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Skin wound-enhanced survival and myelocytopoiesis in mice after whole-body irradiation

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Research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources, National Research Council.

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Skin wounding at 24 h before whole-body, ^{60}Co irradiation of mice raised the $LD_{50/30}$ from 8.09 to 9.71 Gy, resulting in a dose reduction factor of 1.2. Concentrations and quantities of myeloproliferative cells were examined at 3, 7, 10, and 14 days after 7 Gy, skin wounding 24 h before 7 Gy, and in control non-treated mice. Wounding before irradiation provoked an increase in marrow and splenic clonogenic cells that was earlier and greater than that noted for irradiated mice. Supranormal levels of splenic CFU-s

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20. ABSTRACT (continued)

and CFU-c were found in animals wounded before irradiation. M-CFC values were depressed throughout, although greater for combined injured animals than for irradiated mice.

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FROM THE DEPARTMENT OF EXPERIMENTAL HEMATOLOGY (CHAIRMAN: S. J. BAUM), ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE, BETHESDA, MARYLAND 20014, USA.

SKIN WOUND-ENHANCED SURVIVAL AND MYELOCYTOPOIESIS IN MICE AFTER WHOLE-BODY IRRADIATION

G. D. LEDNEY, D. A. STEWART, E. D. EXUM and P. A. SHEEHY

Delayed wound healing and increased mortality were documented in rats and mice subjected to whole-body exposures of midlethal radiation doses (STROMBERG et coll. 1968, LANGENDORFF et coll. 1964). Contrary to this, it appears that wounding before irradiation may enhance survival and only minimally interfere with the processes of wound healing. However, the data regarding this finding are equivocal. In rats wounded 7 to 9 days before a lethal dose of radiation, the survival was not increased (KINNAMON & FAIRCHILD 1965). The wounding of rats 4 days before a midlethal radiation dose resulted in no increase in survival, but survival numbers were increased when wounding preceded radiation exposure by 24 h (STROMBERG et coll.). In mice, a tendency toward enhancement of survival from a midlethal radiation dose occurred in animals wounded at various times up to one month before irradiation (LANGENDORFF et coll.).

Profound perturbations are produced in the myeloproliferative compartments in mice (LEDNEY et coll. 1980) after wound trauma and in man (PHILIP et coll. 1980) after surgical trauma. The changes produced in the proliferative compartments subsequent to trauma are reminiscent of those seen in individuals treated with cytotoxic agents (LEDNEY 1970, MILLAR et coll. 1978) that, when injected at the appropriate time, result in enhanced survival and hematopoietic recovery in irradiated animals. Thus, it was hypothesized that survival and myeloproliferative recovery would be enhanced in animals subjected to a skin wound before irradiation. Both survival and changes in colony-forming cells consistent with survival from radiation were noted in mice wounded before irradiation.

Materials and Methods

Animals. Female mice, (C_3Bl/6 X CBA)F1 Cum BR, were obtained from Cumberland View Farms, Clinton, Tennessee. All mice were acclimated to laboratory conditions in the following way. First, for a period of 2 weeks, the animals were housed in groups of 15 in a quarantined facility until a random sample was found to be free of histologic lesions of common murine diseases and until sterile water bottle cultures of all animals were found to be free of Pseudomonas spp. Secondly, the animals were housed in groups of 5 mice each for 2 weeks before experimentation. The mice were between 10 and 16 weeks old when used. At all times, the mice were kept on a 6 a.m. (light) to 6 p.m. (dark) cycle in filter-covered cages. Wayne Lab-Blox diet was provided throughout the quarantine and experimental time periods. Chlorinated (12 ppm) water was provided after the quarantine period.

Wounding. A 2.0 to 2.5 cm^2 circular wound was cut in the anterior-dorsal skin fold and underlying panniculus carnosus muscle with a steel punch. The punch was cleaned by immersion in 70% ethanol. The wounds were left open to the environment and were not treated in any way. Groups of mice were

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wounded under light Metafane (methoxyflurane, Pittman-Moore, Inc., Washington Crossing, New Jersey) anesthesia between 10 a.m. and 2 p.m., 24 h before or after ^{60}Co irradiation. Such a wound constitutes about 4 per cent of the total skin surface area and is not lethal to the mouse. Groups of irradiated non-wounded mice were subjected to the anesthetic either before or after exposure to radiation.

Irradiation. Mice were placed in Plexiglas restrainers and given whole-body irradiation with 0.4 Gy/min by bilaterally positioned ^{60}Co elements containing 5.18 PBq (140 000 Ci). All irradiations were performed between 10 a.m. and 2 p.m. Dose determinations were made with the use of a 50 ml AFRRI-designed tissue-equivalent ionization chamber calibrated against a National Bureau of Standards ionization chamber. The dose provided within the exposure field varied 3 per cent, as determined by thermal luminescence dosimetry conducted within tissue-equivalent mouse phantoms.

Cell preparations. The spleen and all long bones of the hind legs were removed aseptically from cervically dislocated mice and placed in Roswell Park Memorial Institute (RPMI)-1640 medium (Flow Labs, Rockville, Maryland) on ice (4°C). Bone marrow cells were expulsed by a syringe and a 25-gauge needle. The spleens were minced with scissors in a glass vessel. All cell preparations were passed through 6 to 8 layers of nylon mesh and were washed two times in RPMI-1640.

Colony-forming unit-spleen (CFU-s) assay. The CFU-s assay was performed by intravenous injection of groups of 6 to 8 irradiated mice either with 25×10^4 spleen cells or 25×10^4 bone marrow cells. Endogenous spleen colony formation was obviated by giving B6CBF1 mice 10 Gy of ^{60}Co irradiation at 0.4 Gy/min. Irradiated mice were engrafted with the cells within 4 h of irradiation. The spleens were removed 8 days later and fixed in Bouin's solution for 2 to 4 h, after which the surface colonies were counted independently by three persons. The average number of colonies per spleen was determined from the three counts. The number of CFU-s per 10^6 nucleated cells was determined by multiplying the average number of nodules per spleen by the appropriate factor and then preparing a grand mean from the adjusted values for each treatment group. The total tissue quantity of CFU-s was determined by taking into account the number of CFU-s per 10^6 nucleated cells and the total number of nucleated cells.

Soft-agar clonogenic assays. The colony-forming unit-culture (CFU-c) assay for granulocyte-macrophage progenitor cells and colony-forming cell assay for monocyte-macrophages (M-CFC) were done as follows. A two-layer agar system was used, consisting of a firm 0.5% nutrient agar underlayer containing colony-stimulating activity (CSA) and an overlayer of 0.3% nutrient agar containing either 10^5 spleen cells or 2.5×10^4 bone marrow cells per culture plate. Extracts from the placentae and uteri of pregnant mice (PMUE) were used as the source of CSA. The maximum CSA was observed with a 3.3% concentration (v/v) of PMUE in culture medium plus agar. A single preparation of PMUE was used. Approximately 100 to 200 colonies/10^5 spleen cells derived from normal mice of either strain were measured at this PMUE concentration. Three replicate plates were incubated at 37°C in 5% CO_2. Plates were counted for CFU-c colonies (<50 cells) and clusters (>50 cells) after 10 days of incubation and for M-CFC colonies at 21 days after culture. The number of each colony type per 10^6 cells and the total tissue quantity were determined as described in the section on CFU-s.

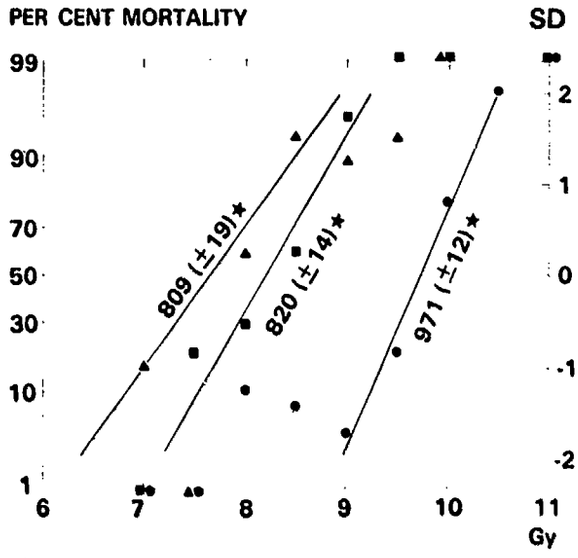

Fig. 1. Per cent 30-day mortality (probits) of mice given a 4 per cent body-surface skin-wound either 24 h before or 24 h after whole-body ^{60}Co irradiation. $LD_{50/30}$ values with attending 95 per cent confidence limits (*) are presented in the figure. Wounded 24 h after irradiation (▲); wounded 24 h before irradiation (●), irradiated controls (■).

Results

Mortality and survival times. The mortality of mice subjected to wound-trauma either 24 h before

Table 1

Mortality and survival times of mice given a 4 per cent body-surface skin-wound either 24 h before or 24 h after whole-body ^{60}Co irradiation

^{60}Co (Gy)	Wound 24 h before irradiation		Wound 24 h after irradiation		Irradiated controls	
	Mortality fraction*	MST±SE**	Mortality fraction	MST±SE	Mortality fraction	MST±SE
7.0	0/31	–	5/31	23.6±1.7	0/31	–
7.5	0/15	–	0/15	–	3/15	23.0±2.1
8.0	5/47	23.0±0.3	26/44	7.1±0.2	14/47	19.6±0.8
8.5	1/13	23.0	14/15	13.6±1.2	9/15	17.8±1.1
9.0	2/47	20.0±8.0	42/47	10.4±0.8	45/47	15.3±0.3
9.5	3/15	20.3±6.0	14/15	11.1±0.9	15/15	14.5±0.7
10.0	37/47	12.2±0.8	47/47	8.4±0.4	47/47	12.6±0.2
11.0	32/32	9.3±0.2	32/32	8.4±0.4	32/32	12.0±0.2

* Mortality fraction = number of animals dying during 30-day period/total number of animals treated.

** MST±SE = mean survival time ±1 standard error.

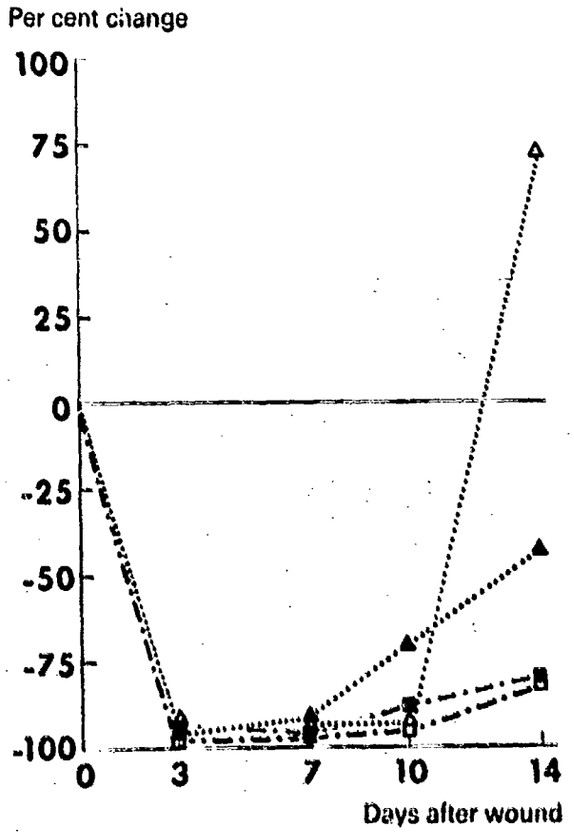

Fig. 2. Per cent change in the nucleated cellularity of the long bones of the legs and the spleens of mice after either a 4 per cent body-surface skin-wound given 24 h before 7 Gy ^{60}Co irradiation or after irradiation only. The line drawn at 0 represents the normal organ cellularity. Normal values are appear in Table 2. Symbols are for irradiation only: marrow ■···■; spleen □–·–□. For wounding before irradiation: marrow ▲···▲; spleen △–·–△.

or 24 h after exposure to ^{60}Co radiation is presented in Table 1 and depicted in Fig. 1. The $LD_{50/30}$ values, as determined from three replicate experiments are: 8.2 Gy for irradiated mice, 8.09 Gy for mice wounded 24 h after irradiation, and 9.71 Gy for mice wounded 24 h before irradiation. Thus the dose reduction factor (DRF) for mice wounded before irradiation was 1.2. The data may be represented by straight lines (Fig. 1), but the slopes of the lines are different from each other.

Swollen cervical lymph nodes, symptomatic of bacterial infections occurred in 6/60 mice wounded before irradiation with 8 or 8.5 Gy. These were never noted either in the other mouse groups wounded before the various radiation doses or in irradiated controls. The mortality data for these two radiation doses are indicated in Fig. 1, but because of their apparent isolated occurrence, the values were not used in the computations for the straight line representing mortality in mice wounded before irradiation.

The mean survival times (MST) of mice dying within a 30-day observation period are presented in Table 1. In mouse groups wounded before irradiation with doses less than 10 Gy, the MST were greater than that for mice given irradiation only. The MST of mice wounded after irradiation were lower than those for mice in the other treatment groups. Swollen cervical lymph nodes, indicative of microbial infections, were noted in all mice that died when wounded after irradiation and this may have ac-

Table 2

Cellularity of long bones of legs and spleens of mice after either wound-trauma and irradiation or irradiation only

Treatment group	Tissue assayed*	Days after wounding			
		3	7	10	14
4 per cent body-surface skin-wound 24 h before 7 Gy ^{60}Co irradiation	Marrow	2.4±0.9**	4.1±0.6	13.7±0.7	26.0±6.5
	Spleen	4.4±2.1	3.8±0.4	4.6±0.7	131.4±9.6
7 Gy ^{60}Co irradiation	Marrow	2.8±1.7	1.8±0.1	5.1±0.8	9.2±0.9
	Spleen	2.1±0.4	3.3±0.4	3.8±1.6	13.8±5.1

* Bone marrow cells were harvested from the paired humeri, femora, and tibiofibular processes. Nucleated cell quantities of normal bone marrow cavities and spleens were $76.2\pm5.6\times10^6$ and $45.7\pm3.8\times10^6$, respectively.

** Values indicated are mean numbers of nucleated cells $\times10^6\pm1$ SE where n=12.

Table 3

Colony-forming unit-spleen of marrow and spleen cells of mice after either wound-trauma and irradiation or irradiation only

Treatment group	Tissue assayed*	Assay method	Days after wounding			
			3	7	10	14
4 per cent body-surface skin-wound 24 h before 7 Gy ^{60}Co irradiation	Marrow	Per 10^6	28±15**	143±19	129±19	217±27
		Content	79±5	873±80	1 780±260	5 710±970
	Spleen	Per 10^6	1	10±3	42±6	90±4
		Content	3±2	37±8	183±18	11 610±470
7 Gy ^{60}Co irradiation	Marrow	Per 10^6	23±6	3±3	68±14	175±20
		Content	91±29	7±7	340±80	1 600±180
	Spleen	Per 10^6	1	1	10±5	96±8
		Content	6±6	2±2	47±26	935±200

* Concentrations of CFU-s in normal marrow and splenic tissues were 601±20 and 81±2 per 10^6 nucleated cells, respectively. The CFU-s contents of the long bones of the legs and splenic tissue of normal mice were 26 680±825 and 3 860±190, respectively. In all instances, n=12.

** Values indicated are mean numbers of CFU-s±1 SE.

counted for the early deaths; this symptom never occurred in irradiated control mice.

Wound healing. Wound closure differed according to the time of irradiation relative to wounding. First, in non-irradiated mice, wound closure was completed in 10 days without formation of a large eschar. In mice wounded before irradiation, wound enclosure occurred in 10 to 12 days beneath an eschar that approximated the initial wound size. The eschars sloughed off between 10 to 20 days after wounding revealing a moist depilated area. During the observation period, that area reduced in size and produced grey hair. In mice wounded after irradiation, the wounds increased in size to a maximum 3 or 4 days after wounding. Wound closure in these animals was delayed about 4 days above that in mice wounded before irradiation. If the mice survived the combined injury, eschar sloughing and hair growth

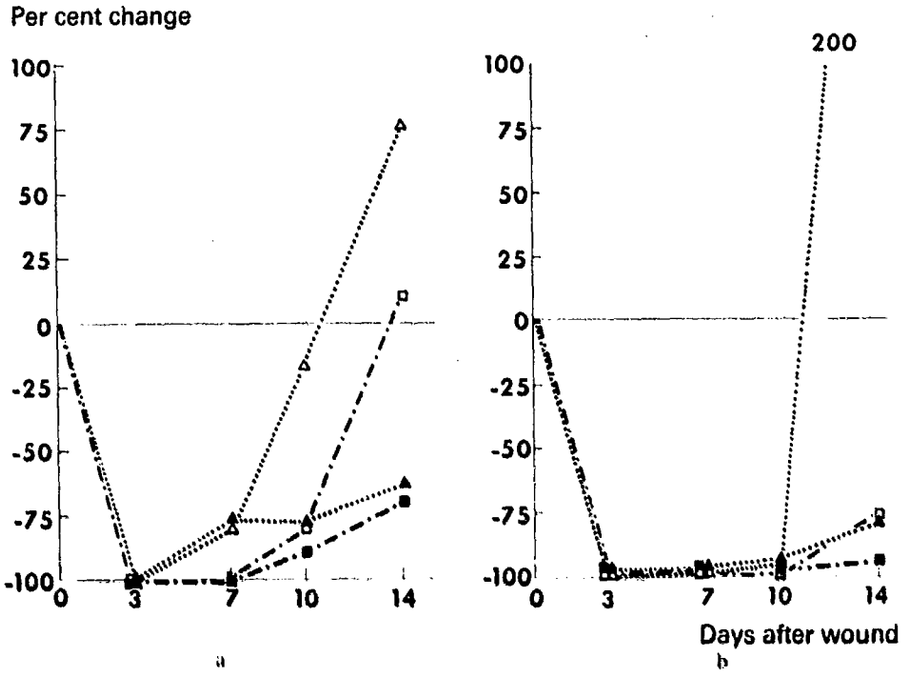

Fig. 3. Per cent change in the colony-forming unit-spleen per 10^5 nucleated cells (a) and organ content of marrow cells from the long bones of the legs and the spleens (b) after either a 4 per cent body-surface skin-wound given 24 h before 7 Gy ^{60}Co irradiation or after irradiation only. The lines drawn at 0 represent the normal concentrations and organ contents. The relative normal values appear in Table 3. Symbols as in Fig. 2.

proceeded about 5 days slower than that for mice wounded before irradiation.

Cellularity. The number of nucleated bone marrow and spleen cells were determined in mice for a 2-week period after the single or combined stresses of 7 Gy and a 4 per cent body-surface skin-wound. The data are presented in Table 2 and in Fig. 2 as a per cent of untreated controls.

Compared with the irradiation controls, the quantity of nucleated cells in the marrow compartments of mice wounded before irradiation started the return to normal numbers earlier (days 7–10) and was two to three times higher than irradiation controls by day 14. Splenic cell numbers in the combined injured mice started to return to normal after day 10 and reached a value tenfold higher than irradiated mice 14 days after wound-trauma.

Colony-forming unit-spleen. The CFU-s assay was used to estimate myelopoietic recovery of the bone marrow and spleen of mice after the single or combined stresses. The CFU-s data appear in Table 3 and are depicted graphically in Fig. 3 as a per cent of control-untreated mice. Compared with the irradiated control mice, wound-trauma before irradiation advanced the time at which noticeable increases in CFU-s concentration were seen from 10 to 7 days. During the test period, the splenic and long bone content of CFU-s was less than 25 per cent of the normal control values, with the exception of the splenic CFU-s of traumatized-irradiated mice on day 14. In those mice, a threefold increase in content occurred and a twofold increase in concentration of CFU-s.

Colony-forming unit-culture assay. Alterations in the CFU-c concentration and content of the marrow and spleen of treated mice are presented in Table 4 and in Fig. 4. Wound-trauma before irradiation was beneficial in terms of a smaller reduction in the CFU-c concentration in the myeloid tissues. Wounding before irradiation also resulted in an earlier and greater return of splenic concentration and content of CFU-c than that measured for the tissues from irradiated mice. In all treatment groups of mice given irradiation, the splenic CFU-c responses followed increases detected first with bone marrow cells.

Monocyte-macrophage colony-forming cells. The M-CFC quantities found in the myeloid tissues of treated mice are presented in Table 5 and Fig. 5. The concentrations and tissue contents of M-CFC were

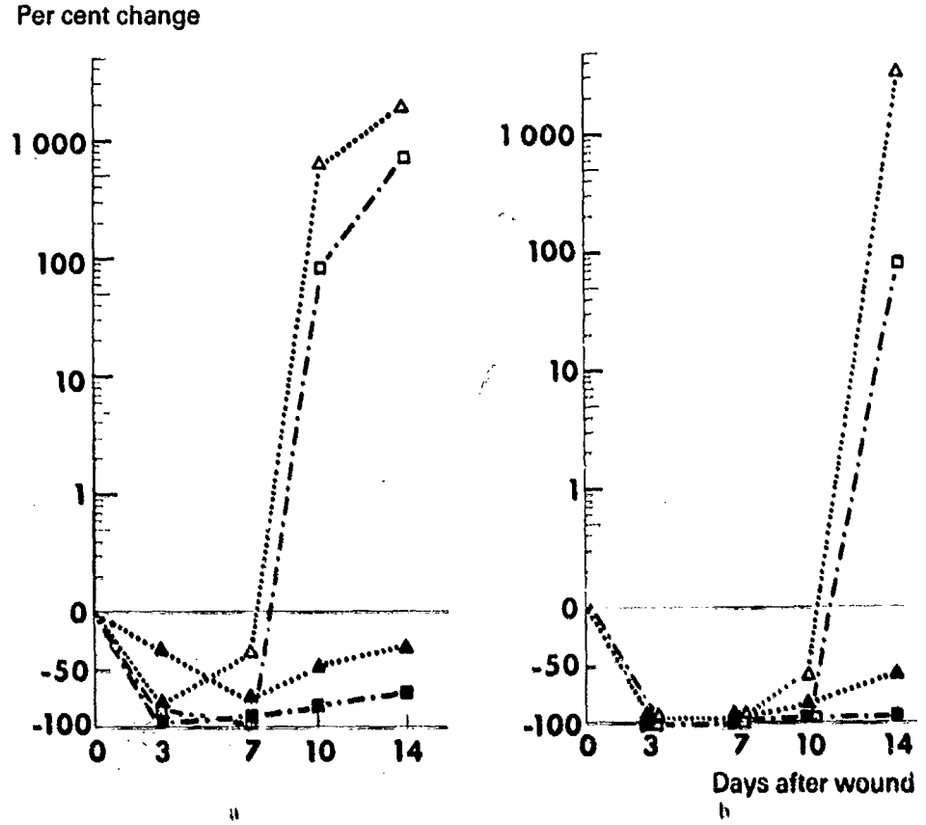

Fig. 4. Per cent change in the colony-forming unit-culture per 10^5 nucleated cells (a) and organ content of marrow cells from the long bones of the legs and the spleens (b) after either a 4 per cent body-surface skin-wound given 24 h before 7 Gy ^{60}Co irradiation or after irradiation only. The lines drawn at 0 represent the normal concentrations and organ contents. The relative normal values appear in Table 4. Symbols as in Fig. 2.

Table 4

Colony-forming unit-culture of marrow and spleen cells of mice after either wound-trauma and irradiation or irradiation only

Treatment group	Tissue assayed*	Assay method	Days after wounding			
			3	7	10	14
4 per cent body-surface skin-wound 24 h before 7 Gy ^{60}Co irradiation	Marrow	Per 10^5	847±127**	323±68	680±63	880±53
		Content	2 309±509	1 429±333	9 373±927	23 194±2 490
	Spleen	Per 10^5	4±1	9±3	100±14	284±22
		Content	11±3	30±10	425±40	36 535±2 160
7 Gy ^{60}Co irradiation	Marrow	Per 10^5	63±21	103±34	211±53	349±52
		Content	114±33	190±63	1 308±321	3 160±424
	Spleen	Per 10^5	2±1	<1	25±10	142±3
		Content	3±2	1	56±21	1 953±224

* Concentrations of CFU-c in normal marrow and splenic tissues were 1 305±71 and 14±1 per 10^5 nucleated cells, respectively. The CFU-c contents of the long bones of the legs and splenic tissues of normal mice were 57 832±2 882 and 1 063±76, respectively. In all instances, n=12.

** Values indicated are mean numbers of CFU-c±1 SE.

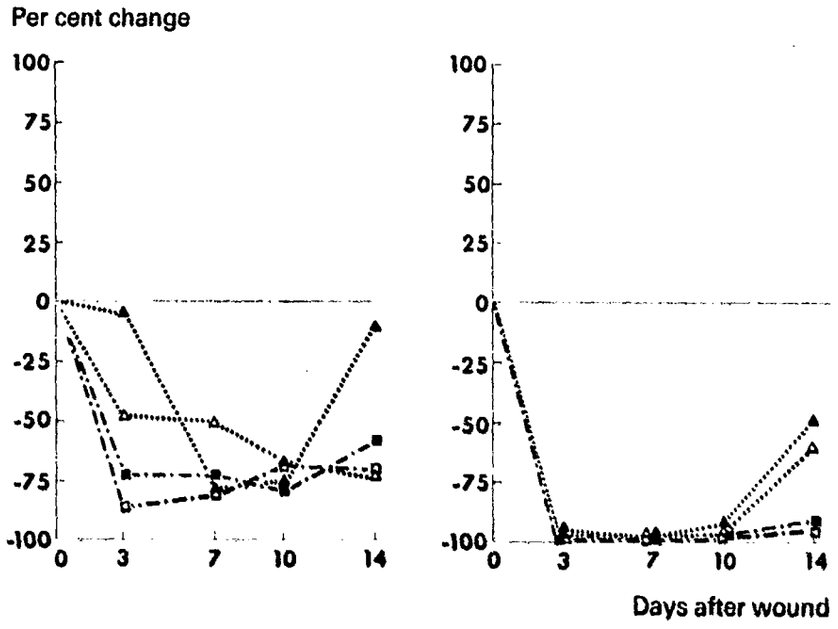

Fig. 5. Per cent change in the macrophage-monocyte colony-forming cells per 10^5 nucleated cells (a) and organ content of marrow cells from the long bones of the legs and the spleen (b) after either a 4 per cent body-surface skin-wound given 24 h before 7 Gy ^{60}Co irradiation or after irradiation only. The lines drawn at 0 represent the normal concentrations and organ contents. The relative normal values appear in Table 5. Symbols as in Fig. 2.

Table 5

Monocyte-macrophage colony-forming cells of marrow and spleen cells after either wound-trauma and irradiation or irradiation only

Treatment group	Tissue assayed*	Assay method	Days after wounding			
			3	7	10	14
4 per cent body-surface skin-wound 24 h before 7 Gy ^{60}Co irradiation	Marrow	Per 10^5	2 203 ± 220**	483 ± 51	583 ± 86	2 037 ± 228
		Content	5 322 ± 736	1 959 ± 227	7 860 ± 1 103	55 875 ± 8 598
	Spleen	Per 10^5	75 ± 15	70 ± 12	45 ± 4	38 ± 7
		Content	273 ± 20	238 ± 39	202 ± 22	8 482 ± 949
7 Gy ^{60}Co irradiation	Marrow	Per 10^5	753 ± 79	627 ± 130	333 ± 56	967 ± 174
		Content	1 467 ± 224	1 142 ± 247	1 798 ± 354	8 482 ± 1 340
	Spleen	Per 10^5	20 ± 7	29 ± 2	44 ± 7	41 ± 3
		Content	38 ± 11	97 ± 8	136 ± 16	461 ± 43

* Concentrations of M-CFC in normal marrow and splenic tissues were 2 319 ± 137 and 141 ± 9 per 10^5 nucleated cells, respectively. The M-CFC contents of the long bones of the legs and splenic tissues of normal mice were 106 711 ± 8004 and 12 607 ± 1687, respectively. In all instances, $n = 12$.

** Values indicated are mean numbers of M-CFC ± 1 SE.

reduced in all treatment situations to quantities less than that found in control-untreated animals. Wound-trauma before irradiation resulted in enhanced M-CFC concentration values on day 3 when compared with irradiated controls. The concentration of marrow M-CFC in mice wounded before irradiation returned to the range of non-treated control mice by day 14, while all other M-CFC values were reduced at least 50 per cent. The content of M-CFC in the myeloid tissues of all mice irradiated

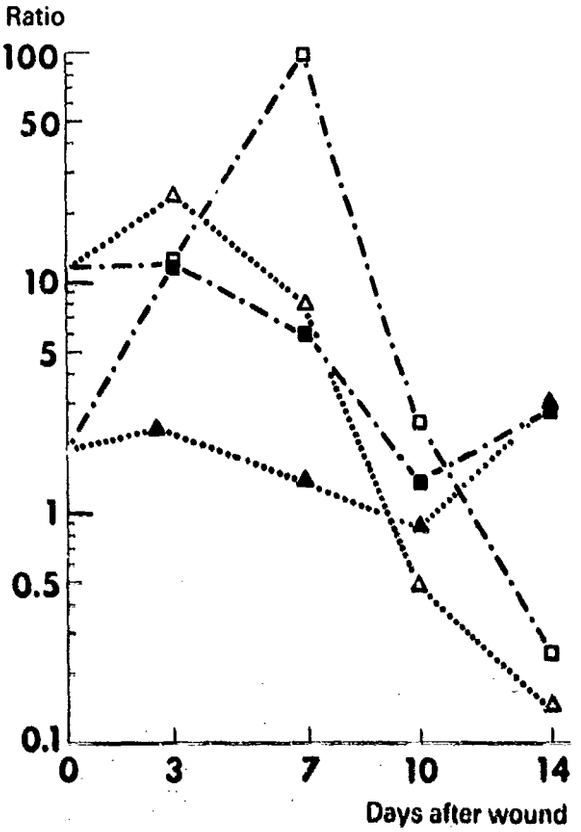

Fig. 6. Ratios of macrophage-monocyte colony-forming cells to colony-forming unit-culture cells obtained with marrow and splenic cells from mice after either a 4 per cent body-surface skin-wound given 24 h before 7 Gy ^{60}Co irradiation or after irradiation only. Ratios were determined by dividing the M-CFC content values listed for the marrow and spleen in Table 5 by the CFU-c content values listed in Table 4. Normal ratios given at time 0 are 10 and 2 for splenic and marrow M-CFC/CFU-c ratios. Symbols as in Fig. 2.

was reduced for the first 10 days. Recovery, to about 50 per cent of non-treated control values, was observed on day 14 in both the spleens and marrow of mice wounded before irradiation.

Discussion

The significant finding of the present investigation is the enhancement of survival from midlethal and lethal doses of radiation in mice wounded 24 h before exposure. This was accompanied by increases in clonogenic myeloid elements that appeared earlier and in greater quantities than those occurring in irradiated control animals.

In surgically traumatized persons, granulocytes and macrophages migrate to the wound site and assist in wound debridement (LEMOVICH & ROSS 1975) and neutralization of foreign bodies such as bacteria (SIMPSON & ROSS 1972). Utilization of these mature cells results in an increased demand for differentiated progeny produced by the myelopoietic centers. Thus, PHILIP et coll. observed an 80 to 90 per cent decrease in the peripheral blood content of CFU-c one day after surgery and a nearly twofold increase 7 days after the operative procedure. Thus, surgical trauma sets into motion myeloproliferative responses which could, if left undamaged or repairable, aid in the enhancement of survival in irradiated mice.

In the present experiments, wounding mice before irradiation appeared to influence the myelocytopoietic compartments in ways known to be associated with enhancement of survival from radiation induced by a variety of treatments. First, in the CFU-s and CFU-c compartments, concentrations of these elements started to return first in the bone marrow and then in the spleen sooner than that for irradiated control mice (cf. Tables 3, 4). Secondly, in the combined injured animal, the marrow and splenic concentrations of CFU-c and M-CFC were not reduced to the levels seen in irradiated mice. These observations, taken together with the findings that myeloid proliferative elements are necessary to enhance survival in irradiated animals (MCCULLOCH & TILL 1964), can account for the enhanced survival of wounded irradiated mice.

In normal mice, the splenic and bone marrow cell ratios of M-CFC/CFU-c (Fig. 6) are about 10 and 2, respectively. In irradiated mice, increases in these ratios on day 3 for marrow (from 2 to 10) and on day 7 for spleen (from 10 to 100) are accounted for by the greater relative losses of CFU-c compared with M-CFC rather than relatively enhanced M-CFC production. Wounding before irradiation resulted in marrow M-CFC/CFU-c ratios at the times tested that varied little about the normal ratio of 2. This is considered to mean that there is uniform loss and recovery of 'normal' production ratios of both cell populations during recovery even from the combined stress condition. In the spleen of the combined injured animal, the ratio is moderately increased during the first week after trauma due to a greater relative loss of CFU-c as compared with normal controls. However, the ratio is decreased during the second week of recovery to one or two per cent of that in normal animals. This is explained by the greater relative increases in CFU-c over M-CFC increases during that time. The reason for this is

unknown but it may be due to (1) the biologic requirement for mature granulocytes to assist against microbial complications and (2) the response to the negative feed back signal of granulocytopenia occurring after irradiation.

It is conceivable that the robust splenic myeloproliferative response in wounded mice in the post-irradiation period could account for the enhancement of survival. Splenic extramedullary myelocytopoiesis in mice and the lack thereof in rats may also be the reasons for the conflicting data surrounding wound-enhanced survival from radiation in rodents. However, previously it was determined that wounding enhances survival equally well in splenectomized, sham-splenectomized mice and in control unoperated mice given 9 Gy radiation (LEDNEY et coll. 1981). This suggests that enhanced survival of mice wounded before irradiation is independent of extramedullary splenic myelocytopoiesis.

The physiologic stimulus of skin wounding and subsequent healing result in a number of changes in the mature cells of the peripheral blood pool (BRYANT 1977). For example, erythrocytes are lost through the wound site, and platelets, through their aggregation and adhesion capacities, attempt to maintain homeostasis (MORENO 1975). Along these lines, in mice, the daily removal of about 20 per cent of the blood volume for 4 consecutive days enhanced survival from midlethal doses of irradiation (MARSH et coll. 1968). However, splenectomy abolished the radiation protective effect.

It is tempting to think that the survival was enhanced by hemorrhage-induced aplasia. However, hematocrits taken one day after wounding (39±3%) were not significantly different from that of control values (42±3%). Additionally, splenectomy did not abolish the enhanced survival seen in wounded and irradiated mice (LEDNEY et coll. 1980).

The mechanism by which wounding seems to stimulate myeloproliferation and survival from radiation is unclear. However, intestinal cell-tight junctions are disrupted in mice and rats after radiation injury (WALKER & PORVAZNIK 1978, PORVAZNIK 1979), and endotoxin, released by the intestinal microflora, may pass through the injured sites into the circulation. Along these lines, endotoxin, when injected shortly before or after irradiation, protects both conventional (SMITH et coll. 1958, AINSWORTH et coll. 1970) and germ-free animals (LEDNEY & WILSON 1965). Additionally, endotoxin is a potent stimulator of colony-stimulating factor (CSF; QUESENBERRY et coll. 1972), and CSF is increased in the serum of conventional mice subsequent to radiation injury (HALL 1969, MORLEY et coll. 1971). CSF, generated by host tissues in response to tissue trauma, may also enhance myelocytopoiesis (METCALF 1977) and this, in conjunction with the endotoxin-stimulated CSF, may account for wound-enhanced survival from radiation. Work is in progress, testing this thesis.

SUMMARY

Skin wounding at 24 h before whole-body ^{60}Co irradiation of mice raised the $LD_{50/30}$ from 8.09 to 9.71 Gy resulting in a dose reduction factor of 1.2. Concentrations and quantities of myeloproliferative cells were examined at 3, 7, 10, and 14 days after 7 Gy, skin wounding 24 h before 7 Gy and in control non-treated mice. Wounding before irradiation provoked an increase in marrow and splenic clonogenic cells that was earlier and greater than that noted for irradiated mice. Supranormal levels of splenic CFU-s and CFU-c were found in animals wounded before irradiation. M-CFC values were depressed throughout, although greater for combined injured animals than for irradiated mice.

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