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Effects of low-dose total-body irradiation on canine bone marrow function and canine lymphoma

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Effects of Low-Dose Total-Body Irradiation on Canine Bone Marrow Function and Canine Lymphoma

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Low-dose total-body irradiation, 150 rad given in 10 fractions over 5 weeks, is a useful treatment modality for favorable-prognosis lymphomas. Little is known, however, about the effects of this regimen on normal bone marrow. Six healthy beagle dogs and 5 dogs of various breeds with lymphoma were treated with total-body irradiation. Three of the 5 lymphomatous dogs achieved remissions of limited duration. No changes in the hemograms or in bone marrow cellularity (as assessed by needle marrow biopsies) could be detected during or after treatment. Bone marrow progenitor cells were studied weekly during treatment and for 4 weeks thereafter using in vitro growth assays for GM-CFC and M-CFC. These studies demonstrated significant reductions ($P < 0.001$) of granulocyte and macrophage progenitor cells with subsequent recovery toward normal pre-irradiation and sham irradiation values. Two additional dogs were injected with sublethal doses of *Salmonella typhosa* endotoxin 2 weeks after completion of the irradiation regimen. Their bone marrow GM-CFC responses were dramatically blunted compared to nonirradiated controls whereas their peripheral leukocyte responses and serum CSF levels were comparable to nonirradiated controls. These studies suggest that total-body irradiation may induce bone marrow injury that may be clinically significant if patients so treated are further stressed by infections or myelosuppressive drugs.
Key words: Canine – TBI – Bone marrow – GM-CFC

Low-dose total-body irradiation (TBI) is an increasingly popular treatment modality for favorable-prognosis lymphomas with therapeutic responses comparable to conventional chemotherapy regimens (1–5). Despite increasing use of TBI as therapy for lymphoma, little research has investigated potential damage to the patient's normal bone marrow after repeated exposure to low-dose gamma irradiation. It has been reported that low-level radiation exposures result in increased risk of subse-

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quent development of leukemia (6). In addition, granulocyte-macrophage colony-forming cell (GM-CFC) assays in dogs have been described following single-dose gamma irradiation exposures (7) and continuous, low-dose irradiation (8). In both cases, reductions of GM-CFC are described. It is the purpose of this study to use a canine model to obtain information concerning possible damage and recovery of the hematopoietic system from a fractionated low-dose total-body gamma irradiation program paralleling human clinical regimens.

The principal morbidity with this regimen is marrow suppression, mainly thrombocytopenia. Severe thrombocytopenia, although uncommon, usually occurs several weeks after completion of therapy, associated with marrow tumor or splenomegaly (9). This suggests that significant myelosuppressive events may occur during TBI that are not reflected in routine peripheral blood cell counts. To investigate the effects of fractionated TBI on bone marrow, canine marrow progenitor cell populations were evaluated by in vitro colony-forming cell assays. The dog model was chosen because of its similarity to man in both radiosensitivity (10) and histologic appearance of lymphomas (11).

MATERIALS AND METHODS

Animals. Healthy, purebred adult, female beagles, weighing 10 to 15 kg, and lymphomatous dogs of all breeds were used in the study. Dogs with suspected spontaneous lymphoma were referred by practicing veterinarians. A diagnosis was established via a lymph node biopsy, and stage of the disease was evaluated using clinical signs, bone marrow biopsies, and hematologic parameters. Those accepted for the study were then treated to eliminate parasitic infestations

and were immunized against distemper, hepatitis, and rabies. Informed consent was obtained from owners of the lymphomatous dogs accepted for the radiotherapy program. All dogs were housed in temperature-controlled rooms in individual stainless steel cages and were fed kibbled laboratory dog food, supplemented once weekly with a high-protein canned-meat ration. Water was provided ad libitum. The majority of dogs with lymphoma remained under the care of their owners and were treated on an out-patient basis. All dogs with lymphoma were examined weekly by a veterinarian.

Experimental procedure. Plexiglas containers were used to position the nonanesthetized dogs perpendicularly to parallel-opposed ^{60}Co sources delivering a midline tissue dose of 9 rad/min. A dose of 15 rad was administered twice weekly for 5 weeks for a total cumulative dose of 150 rad. This schedule is similar to that prescribed in many human clinical regimens.

Rib bone marrow aspirations were obtained from normal-irradiated and lymphomatous-irradiated dogs as well as from a normal nonirradiated control dog as a pretherapy specimen. This was done weekly for the 5 weeks of irradiation and weekly for 4 weeks thereafter. Dogs were anesthetized with Surital (Parke-Davis, A. J. Buck & Son, Baltimore, Maryland) before aspiration of bone marrow from their lateral ribs and Jamshidi bone marrow needle biopsies (Kormed, Minneapolis, Minnesota) from the dorsal iliac crests. Mononuclear marrow cells were separated from heparinized aspirates mixed with an equal volume of McCoy's 5a medium (GIBCO, Grand Island, New York) using Ficoll-Hypaque (Pharmacia, Piscataway, New Jersey). They were then washed in medium with 5% fetal calf serum and a 1% antibiotic-antimycotic mixture (100× GIBCO, Grand Island, New York). The mononuclear marrow cells were then resuspended in fresh, chilled medium and then counted and assayed in the various progenitor cell culture systems. One aliquot of peripheral blood was obtained weekly for a complete blood count (CBC) and for serum to be tested for the presence of colony-stimulating factor (CSF). Bone marrow smears were made to determine myeloid-erythroid (M:E) ratios, scoring neutrophils, bands, metamyelocytes, myelocytes, promyelocytes and myeloblasts as myeloid cells and orthochromic normoblasts, polychromatophilic normoblasts, basophilic normoblasts, and pronormoblasts as erythroid cells. The bone marrow

TABLE 1

Hematologic parameters[a] in normal dogs undergoing fractionated, low-dose total-body irradiation

Time (weeks)	Cumulative dose (rad)	Hct	WBC	Platelets $\times 10^3$	M:E
0	0	$45_8 \pm 2.7$	$7086_6 \pm 980$	$295_5 \pm 39$	$1.05_6 \pm 0.05$
1	30	$42_8 \pm 2.1$	$6157_6 \pm 532$	$311_5 \pm 38$	$1.10_6 \pm 0.08$
2	60	$42_8 \pm 1.5$	$6733_6 \pm 536$	$288_6 \pm 31$	$1.01_6 \pm 0.08$
3	90	$42_8 \pm 1.7$	$6450_6 \pm 742$	$341_5 \pm 56$	$0.90_6 \pm 0.09$
4	120	$44_6 \pm 1.4$	$6150_6 \pm 378$	$323_3 \pm 79$	$0.80_3 \pm 0.12$
5	150	$43_8 \pm 1.1$	$5880_6 \pm 882$	$270_5 \pm 73$	$1.09_6 \pm 0.13$
6		$43_8 \pm 1.0$	$5350_6 \pm 332$	$200_5 \pm 48$	$0.93_4 \pm 0.19$
7		$44_6 \pm 1.4$	$6650_6 \pm 994$	$267_6 \pm 43$	$0.68_5 \pm 0.06$
8		$45_6 \pm 1.1$	$7450_6 \pm 1092$	$304_6 \pm 26$	$0.90_5 \pm 0.12$
9		$46_8 \pm 1.1$	$6130_6 \pm 364$	$257_8 \pm 39$	$0.84_5 \pm 0.05$

[a] Hct = hematocrit (%), WBC = white blood cell count (/mm³), M:E = myeloid:erythroid ratio. These and the platelet count (/mm³) are expressed as mean values (±SEM) for several experiments. Subscript = number of separate experiments.

biopsies were decalcified and histologic sections were prepared for assessment of cellularity and the presence of tumor.

Granulocyte-macrophage colony-forming cell (GM-CFC) and macrophage colony-forming cell (M-CFC) assays. Bone marrow-derived GM-CFC (12) and M-CFC (13) were assayed as described. Serum obtained from a dog 4 h after an intravenous injection of 50 µg of *Salmonella typhosa* endotoxin (LPS) (Bacto Lipopolysaccharide W, Difco Laboratories, Detroit, Michigan) was used as a source of CSF (14). A double-layer agar system was used with 15% CSF and 15% normal human serum as an enhancing factor (15) in the lower layer. Culture medium was substituted for endotoxin serum to detect endogenous colony formation which was less than 4/10⁵ cultured cells. Marrow cells were plated in quadruplicate in 35-mm plastic petri dishes at 10⁵ nucleated cells/dish and incubated at 37°C in a humidified 7% CO_2 in air atmosphere. Colonies with greater than 50 cells were scored as being derived from GM-CFC after 9 days of incubation and as being derived from M-CFC after 25 days of incubation.

Endotoxin stress. During the second week of recovery from our TBI regimen, 2 additional normal-irradiated dogs and three nonirradiated control dogs each received an iv injection of 10 µg of LPS. Bone marrow aspirates, peripheral blood counts and serum were obtained from each anesthetized animal before LPS injection and for 4 consecutive days thereafter. The separated

mononuclear marrow cells were assayed for content of GM-CFC and the serum for CSF.

Assessment of colony-stimulating activity. Sera collected from normal-nonirradiated, normal-irradiated, lymphomatous-irradiated, and LPS-injected dogs were evaluated for CSF activity. Test sera, 0.15 ml, were used in the lower layer as the only source of CSF in the two-layer agar system described above. Bone marrow from a single normal dog was then plated in the upper agar layer as target cells. CSF was expressed as number of colonies/plate. All experiments were done in triplicate.

Statistical analysis. Results are expressed as mean values + SEM of replicate experiments with the exception of the endotoxin challenge data where individual experimental values are reported. Differences of significance among means were determined by Student's *t* test.

RESULTS

Pathology. Six healthy beagle dogs and 5 dogs of various breeds with diffuse poorly differentiated lymphomas underwent TBI. One normal-nonirradiated beagle served as a sham-irradiated control. No adverse pathological effects were observed from TBI in any of the animals, and 3 of the 5 treated lymphomatous dogs achieved complete re-

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TABLE 2

Canine bone marrow GM-CFC[a] and M-CFC during and after fractionated, low-dose total-body irradiation

Time (weeks)	Cumulative dose (rad)	Normal irradiated		Normal sham irradiated		Lymphomatous
		GM-CFC	M-CFC	GM-CFC	M-CFC	GM-CFC
0	0	$84_5 \pm 6.5$	$110_4 \pm 6.7$	74	113	$65_5 \pm 18.6$
1	30	$48_6 \pm 5.6$	$36_4 \pm 15.6$	63	83	58_1
2	60	$23_5 \pm 9.9$	$34_4 \pm 10.1$	83	95	$29_5 \pm 13.3$
3	90	$26_5 \pm 10.1$	49_2	64	101	$43_1 \pm 27.1$
4	120	$11_3 \pm 4.6$	$6_3 \pm 3.1$	101	73	$23_4 \pm 10.0$
5	150	$17_6 \pm 7.2$	$49_3 \pm 17.8$	76	85	$12_4 \pm 3.1$
6		$22_1 \pm 11.4$	N.D.	91	74	N.D.
7		$40_1 \pm 5.3$	82_2	111	122	$43_4 \pm 7.2$
8		$46_6 \pm 7.2$	$50_4 \pm 2.4$	71	82	54_1
9		$66_1 \pm 5.3$	64_2	76	81	41_1

[a] GM-CFC = granulocyte-macrophage colony-forming cell; M-CFC = macrophage colony-forming cell. Both are expressed as mean number of colonies/10^5 nucleated cells. Subscript = number of separate experiments, one dog per experiment. N.D. = not determined.

missions of about 12 weeks average duration. Two lymphomatous dogs experienced rapidly progressive lymphoma with marrow replacement during TBI and were euthanatized at the owners' request on completion of treatment. As shown in Table 1, the nadir for mean values for peripheral blood counts was reached at 1 week post exposure while bone marrow M:E ratios and cellularity remained within normal limits (no significant difference) throughout the study for all the normal dogs.

GM-CFC and M-CFC activity. Bone marrow GM-CFC and M-CFC mean values for 6 normal-irradiated beagles, 5 lymphomatous-irradiated dogs, and a sham-irradiated dog are shown in Table 2. There were significant reductions in both GM-CFC and M-CFC colony formation ($P < 0.001$) compared to pretreatment and control values after 90 rad total cumulative dose in all animals, followed by gradual recovery toward normal values. It is of interest, as previously noted, that no associated changes in peripheral blood counts, marrow differentials, or marrow cellularity could be detected to parallel this reduced progenitor cell population. CSF potency was monitored at various times during all experiments to ensure that CSF remained at pretreatment levels.

Endotoxin stress. The bone marrow GM-CFC responses of 2 normal-irradiated beagles to LPS at 2 weeks after TBI is compared to the LPS response of normal-nonirradiated beagles in Table 3. Rather than the large rise in GM-

TABLE 3

Canine GM-CFC[a] response to endotoxin 2 weeks following low-dose total-body irradiation

Percent of pre-endotoxin GM-CFC values

	Hours after endotoxin				
	0	24	48	72	96
Irradiated dog 1	100	96	19	85	18
Irradiated dog 2	100	87	94	178	90
Nonirradiated dogs[b]	100	96	180	150	290

[a] GM-CFC = granulocyte-macrophage progenitor cell in culture.

[b] Average values for three normal, nonirradiated dogs.

CFC concentration demonstrated by the control at 72 h after LPS, the previously irradiated dogs showed a markedly diminished response to LPS. However, the peripheral leukocyte response in these dogs was normal. Mean values showed a marked rise in leukocytes and neutrophils to peak values at 48 h after LPS injection. The peripheral neutrophils in the irradiated dogs rose from an average 2-week post-irradiation level of 4730–9270/mm³ 48 h after the LPS, while the normal, nonirradiated dogs showed a rise in neutrophil values from 5848–11,736/mm³.

CSF assay. No variations in serum CSF from any of the dogs exposed to the irradiation regimen could be detected. The previously irradiated dogs responded with a marked increase (232% and 365% for dogs 1 & 2, respectively) in the level of serum CSF measured 6 h after LPS challenge 2 weeks after TBI. This response was comparable to that of the normal, nonirradiated dogs (average 360% increase) as well as published data (12).

DISCUSSION

Bone marrow progenitor cells were evaluated weekly during TBI and continued for 4 weeks thereafter. In vitro soft agar cultures were used to measure GM-CFC and M-CFC activity. These studies demonstrated significant reductions in granulocyte-macrophage progenitor cells followed by a gradual recovery toward normal values. Peripheral blood CBC and marrow cellularity had remained unchanged during the irradiation regimen.

Three of the 5 lymphomatous dogs that received TBI obtained remissions of their disease following therapy. Un-

fortunately, these responses were brief. With the exception of the 2 dogs with progressive marrow tumor that did not respond to TBI, no differences between the progenitor cell assays or responses to TBI could be detected between the lymphomatous and the normal-irradiated dogs.

Although the effectiveness of TBI for canine lymphoma is of interest, the marked reduction of marrow progenitor cells during the fractionated TBI is far more intriguing. Apparently a bone marrow injury is induced by this treatment, which is not reflected in peripheral counts or marrow cellularity. The simplest explanation for the apparent paradox of a normal CBC and marrow cellularity associated with decreased marrow progenitor cell values may involve adequate progenitor cells to maintain a steady state but also diminished reserves to support a state of marrow stress such as infection or myelosuppressive drugs.

To test the bone marrow response to a granulopoetic stress following fractionated TBI, 2 normal-irradiated and three normal-nonirradiated dogs were injected with LPS. The irradiated dogs were 2 weeks post-TBI and had recovered their GM-CFC values to within pretreatment levels. As demonstrated in Table 3, the bone marrow GM-CFC responses of the irradiated beagles were markedly reduced compared to the nonirradiated animals. Thus, although the irradiated beagles had shown progressive recovery of GM-CFC toward pre-TBI levels, their bone marrows were unable to muster GM-CFC responses appropriate to the LPS stress as suggested by our controls and previously reported studies (12). This is in spite of a normal increase in circulating levels

of CSA in the irradiated, endotoxin-injected dogs. Since the GM-CFC response to LPS was evaluated only at 2 weeks post-TBI, further studies are needed to determine how long this defect persists, and therefore if it forms a component of radiation-induced residual injury to the hematopoietic system (16,17).

In conclusion, fractionated TBI has been shown to induce remissions in canine lymphoma. Profound reductions of canine marrow progenitor cells occurring with TBI could interfere with the marrow's ability to respond to further stress. The poor GM-CFC response to LPS stress following TBI supports this conclusion. This bone marrow injury may be clinically significant if patients treated with TBI are further stressed by infections or myelosuppressive drugs.

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REFERENCES

1. Carabell SC, Chaffey JT, Rosenthal DS, Moloney WC, Hellman S. Results of total body irradiation in the treatment of advanced non-Hodgkin's lymphomas. Cancer 1979; 43:994–1000.

2. Brereton HD, Young RC, Longo DL et al. A comparison between combination chemotherapy and total body irradiation plus combination chemotherapy in non-Hodgkin's lymphoma. Cancer 1979;43:2227–31.

3. Choi NC, Timothy AR, Kaufman SD, Carey RW, Aisenberg AC. Low dose fractionated whole body irradiation in the treatment of advanced non-Hodgkin's lymphoma. Cancer 1979;43:1636–42.

4. Rostum AY, Peckham MJ. Total body irradiation in advanced non-Hodgkin's lymphoma. Europ J Cancer 1977;13:1241–9.

5. Qasim MM. Blood and bone marrow response following total body irradiation in patients with lymphosarcomas. Europ J Cancer 1977;13:483–7.

6. Bizzozero OJ, Johnson KG, Ciocco A, Kawasaki S, Toyoda S. Radiation-related leukemia in Hiroshima and Nagasaki 1946–1964, II Observations on type-specific leukemia, survivorship, and disease behavior. Ann Intern Med 1967;66:522–30.

7. Wilson FD, Stitzel KA, Klein AK et al. Quantitative response of bone marrow colony-forming units (CFU-C and PFU-C) in weanling beagles exposed to acute whole-body irradiation. Radiat Res 1978;74:289–97.

8. Norris WP, Tyler SA, Sacher GA. An interspecies comparison of responses of mice and dogs to continuous Co-60 gamma irradiation. Biological and Environmental Effects of Low Level Radiation, Vienna, International Atomic Energy Commission, 1976.

9. Thar TL, Million RR. Total body irradiation in non-Hodgkin's lymphoma. Cancer 1978; 42:926–31.

10. Norris WP, Fritz TE, Rehfeld CE, Poole CM. The response of the beagle dog to cobalt-60 gamma radiation: Determination of the LD50 (30) and description of associated changes. Radiat Res 1968;35:681–708.

11. Jarrett WFH, Mackey LJ. Neoplastic diseases of the haematopoietic and lymphoid tissues. Bull Wld Hlth Org 1974;50:21–34.

12. MacVittie TJ, Walker RI. Endotoxin-induced alterations in canine granulopoiesis: Colony-stimulating factor, colony-forming cells in culture, and growth of cells in diffusion chambers. Exp Hematol 1978;6:613–8.

13. Porvaznik M, MacVittie TJ. Detection of gap junctions between the progeny of a canine

macrophage colony-forming cell in vitro. J Cell Biol 1979;82:555-64.

4. Quesenberry PJ, Morley AA, Rickard KA, Garrity D, Howard D, Stohlman F Jr. Effect of endotoxin on granulopoiesis and colony stimulating factor. N Engl J Med 1972; 286:227-32.

15. Metcalf D, MacDonald HR, Chester HM. Serum potentiation of granulocyte and macrophage colony formation in vitro. Exp Hematol 1975;3:261-73.

16. Baum SJ, Alpen EL. Residual injury in the erythropoietic system of the rat by periodic exposure to x-irradiation. Radiat Res 1959; 11:844-60.

17. Gong JK, MacVittie TJ, Vertalino JE. A method for determining residual injury in the hematopoietic system of the x-irradiated rat. Radiat Res 1969;37:467-77.

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