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Survival and hematopoietic recovery in mice after wound trauma and whole-body irradiation

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Skin wounding 24 h before but not 24 h after whole-body ^{60}Co irradiation results in enhanced survival (DRF 1.2). Slopes of survival curves, dose-response survival patterns, and morphologic observations support the idea that infectious complications lead to early death (8-14 days) in most mice wounded after irradiation and in late death (>21 days) in most mice wounded before irradiation. Wounding before irradiation produced increases in both marrow and splenic clonogenic cells, and they commenced earlier and reached

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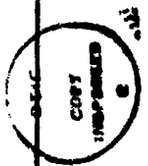
20. ABSTRACT (continued)

greater quantities than those found for irradiated mice. E-CFU quantities were increased by wounding before radiation. Spleen cells obtained from mice 1-2 weeks after the combined injury were found to rescue more lethally irradiated mice than spleen cells from mice only irradiated. Hydroxyurea eradication of trauma-induced endogenous spleen colonies supports the idea that wounding before radiation enhances survival and hematopoietic recovery by stimulating resting hematopoietic clonogenic cells into DNA synthesis.



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SURVIVAL AND HEMATOPOIETIC RECOVERY IN MICE AFTER WOUND TRAUMA AND WHOLE-BODY IRRADIATION

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Increased mortality is observed in rodents subjected to skin wound trauma after midlethal doses of radiation (1,2). In animals receiving lethal doses of radiation before skin wounding, survival times are decreased compared to irradiated contols. Contrary to the decreased survival noted in rodents subjected to wounding after radiation, wounding before irradiation may increase survival. However, the data supporting this are equivocal. In rats, survival was increased only slightly (2) or not at all (3) in animals wounded before midlethal radiation doses. In mice, a small increase in the number of survivors was noted when midlethal irradiation followed wounding (2). The enhanced survival from radiation noted in surgically traumatized animals may be related not only to the species of rodent used, but also to the genetic stain, as described (4). Contrary to the above reports, previous investigations from this laboratory support the idea that wound trauma before lethal whole-body irradiation augmented survival in mice (5,6). The purposes of this report are: a) to summarize some of our findings using a mouse model of wound trauma that enhances survival from radiation; and b) to provide data supporting the hypothesis that wound trauma before either midlethal or lethal radiation doses enhances survival by stimulating hematopoiesis and advancing hematopoietic recovery.

MATERIALS AND METHODS

ANIMALS. Female 5-week-old (C57BL/6 x CBA)F_1 Cum BR mice were obtained from Cumberland View Farms, Clinton, TN. All mice were acclimated to laboratory conditions in the following way. First, the animals were quarantined for a period of 1-2 weeks, in groups of 20. Animals used were those from groups found to be free of pseudomonas sp and histologic lesions of common murine disease. Secondly, the animals were housed in groups of five 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)

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cycle in filter-covered cages. Wayne Lab-Blox diet was provided throughout the quarantine and experimental periods. Chlorinated (10 ppm) water was provided after the quarantine period.

WOUNDING. Groups of mice were wounded under light Metafane (methoxyflurane, Pittman-Moore, Inc, Washington Crossing, NJ) anesthesia between the hours of 10 a.m. and 2 p.m. A 2.0-2.5 cm^2 circular wound was cut in the anterior-dorsal skin fold and underlying panniculus carnosus muscle (between the scapulae) with a steel punch. The punch was cleaned after the wounding of each mouse by immersion in 70% ethanol. Such a wound constitutes about 4% of the total skin surface area and is not lethal to the mouse. The wounds were left open to the environment and were not treated in any way. Subsequent to the wounding procedure, all mice were placed in sanitized cages that contained an autoclaved, commercially available, hardwood chip bedding (Ab-Sorb-Dri, Maywood, NJ). Groups of irradiated nonwounded 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 at 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 by 3%, 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 RPMI-1640 medium (Flow Labs, Rockville, MD) on ice. 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 6-8 layers of nylon mesh and then washed three times in RPMI-1640 at 250 g and suspended in RPMI-1640. Viability estimates and nucleated cell counts were performed in a hemocytometer with 0.2% trypan blue dye and Turk's solution. The total nucleated cellularity (TNC) was determined for each tissue. Cell dilutions for the assays were done with RPMI-1640.

COLONY-FORMING UNIT-SPLEEN (CFU-S) ASSAY. The CFU-s assay was used to determine the concentration and quantity of pluripotent cells in the spleen and in the paired long bones of the legs. Mice, numbering 6-8 animals per group, were injected i.v. with either 2.5×10^5 spleen cells or 2.5×10^4 marrow cells within 4 h of irradiation. Endogenous CFU formation was obviated by 10 Gy ^{60}Co radiation given at 0.4 Gy/min. The spleens were removed 8 d later and fixed in Bouin's solution for 2-4 h. The surface colonies were counted independently by three persons, and the average number of CFU-s on each spleen was determined from the three counts. The number of CFU-s per 10^5 nucleated spleen and marrow 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 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. However, the total tissue contents of CFU-s are not presented since the qualitative changes observed after the various treatments were similar, whether the data were expressed as a relative or absolute value.

SOFT-AGAR CLONOGENIC CELL ASSAY. In vitro assays for granulocyte-macrophage colony-forming cells (GM-CFC) and monocyte macrophage colony-forming cells (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 10^6 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 10 to 20 colonies/10^6 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 GM-CFC colonies (>50 cells) and clusters (<50 cells) 10 days after culture and for M-CFC colonies 21 days after culture. The number of each type of colony per 10^6 cells and the total tissue quantity were determined as described in the section on CFU-s.

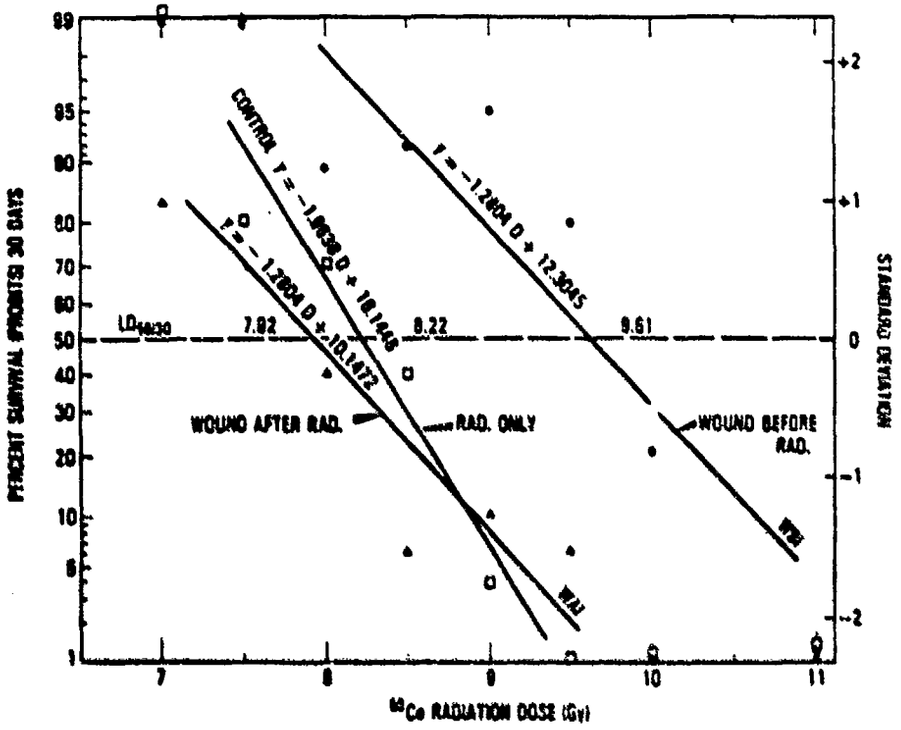
EXPERIMENTAL DESIGN AND STATISTICAL TESTING. Survival data for all irradiated mice were analyzed using quantal response methods (7). Probit lines were fit to each set of data separately, using an appropriate line transformation of dose to insure an increasing dose metameter. Chi square analysis for linearity and parallelism of the data were made. In hematopoietic recovery studies, clonogenic cell assays were done with spleen and bone marrow cells harvested from mouse groups on days 3, 7 , 10, and 14 after wounding. The treatment groups included: a) mice wounded 24 h before 7.0 Gy irradiation, and b) mice given only 7.0 Gy radiation. Based on the radiation dose survival response studies, hematopoietic recovery was expected in a majority of mice after either treatment. Thus, sufficient cells were available for assay purposes. Four replicate experiments were done over a 6-month period. Each replicate contained the pooled tissues from each of three mice. The hematopoietic clonogenic cell recovery data presented in this report are expressed only as relative concentration changes. Changes in clonogenic cell concentrations were mirrored by similar changes in the absolute population of cells.

RESULTS

SURVIVAL FRACTIONS AND SURVIVAL TIMES. The percent survival of mice given a skin wound either 24 h before or 24 h after exposure to ^{60}Co radiation is depicted in Fig 1. Tests for parallelism of probit lines between control irradiated mice and mice wounded before or after

radiation were as follows. A significant difference in slopes ($p = 0.019$) was found between the irradiated controls and mice wounded after irradiation. No difference in slopes ($p = 0.217$) was found between control irradiated mice and animals wounded before irradiation. This is explained by the variability produced by the late deaths of a few mice at some of the radiation doses. The estimated slopes for mice wounded before or after radiation were nearly identical ($p = 0.967$). Thus, a pooled estimate of common slope was made, and the probit lines for these two groups were plotted with this slope. The difference between this slope and the one for the controls indicates that wounding produces a mouse that is on the average less sensitive to changes in radiation dose. In addition, mice wounded before irradiation showed enhancement of survival indicated by a DRF of 1.2.

FIGURE 1



Percent (probits) 30-day survival of wounded-irradiated mice. Slopes for each treatment are presented in each line, as are the corresponding $LD_{50/30}$ radiation doses in Gray (Gy) units.

The overall mean survival times for all mouse groups wounded after irradiation and dying during the 30-day observation period was 11.8 ± 2.1 days. This compares to the 16.0 ± 1.5 days for all mouse groups only irradiated and 18.0 ± 2.4 days for all mouse group wounded before irradiation. Swollen cervical lymph nodes, symptomatic of microbial

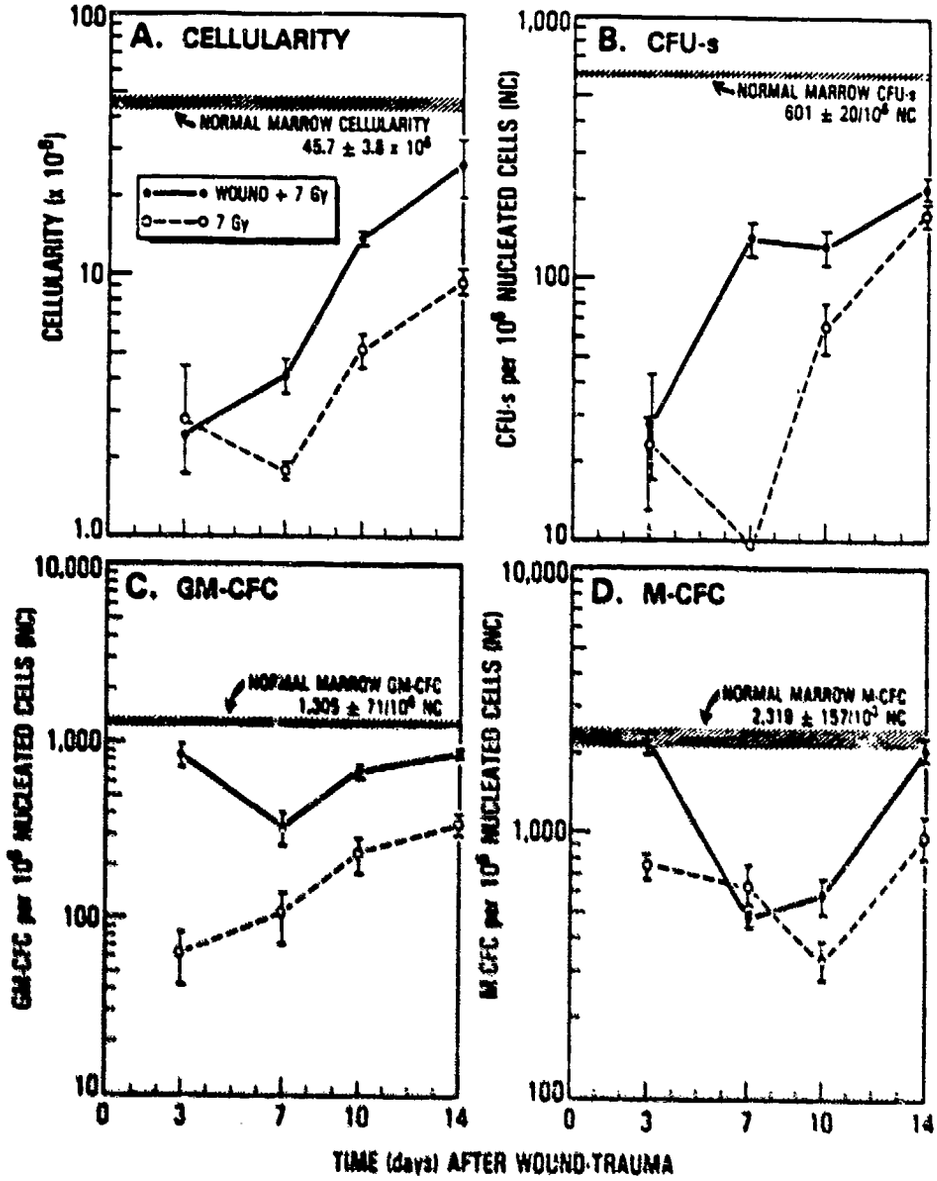
infections, were observed in all mice that died subsequent to wounding after irradiation. Similarly, this symptomatology was noted in those animals that died approximately 3 weeks after wounding followed by irradiation. Lymph node involvement was not detected in mice given radiation only.

WOUND HEALING. Wound closure differed according to the time of irradiation relative to wounding. First, in normal nonirradiated mice, wound healing was completed in 10 days without large eschar formation. In mice wounded before irradiation, wound enclosure occurred in 10-12 days beneath an eschar that approximated the initial wound size. The eschars sloughed off 10-20 days after wounding, revealing a moist depiliated area. During the observation period, that area reduced in size, and gray hair grew from it. In mice wounded after irradiation, the wounds increased in size to a maximum 3-4 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 occurred about 5 days after that for mice wounded before irradiation.

BONE MARROW CELLULARITY AND CLONOGENIC CELL ASSAYS. The survival recorded for mice wounded before irradiation prompted investigations into the character of hematopoietic recovery in these animals. Thus, the marrow cellularity and concentrations per 10^6 nucleated cells of CFU-s, GM-CFC, and M-CFC are presented in Fig 2. In the CFU-s compartment, skin wounding at 24 h before 7 Gy of irradiation resulted in the return toward normal values by day 7. The increase in the CFU-s compartment of wounded-irradiated mice preceded that seen in irradiated control mice by 3 days. GM-CFC quantitites were significantly higher ($P<0.01$) at all times points tested in wounded irradiated mice compared to that for animals given radiation only. In irradiated control mice, the lowest level of GM-CFC was seen on day 3 after irradiation with increases toward normal levels of GM-CFC detected on each successive time period tested thereafter. The M-CFC population in wounded irradiated mice was within the normal control range on day 3 after wounding. The nadir of M-CFC in wounded irradiated animals was found on day 7, and by day 14, that cell population had returned to the normal control level. In irradiated control animals, the M-CFC population was reduced by about 75% from that of controls during the first 10 days of the study. M-CFC recovery toward normal was not seen until 14 days after irradiation.

SPLENIC CELLULARITY AND CLONOGENIC CELL ASSAYS. The splenic cellularity and concentrations per 10^6 nucleated cells of CFU-s. GM-CFC, and M-CFC are depicted in Fig 3. Splenic hypocellularity was observed in both wounded irradiated animals and in irradiated mice the first 10 days after treatment. However, wounding elicited a hypercellularity response 2 weeks after irradiation. The quantity of cells at that time was tenfold greater than that for irradiated control animals. Exponential increases in the number of detectable CFU-s were seen in both treatment groups of mice. However, this increase started 3-4 days earlier in wounded irradiated mice (days 3-7) than in irradiated

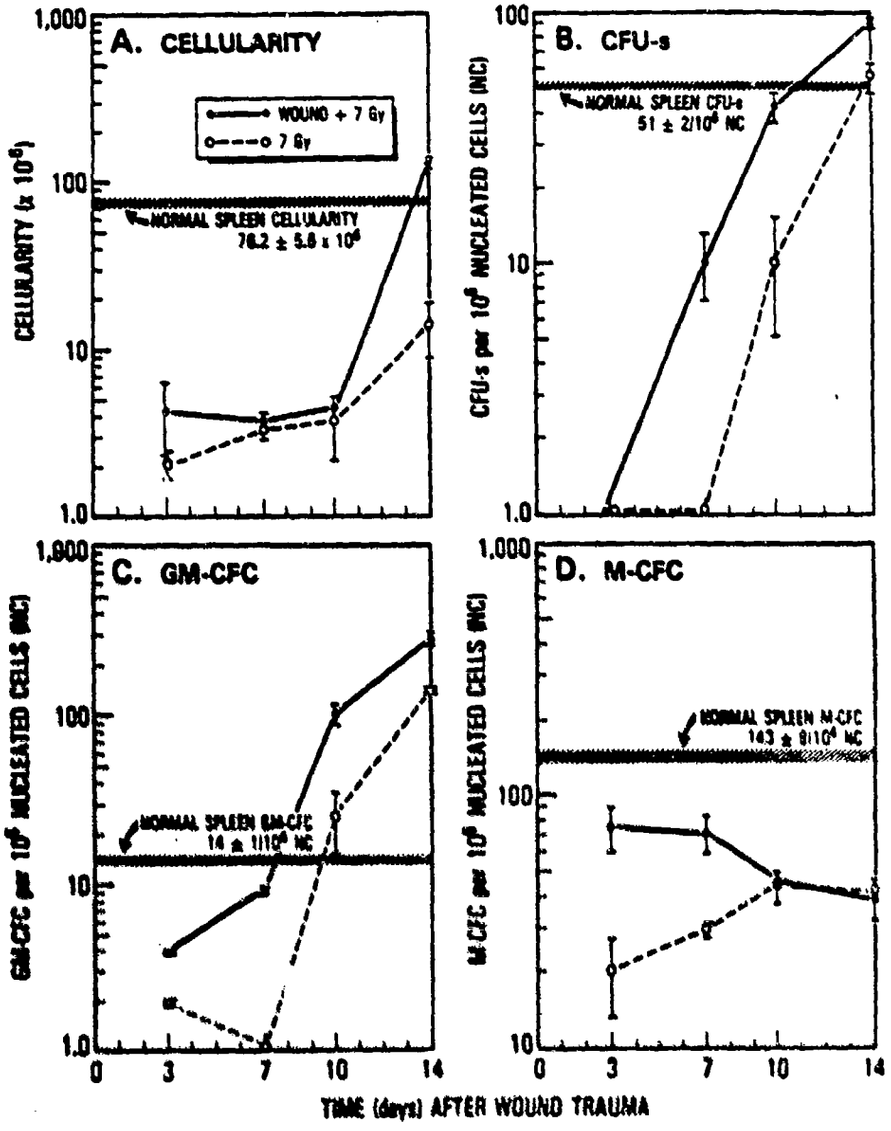
FIGURE 2



Semilogarithmic plots of bone marrow cellularity (A) and concentrations per 10^6 nucleated cells (NC) of (B) CFU-s (C) GM-CFC, and (D) M-CFC. Each point represents the mean value obtained from 6 replicate experiments ± S.E.

control animals (days 7-10). GM-CFC quantities for both the combined injured and the irradiated control mice were significantly less than normal values the first week after treatment. Wounding before irradiation resulted in significantly more detectable GM-CFC at each time point examined than that seen for irradiated-only mice. Additionally,

FIGURE 3



Semilogarithmic plots of splenic cellularity (A) and concentrations per 10^5 nucleated cells (NC) of (B) CFU-s, (C) GM-CFC, and (D) M-CFC. Each point represents the mean value obtained from 4 replicate experiments ± S.E.

a return toward normal splenic GM-CFC values was seen sooner (days 3-7) in wounded irradiated mice than in irradiated controls (days 7-10). A GM-CFC hypercellularity was noted in both treatment groups during the second week after treatment. Splenic M-CFC values for both wounded irradiated and irradiated-only mice were significantly

depressed below normal quantities for the entire test period. However, the splenic M-CFC quantities for mice wounded before irradiation were significantly increased during the first week after injury compared to the irradiated controls. The quantities of splenic M-CFC were the same in both treatment groups during the second week after the treatments.

ENDOGENOUS CFU AND SURVIVAL STUDIES. Endogenous (E) CFU were determined in wounded mice both 8 and 12 days after irradiation. Radiation doses used approximated the $LD_{0/30}$ (7 Gy) and the $LD_{100/30}$ (9Gy) for $B6CBF_1$ female mice. Concurrent 30-day survival studies were done with other mouse groups treated at the same time as the mice for the E-CFU investigations. Additional E-CFU and survival studies were done in mouse groups injected with hydroxyurea (HU). HU was injected i.p. into the mice at the rate of 1 mg/g body weight at 20 h after wounding and/or 4 h before irradiation. This dosage and injection schedule was previously determined (8) to be effective in blocking the appearance of E-CFU in wounded, irradiated mice.

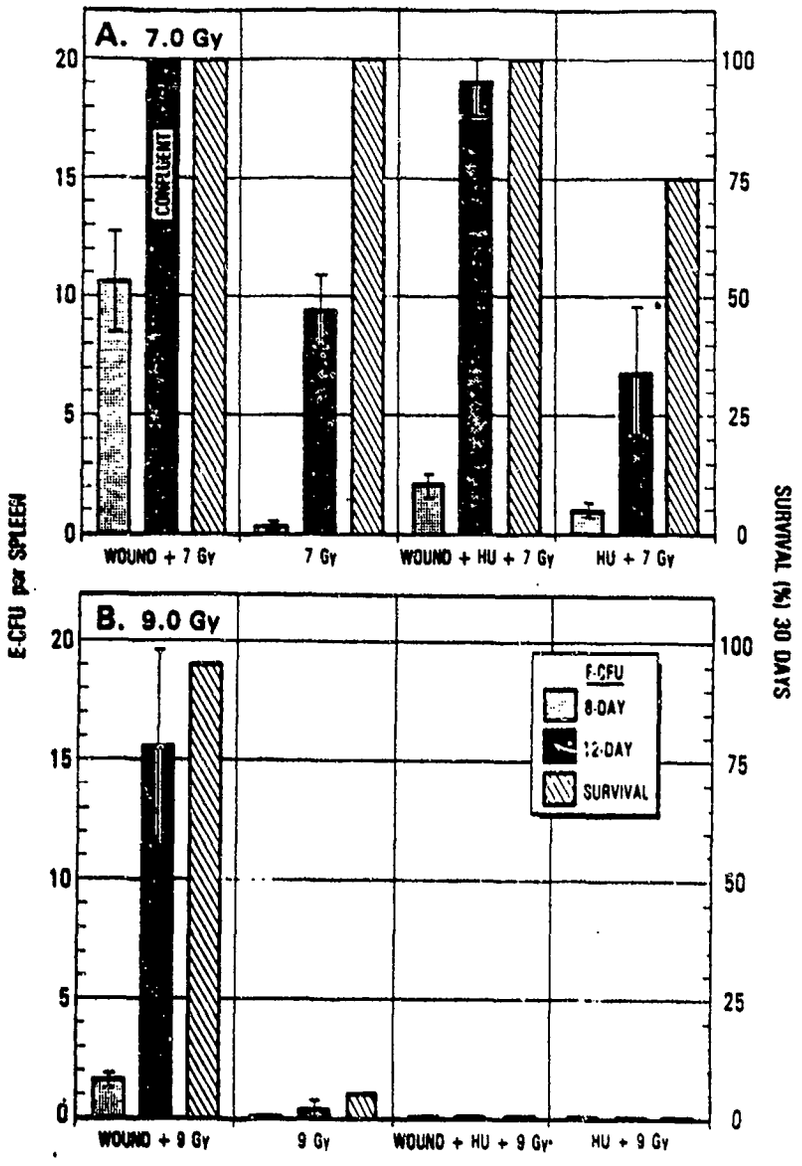
The E-CFU and survival data obtained at 7 Gy and 9 Gy irradiation for four treatment groups are presented in Fig 4. Control groups of mice either wounded, given HU, or injected with HU followed by wounding all survived, and are not presented in the figure. At both radiation doses, wounding before irradiation resulted in increased numbers of 8-day and 12-day E-CFU, compared to that seen in animals given radiation only. At the $LD_{100/30}$ for irradiation-only mice, the increased 8-day and 12-day E-CFU quantities can be compared to the 95% 30-day survival obtained in a similarly treated group of mice.

HU treatment of wounded mice at 4 h before 7 Gy irradiation resulted in decreased E-CFU quantities and a complete reduction in that cell compartment in wounded mice given HU before 9 Gy. HU treatment resulted in some reduction in survival percentage of mice only exposed to 7 Gy and completely obviated survival in all wounded irradiated or irradiated control mice given 9 Gy.

Size differences between some of the 8-day and 12-day E-CFU were noted. Colony size in 8-day E-CFU ranged between 0.5 and 2.0 mm. Similar sized E-CFU were observed for 12-day E-CFU, but about 10-40% of each spleen's colonies were about 3.5 mm in size. This observation could be accounted for by postulating either that: a) the size differences were due to cellular proliferation differences between the colonies, resulting in different colony sizes; or b) more than one type of cell could produce endogenous spleen colonies. The latter idea was previously posited as an explanation for the discrepancy in numbers and location seen in 7-8 day versus 9-12 day colonies formed in irradiated mice injected with low quantities of syngeneic marrow cells (9). These authors suggested that early colonies had limited potential and were akin to in vitro grown burst-forming unit-erythroid (BFU-E) while older colonies were pluripotential in nature. If this were indeed the case, then the cells from spleens containing these colonies might have different capabilities of rescuing lethally

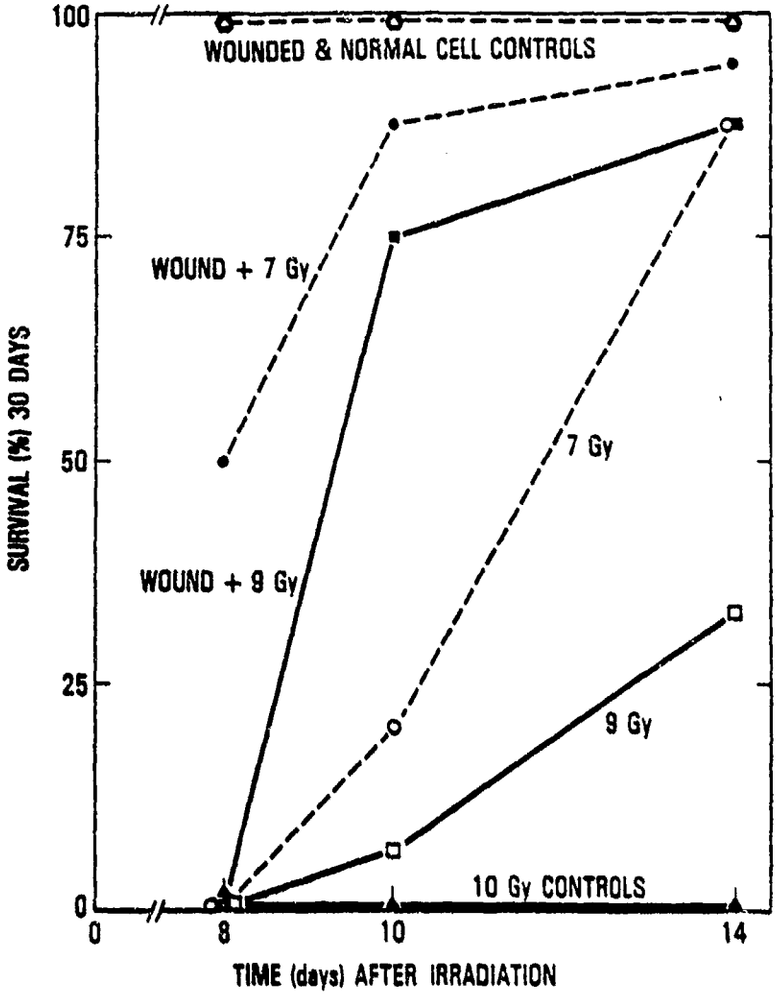
irradiated mice. This idea was tested in a series of transplantation studies.

FIGURE 4



Histogram depicting endogenous colony-forming unit spleen (E-CFU) at 8 and 12 days and percent 30-day survival in B6CBF1 female mice given (A) 7 GY and (B) 9 Gy. Hydroxyurea (1 mg/g body weight) was injected i.p. into mice 4 h before irradiation. Mice were wounded at 24 h before irradiation. Mean values are indicated ± S.E.

FIGURE 5



Percent 30-day survival of mice irradiated with 10 gy and engrafted with 2.5×10^6 spleen cells obtained from donor animals at 8, 10, or 14 days after either 7 Gy or 9 Gy. Combined-injured donor mice were wounded at 24 h before irradiation. All mice engrafted with spleen cells from normal mice or wounded mice survived. Since these data are similar only one curve labeled "Normal Cell Controls" is used in the figure. All 10-Gy irradiated control mice died. Note that 2/12 prospective donor mice given 9 Gy died by day 14. Percent survival recorded with cells from these mice is based on 2 survivors of 6 animals able to be engrafted. All other data points based on N = 16 from 2 replicate experiments.

TRANSPLANTATION STUDIES. Presented in Fig 5 are the 30-day survival data of mice given 10 Gy of radiation and 2.5 x 10^6 $B6CBF_1$ female nucleated spleen cells. Mice were engrafted with spleen cells from other mouse groups: a) untreated controls and wounded only; b) wounded 24 h prior to either 7 or 9 Gy; or c) irradiated only with either 7 or 9 Gy. The survival of control mice irradiated with 10 Gy and control irradiated mice engrafted with 2.5 x 10^6 spleen cells from both wounded and untreated control mice is also depicted. In Fig 5, one line is used to indicate the survival of mice engrafted with spleen cells from untreated controls and wounded only animals. Spleens were taken at 8, 10, and 14 days after irradiation; cell suspensions were prepared and transplanted into irradiated mice.

The capability of spleen cells from injured mice to rescue irradiated mice depended on the three factors of wound trauma, radiation dose, and the time point when cells were harvested after the combined injuries. Wounding prospective donor animals before irradiation evoked an earlier and greater survival percentage in irradiated transplanted mice than cells from mice only irradiated. Additionally, survivors were obtained both earlier and in greater numbers when prospective wounded donors were given 7 Gy than those given the 9 Gy dose. Thus, while spleens of mice examined at various times after irradiation may harbor E-CFU formed either by cells with limited or multipotential capacities or both, a standard number of spleen cells engrafted into lethally irradiated mice is capable of producing hematopoietic rescue. It is well established that cells with multipotential capacity are necessary to rescue irradiated animals. Thus, our data point to the idea that in a standard size inoculum and at various time intervals after experimental treatment, sufficient multipotential cells are present to bring about survival from lethal radiation injury. Whether these cells were solely responsible for E-CFU formation at the times tested is a moot point.

All prospective wounded, irradiated, and combined-injured donor mice survived until used for the transplantation studies on days 8 and 10 after treatment. However, most prospective donor mice given 9 Gy only were dead by day 14 after irradiation. The survival fraction (2 survivors/6 engrafted) on day 14 is based on a small number (2 of 12) of animals that survived 9 Gy until that time. Thus, these donors did not represent the effect seen in the majority of mice.

DISCUSSION

In the experiments reported here, wounding mice before irradiation appeared to influence the hematopoietic compartments in ways known to enhance survival from radiation. First, in the CFU-s and GM-CFC compartments, concentrations of these elements started to return toward normal first in the bone marrow and then in the spleen, sooner than that for irradiated control mice. Secondly, in the combined-injured animal, the marrow and splenic concentrations of GM-CFC and M-CFC were not reduced to the levels seen in irradiated mice. These observations, taken together with the findings that myeloid prolifer-

ative elements are necessary to enhance survival in irradiated animals can account for the enhanced survival of wounded irradiated mice.

The $LD_{50/30}$ of mice wounded after irradiation and of irradiated control mice were not significantly different. This is surprising, since a number of investigators reported that wounding after irradiation added significantly to the mortality incidence (1,2). The use of clean wounding techniques and the animal husbandry described earlier may account for the observations recorded here. Nonetheless, infections influenced survival fractions and survival times in nearly all wounded animals that died after radiation exposure. It is conceivable that the splenic myeloproliferative response in wounded mice in the post-irradiation period could account for the enhancement of survival. Splenic extramedullary hematopoiesis occurs in normal and physiologically stressed mice. However, in rats, splenic hematopoiesis is severely reduced. This difference in extramedullary hematopoiesis between these species may help explain the conflicting data surrounding wound-enhanced survival from irradiation in rodents. However, it was previously determined that wounding enhances survival equally well in splenectomized, sham-splenectomized mice and in control unoperated mice given 9 Gy radiation (6). This suggests that enhanced survival of mice wounded before irradiation is independent of extramedullary splenic hematopoiesis.

The mechanism by which wound trauma enhances survival and hematopoietic recovery after exposure to radiation is of interest. Adrenalcorticosteroids may be involved in the physiologic adaptation processes instituted by the host after wound trauma (10) and in the combined-injured animal (11). In the latter report (11), wounding mice 48 h before 500 R (a sublethal dose of radiation) resulted in twofold to threefold increases in the adrenal content of corticosteroids. No attempt was made to correlate corticosteroid levels in wounded mice exposed to lethal doses of radiation. In the wound trauma studies (10), adrenalectomy of both normal and wounded male mice resulted in a twofold to threefold increase in CFU-s over that for intact normal and wounded animals (10). Importantly, however, adrenalectomy elicited a twofold increase in CFU-s response only in normal female mice and not in wounded female mice. This observation points to the idea that the clonogenic cell responses reported here may be dependent on the sex of the wounded individual. This idea was tested with groups of male and female mice given equivalent $LD_{0/30}$ and LD 100/30 radiation doses. E-CFU were counted 8 days after irradiation, and these data are presented in Table 1.

Wounding at 24 h before either of the radiation dose ranges resulted in increased quantities of E-CFU in both male and female mice over that seen in irradiated control animals. However, a distinct sex-based difference was seen in that the E-CFU responses of female wounded irradiated mice were statistically higher ($P<0.01$) than that for male wounded irradiated animals. Quantitative differences in E-CFU notwithstanding, 90-95% of all wounded male and female mice survived after exposure to approximately $LD_{100/30}$ radiation doses.

Thus, the quantitative differences in E-CFU between sexes does not coincide with different survival percentages for wounded irradiated mice.

TABLE 1
Endogenous CFU in male and female mice wounded 24 hours before irradiation

TREATMENT[a,b]	$B6CBF_1$ (Female)	$B6CBF_1$ (Male)
Wounded 24 hr before 700-750 rad	14.1 ± 1.5[c]	5.1 ± 0.6
700-750 rad only	0.5 ± 0.1	1.2 ± 0.3
Wounded 24 hr before 900-950 rad	1.7 ± 0.4	0.4 ± 0.2
900-950 rad only	0	0

a Female mice - 700 or 900 rad; male mice 750 or 950 rad.
b N = 16 in all groups.
c Mean number of E-CFU (mean ± 1 S.E.) counted 8 days after irradiation. Statistically very significantly different ($P<0.01$).

The pathophysiologic stimulus of skin wounding and subsequent healing results in a number of changes in the mature cells of the peripheral blood pool (12). For example, erythrocytes are lost through the wound site, and platelets, through their aggregation and adhesion capacities, attempt to maintain homeostasis (13). Along these lines, in mice, the daily removal of about 20% of the blood volume for 4 consecutive days enhanced survival from midlethal doses of irradiation (14). Thus, it is tempting to think that the survival recorded here was enhanced by hemorrhage-induced aplasia. However, hematocrits taken 1 day after wounding (39 ± 3%) did not differ significantly from that of control values (42 ± 3%). Additionally, in our studies, splenectomy does not abolish the enhanced survival seen in wounded and irradiated mice (6), whereas splenectomy abolished the radioprotective effect in phlebotomized mice (14).

Another pathophysiological consequence of wound trauma in mice is the synthesis and release into the circulation of an acute-phase substance known as C-reactive protein (CRP)(15). Depending on the initial wound size, CRP amounts are increased 25% above control values (17-24 ug/ml serum) the first day after wounding, rising to onefold to twofold increases 2-4 days after trauma. Serum CRP levels return to the control value range by day 5 after wounding. In in vitro studies, CRP was implicated in the regulation of M-CFC (16). In the combined injury model used here, CRP quantities are elevated at the time of

irradiation because wounding had occurred 24 h previously. Thus, from our in vivo data and the in vitro work previously mentioned, it is tempting to think that CRP may be one substance involved in the modulation of the M-CFC compartments of the combined-injured mouse.

In addition to the ideas already discussed, other mechanisms may be involved in wound-enhanced survival and hematopoietic recovery. For example, intestinal cell-tight junctions are disrupted in mice and rats after radiation injury (17,18), and endotoxin, released by the intestinal microflora, may pass through the injured sites into the circulation. In agreement with this, endotoxin, when injected shortly before or after irradiation, protects both conventional (19,20) and germ-free animals (21). Additionally, endotoxin is a potent stimulator of colony-stimulating factor (CSF) (22), and CSF is increased in the serum of conventional mice after radiation injury (23,24). CSF, generated by host tissues in response to tissue trauma, may also enhance myelocytopoiesis (25), and this, in conjunction with the endotoxin-stimulated CSF, may account for wound-enhanced survival from irradiation. In support of this, we have found a serum-borne factor in mice after wound trauma that promotes GM-CFC growth of normal mouse cells. These data are the subject of a pending report (15).

SUMMARY

Skin wounding 24 h before but not 24 h after whole-body ^{60}Co irradiation results in enhanced survival (DRF 1.2). Slopes of survival curves, dose-response survival patterns, and morphologic observations support the idea that infectious complications lead to early death (8-14 days) in most mice wounded after irradiation and in late death (>21 days) in most mice wounded before irradiation. Wounding before irradiation produced increases in both marrow and splenic clonogenic cells, and they commenced earlier and reached greater quantities than those found for irradiated mice. E-CFU quantities were increased by wounding before radiation. Spleen cells obtained from mice 1-2 weeks after the combined injury were found to rescue more lethally irradiated mice than spleen cells from mice only irradiated. Hydroxyurea eradication of trauma-induced endogenous spleen colonies supports the idea that wounding before radiation enhances survival and hematopoietic recovery by stimulating resting hematopoietic clonogenic cells into DNA synthesis.

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