoietic origin. Indeed, previous studies in dogs and mice showed that long-term toxicity was significantly reduced in animals that received fractionated TBI.[13-15] Also, at least at the low dose rate of 10 cGy/min studied, fractionated and single-dose TBI had comparable marrow toxicity in dogs.[16] However, it was disappointing to see that fractionation spared the cells of the immune system, and a significantly higher proportion of dogs receiving 450, 600, 700, 800, and 920 cGy of fractionated TBI at 7 cGy/min rejected marrow grafts from DLA-identical littermates than of those receiving equivalent total doses of single-dose TBI.[17,18]

The current study re-explored the effects of single- versus fractionated-dose TBI on immunosuppression by using a 10 times higher dose rate than used in the previous study. We reasoned that TBI administered at 70 cGy/min would be more toxic to lymphocytes and, thus, more immunosuppressive than TBI administered at 7 cGy/min. Furthermore, we hoped that, because of the dose-rate–related increase in toxicity, DNA repair in lymphocytes during interfraction intervals would be impaired significantly and, thus, provide immunosuppression with fractionated TBI that was equivalent to that of single-dose TBI. A TBI dose of 450 cGy was chosen, which is inadequate for conditioning dogs for allogeneic DLA-identical transplants when delivered at a rate of 7 cGy/min. We found that almost all dogs had sustained allogeneic marrow engraftment when 450 cGy TBI was administered as a single dose at 70 cGy/min. However, fractionated TBI at 70 cGy/min was significantly less effective than single-dose TBI in conditioning dogs for allografts and could not be shown to be better than TBI administered at the low dose rate of 7 cGy/min.

MATERIALS AND METHODS

Litters of beagles, walker hounds, harriers, and pit bull-beagle crossbreeds were either raised at the Fred Hutchinson Cancer Research Center (Seattle, WA) or purchased from commercial kennels in the state of Washington. The dogs weighed from 6.4 to 17.5 (median, 9.8) kg and were between 6 and 20.5 (median, 7) months old. They were watched for disease for at least 2 months before entering the study. All were immunized for distemper, leptospirosis, hepatitis, and parvovirus. Research was performed according to the principles outlined in the Guide for Laboratory Animal Facilities and Care, prepared by the National Academy of Sciences, National Research Council. The research protocol was approved by the Insti-

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tutional Animal Care and Use Committee of the Fred Hutchinson Cancer Research Center.

Twenty-nine littermate donor/recipient pairs were chosen on the basis of identity for the serologically detectable canine histocompatibility antigens DLA A and B, and of mutual nonreactivity of their peripheral blood mononuclear cells in mixed leukocyte culture, complemented by restriction fragment-length polymorphism assays for canine major histocompatibility complex class II genes.[19-21] Data on 10 of the donor/recipient pairs, marked in Table 1, have been previously reported. They are presented here for purposes of comparison. Donor/recipient pairs were of opposite sex in all cases to permit cytogenetic evaluation of marrow and peripheral blood cells for chimerism after transplantation.

Marrow for transplantation was obtained under general anesthesia by needle aspirations from humeri and femora, as described.[22] Recipients received TBI from two opposing ^{60}Co sources, as described in detail,[13,23] with 6-hour minimum interfraction intervals. TBI was administered to unanesthetized dogs housed in a polyurethane cage placed transversely midway between two opposing ^{60}Co sources. The cage was narrow with adjustable side walls, assuring that dogs were perpendicular to the beam. Beam intensity over the entire cross-sectional area of the cage was greater than 90% of the central axis intensity. The dose rate in air at the midpoint of the cage was determined with a 0.6-cm^3 ionization chamber (Keithley Instruments, Inc, Cleveland, OH) and an electronmeter system (Keithley Instruments). The midplane tissue-absorbed dose in gray (Gy), as determined by implanted lithium fluoride thermoluminescent dosimeters, was numerically approximately 76% of the exposure in air at the center of the cage measured in roentgens. The midplane dose rates were 7 and 70 cGy/minute, respectively. The thermoluminescent dosimeters contained lithium fluoride phosphor in disks (Teflon), and were read out with a reader (Teledyne Isotopes, Westwood, NJ) calibrated over a range of 1 to 50 Gy. Details on uniformity and standardization of the opposing ^{60}Co sources have been previously published.[24]

Recipients received between 1.1 and 4.4 (median, 3.3) $\times$ 10^8 marrow cells/kg by intravenous (IV) infusion within 4 hours of the last dose of TBI. The day of marrow infusion was designated day 0. Dogs did not receive postgrafting immunosuppression. Postgrafting care has been previously described.[22] In addition, all dogs received oral antibiotics, neomycin sulfate, and polymyxin B sulfate, three times daily beginning on day -5 through the day on which the granulocyte counts reached 500/μL postgrafting.

Four groups of recipients were studied, as shown in Tables 1 and 2. All received a total dose of 450 cGy of TBI. In 7 dogs, this was administered as a single dose at a rate of 7 cGy/min. Results in 5 of the 7 have been previously reported.[17] In 5 dogs, the dose was divided into four fractions of 112.5 cGy each and administered at a rate of 7 cGy/min.[18] Seven dogs received a single dose at 70 cGy/min, and 10 dogs received four fractions of 112.5 cGy each at 70 cGy/min. Marrow engraftment was assessed by increases in granulocyte and platelet counts after the postirradiation nadir, histologic features of the marrow from biopsy or autopsy specimens, documentation of cells with donor karyotype in specimens from peripheral blood and marrow,[25] and development of graft-versus-host disease (GVHD). Graft rejection was defined as failure of sustained recovery of granulocyte or platelet counts of donor origin after the postirradiation nadir, along with extreme marrow hypocellularity at autopsy, or declining counts after initial engraftment followed by reappearance of cells with host karyotype and absence of clinical and histologic features of GVHD.

RESULTS

Significant nonhematopoietic toxicities were not seen in any of the recipients, regardless of whether TBI was delivered at 7 or 70 cGy/min. Dogs did not develop diarrhea and maintained adequate oral fluid and food intake without weight loss, so there was no need for parenteral fluid or electrolyte support.

Group 1. Dogs receiving single-dose TBI at 7 cGy/min. Tables 1 and 2 summarize the data. Two of the 7 dogs failed to show recovery of peripheral blood cell counts after the postirradiation nadir and died on day 20 from infection with aplastic marrow. Five dogs showed increasing granulocyte counts along with platelet counts. By day 19, counts began to decline and reached very low levels by day 29. One of the dogs died with pneumonia on day 30 whereas the other four showed complete recovery of their peripheral blood cell counts, as shown in Fig 1A. Evaluable cytogenetic data showed that the peripheral blood cell findings were consistent with an initial hematopoietic recovery originating from grafted donor marrow cells, which was followed by rejection, and then by recovery of host hematopoietic cells. Only one of the five dogs failed to show a second nadir in counts, and this dog's hematopoiesis was entirely of donor origin (D596).

Group 2. Dogs receiving fractionated TBI at 7 cGy/min. Tables 1 and 2 summarize the data. Two of the 5 dogs died on days 17 and 18 with pneumonia; their autopsy marrows showed absent cellularity. The allogeneic graft was presumably rejected outright. In one, cytogenetics on day 14 showed host-type cells. The three remaining dogs showed initial evidence of increasing counts, followed by a second nadir, and followed by complete hematopoietic recovery that was entirely of host origin, as evidenced by cytogenetic studies.

Group 3. Dogs receiving single-dose TBI at 70 cGy/min. Seven dogs were so treated (Tables 1 and 2). All showed prompt hematopoietic engraftment. In 6 of the 7, donor-type hematopoiesis persisted, as evidenced by cytogenetic studies of marrow and peripheral blood cells, whereas 1 dog (D263) appeared to have rejected the marrow graft, as judged by the pattern of granulocyte changes. This dog developed a pneumonia during the second granulocyte nadir and died on day 42 with a moderately cellular marrow. Granulocyte counts of dogs with engraftment are shown in Fig 1B. One of the dogs with engraftment (D257) had severe acute GVHD. This dog died on day 37 from pneumonia with cellular bone marrow.

Group 4. Dogs administered fractionated TBI at 70 cGy/min. Ten dogs were so treated (Tables 1 and 2). All showed initial evidence of increasing peripheral blood white blood cell counts. In three animals, donor-type hematopoiesis persisted without a second granulocyte nadir. In 7 dogs, a second nadir was seen that was similar to the one illustrated in Fig 1A, followed by complete hematopoietic recovery of host type in 4 and by death from infection between days 19 and 21 in 3 dogs.

Comparison of results in the various experimental groups. Table 2 summarizes the results. At the low dose rate of 7 cGy/min, 450 cGy of TBI proved virtually ineffective to condition dogs for DLA-identical marrow grafts, regardless of whether the irradiation was administered as a single dose or in fractions. With a dose rate of 70 cGy/min, 6 of 7 dogs receiving single-dose TBI showed sustained donor-type

Table 1. Marrow Grafts From DLA-Identical Littermates After 450 cGy TBI Delivered at Either 7 or 70 cGy/min as a Single Dose or in Three Fractions

Group	Dose Rate (cGy/min)	TBI Schedule	Recipient No.	Increase in WBC Count*	Sustained Allogeneic Graft	Rejection	Autologous Recovery	Survival (ds)	Cause of Death	Marrow Cellularity at Autopsy	BM + PBL Cytogenetics† (days postgrafting)
1	7	Single dose	C224‡	Yes	No	Yes	Yes	189	Sod Pent	100	ND
			C223‡	Yes	No	Yes	NE	20	Infection	5	ND
			C384‡	Yes	No	Yes	Yes	147	Sod Pent	100	D/H-13; H/D-21, 27; H-90
			C381‡	Yes	No	Yes	Yes	140	Sod Pent	100	H-26, 73, 138
			C431‡	Yes	No	Yes	Yes	30	Pneumonia	10	NE
			D591	No	No	Yes	No	20	Pneumonia	10	NE
			D596	Yes	Yes	No	No	166	Sod Pent	100	D/H-25; D-166
2	7	4 × 112.5 cGy	C569‡	Yes	No	Yes	Yes	125	Sod Pent	100	D/H-16; H/D-27; H-41, 156
			C588‡	No	No	Yes	No	17	Infection	0	H-14
			C589‡	Yes	No	Yes	No	18	Pneumonia	0	NE
			C627‡	Yes	No	Yes	Yes	79	Sod Pent	100	H-29, 40, 70
			C633‡	Yes	No	Yes	Yes	75	Sod Pent	100	H-14, 37, 59
3	70	Single dose	D255	Yes	Yes	No	No	143	Sod Pent	100	D/H-55; D-77, 143
			D256	Yes	Yes	No	No	339	Sod Pent	100	D/H-58, 76, 144; D-213, 337
			D257	Yes	Yes	No	No	37	Sod Pent	100	D/H-37
			D262	Yes	Yes	No	No	56	Sod Pent	100	D/H-40, 55
			D263	Yes	No	Yes	Yes	42	Pneumonia	15-30	NE
			D390	Yes	Yes	No	No	113	Sod Pent	100	D-103
			D427	Yes	Yes	No	No	68	Sod Pent	100	D-68
4	70	4 × 112.5 cGy	D422	Yes	Yes	No	No	223	Sod Pent	100	D-40; D/H-97; D-223
			D458	Yes	No	Yes	Yes	94	Sod Pent	100	H-48, 73
			D462	Yes	No	Yes	No	22	Septicemia	—	NE
			D465	Yes	Yes	No	No	124	Sod Pent	100	D/H-45
			D486	Yes	Yes	No	No	137	Sod Pent	100	D/H-40; D-108
			D487	Yes	No	Yes	Yes	88	Sod Pent	100	H-32
			D500	Yes	No	Yes	No	21	Pneumonia	—	NE
			D519	Yes	No	Yes	Yes	131	Sod Pent	100	H-82
			D520	Yes	No	Yes	No	19	Pneumonia	0	NE
			D535	Yes	No	Yes	Yes	111	Sod Pent	100	H-58

Abbreviations: WBC, white blood cell; H, host origin; D, donor origin; D/H, mixed chimerism, donor cells predominate; H/D, mixed chimerism, host cells predominate; BM, bone marrow; PBL, phytohemagglutinin-stimulated peripheral blood lymphocytes; NE, not evaluated; Sod Pent, dog killed with sodium pentobarbital at completion of the study.

* After the postirradiation nadir.

† On the average, 62 metaphases were analyzed per dog; only spreads with 78 chromosomes were analyzed.

‡ Data on these dogs were previously reported.[14,15]

Table 2. Engraftment and Graft Rejection in Dogs Receiving 450 cGy TBI and Marrow Grafts From DLA-Identical Littermates

Group	Dose Rate (cGy/min)	TBI Schedule	No. of Dogs: Allogeneic Engraftment Yes	No	Total*
1	7	Single dose	1	6	7
2	7	4 × 112.5 cGy	0	5	5
3	70	Single dose	6	1	7
4	70	4 × 112.5 cGy	3	7	10

* Statistical comparisons by exact computer simulation test for differences in proportions. Group 1 *v* group 2: $P = .51$. Group 1 *v* group 3: $P = .01$. Group 3 *v* group 4: $P = .03$. Group 2 *v* group 4: $P = .24$.

engraftment, a result that was significantly different ($P = .01$) from that of dogs receiving a single dose of TBI at 7 cGy/min. Single-dose TBI at 70 cGy/min also proved to be superior to fractionated TBI administered at 70 cGy/min in conditioning dogs for engraftment ($P = .03$). Although the observed engraftment in dogs receiving fractionated TBI at 70 cGy/min was better than that among dogs receiving fractionated TBI at 7 cGy/min (30% *v* 0%), the difference was not large enough to achieve statistical significance in these small samples ($P = .24$).

DISCUSSION

The current study was conducted with an otherwise lethal TBI regimen of 450 cGy, delivered at a dose rate of 70 cGy/min that was 10-fold higher than the dose rate previously used. The study was prompted by previous observations that, whereas marrow toxicity of single- versus fractionated-dose TBI was equivalent at low dose rates of 7 to 10 cGy/min, fractionated TBI was significantly less immunosuppressive when assessed by the criterion of sustained DLA-identical marrow engraftment.[17,18] These data suggested a different DNA repair capacity for cells of the lymphoid as compared with the myeloid hematopoietic systems with dose fractionation, at least when delivered at low dose rates. The current study was based on the expectation that TBI delivered at 70 cGy/min would be more toxic than at 7 cGy/min and that, in direct relation to the dose-rate–related increase in toxicity, DNA repair in interfraction intervals would be slowed to a point that fractionated- and single-dose TBI at 70 cGy/min produced equivalent immunosuppression.

These expectations were only met in part. Single-dose TBI at 70 cGy/min was significantly more immunosuppressive than TBI administered at 7 cGy/min, a finding that is consistent with previous data in allografted and xenografted mice.[26] However, even at the high dose rate of 70 cGy/min, current results do not agree with the concept that sublethal repair of lymphoid cells is minimal after fractionated TBI, and they show that the effects on the lymphoid system of single versus fractionated TBI are not equivalent, even when the radiation is delivered at a relatively high dose rate. Our previous studies at the low dose rate of 7 cGy/min showed that only at 920 cGy total dose of fractionated TBI did virtually all dogs engraft.[18]

Very little data exist in the literature in regard to immunosuppressive effects of fractionated- versus single-dose TBI. In agreement with our data, one group of investigators studying DLA-haploidentical littermate canine marrow grafts described greater efficiency when 1,350 cGy total dose was administered in three fractions over a regimen involving 15 fractions.[27] The dose rate in that study was 4.2 cGy/min. In another study, the dose necessary for consistent engraftment of rat marrow in irradiated mice increased from 800 cGy with a single fraction to 1,500 cGy with five daily fractions.[28] Thus, the available experimental data support the opinion expressed by a minority of clinical investigators that single-dose TBI is more efficient than TBI administered in fractions to condition patients for successful grafts of T-cell–depleted HLA-identical sibling marrow.[29] It would appear that treatment strategies for use of T-cell–depleted and also of HLA-nonidentical marrow grafts need to be reassessed in view of the experimental data and the clinical observations.

Results in this canine model were obtained with a TBI dose that is almost uniformly lethal in the absence of treatment by either hematopoietic growth factors or infusion of autologous marrow.[16,30] Thus, results with marrow allografts

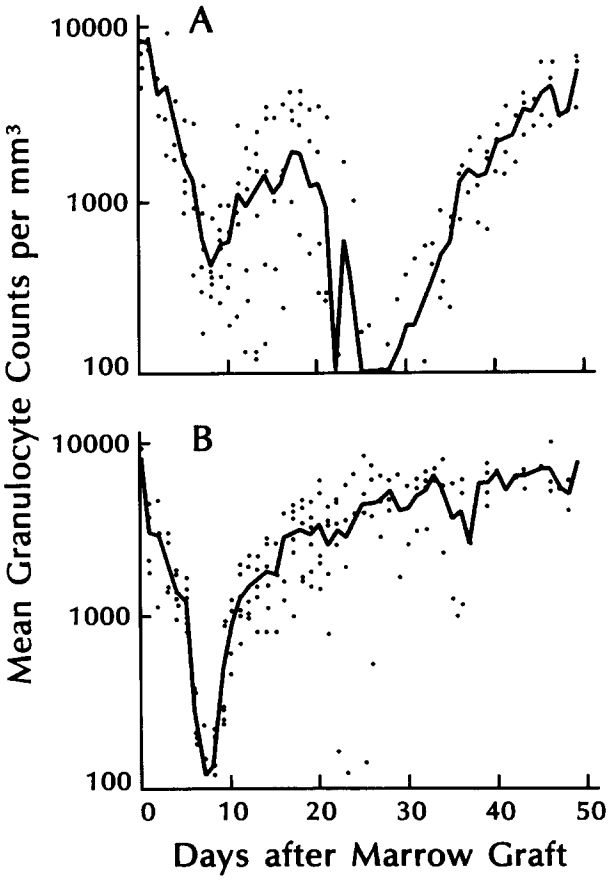

Fig 1. Granulocyte counts in dogs receiving 450 cGy TBI and marrow grafts from DLA-identical littermates. (A) Counts from 6 dogs in group 1 that rejected the graft. (B) Counts from 6 dogs in group 3 with sustained engraftment. (——) Mean values; (●) individual counts.

have relevance to the management of radiation accident victims. Present and previous[17,18] data show that marrow grafts from DLA-identical littermates are useful in extending the survival of dogs exposed to high doses of TBI ranging from 450 to 920 cGy. Sixty-eight percent of dogs in these studies survived (45 of 66 dogs). This included 21 of the 24 dogs (86%) with successful allografts, and 24 of the 42 dogs (57%) that survived with autologous hematopoietic recovery after rejection of the allograft. In the latter setting, survival is likely to be due to the extended hematopoietic support provided by the transient allograft. Similar findings have been made by other investigators.[30-34] The absence of acute or chronic GVHD in most present dogs with sustained allografts may have been related to a transient state of mixed host/donor chimerism, known to facilitate graft-host tolerance in murine studies.[35] Results imply that HLA-identical marrow grafts should be considered in treatment of victims of potentially fatal radiation accidents. To some extent, the survival of radiation accident victims can be improved by the use of recombinant hematopoietic growth factors; however, transplants are useful over higher exposure ranges than are growth factors. Studies with either canine recombinant granulocyte stimulating factor or c-*kit* ligand show that "rescue" from marrow death is possible after 400 cGy TBI dose, but less so after 500 cGy, and not at all after 600 cGy.[16,36-38]

We conclude that 450 cGy single-dose TBI delivered at a dose rate of 70 cGy/min is significantly more immunosuppressive than the same total dose delivered at 7 cGy/min. In agreement with previous findings made at lower dose rates but at higher total doses,[18] fractionated TBI delivered at 70 cGy/min is significantly less immunosuppressive than the same dose of single-dose TBI delivered at the same high dose rate. Results are consistent with the notion that significant DNA repair in lymphoid cells is possible during interfraction intervals, even at the high dose rate of 70 cGy/min. In agreement with previous data, the study showed that genotypically DLA-identical marrow grafts improved the dogs' survival after otherwise lethal TBI, either because of transient allogeneic marrow support, thereby allowing time for autologous recovery to occur, or because of permanent reconstitution of hematopoiesis by allogeneic cells.

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