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As the exposure to ionizing radiation increases during a nuclear event, symptoms of illness which appear first are caused by injury to the most radiosensitive blood cell forming system in the bone marrow. The precise mechanism of the interaction of radiation on cellular constituents must be understood before meaningful therapeutic means for the enhancement of recovery can be developed. Steel-Dickie mice which have a genetic defect were selected for a model to determine →

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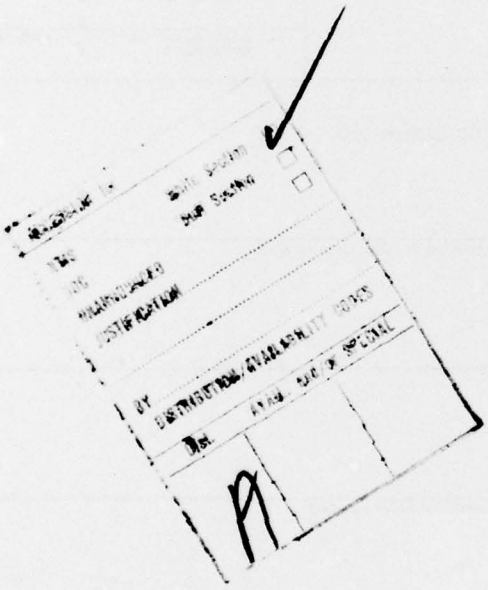
20. ABSTRACT (continued)

some of the interaction of ionizing radiation with bone marrow cells. Therefore, population sizes of high self-renewal potential stem cells, i.e., colony forming units and low self-renewal potential stem cells, i.e., transient endogenous colony forming units in Steel (Sl/Sl^d) mice and their normal congenic littermates were measured and compared. By correcting for differences in the seeding efficiency "f", it was possible to demonstrate that Sl/Sl^d mice suffer a deficiency of both stem cell populations. It is concluded that the defective stromal tissue of the Sl/Sl^d mouse does not support normal size stem cell populations. However, as noted in the discussion, it remains an open question as to whether the defective stromal tissue supports normal differentiation at the stem cell level. This question will be investigated in a follow-up study. Nonetheless, the present study has demonstrated the important role of short-range proliferation factors as opposed to long-range humoral factors in the regeneration of blood forming tissue in the postirradiated animal.



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INTRODUCTION

Peripheral blood colony forming units (CFU) can be distinguished from marrow CFU by their low self-renewal potential.[8] Because Sl/Sl^d mice suffer a deficiency of peripheral blood CFU,[7] it was hypothesized that mutations at the Steel loci disrupt erythropoiesis very early in the erythron, perhaps at the point of commitment of multipotent CFU to the erythrocytic cellular line of differentiation. If this hypothesis is correct, then one would expect an equivalent number of high self-renewal potential CFU, but not low self-renewal potential transient endogenous colony forming units (TE-CFU)[2] in Sl/Sl^d mice and their normal (+/+) littermates. Therefore, in the present study CFU and TE-CFU population sizes in Sl/Sl^d and +/+ mice were measured and compared.

METHODS

Mice. Experimental B6WCF1 mice of genotypes +/+ and Sl/+ were raised at the AFRRI by mating C57BL/6J-+/+ females and WC/Re-Sl/+ males. Experimental WCB6F1 mice of genotypes +/+ and Sl/Sl^d mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. Recipient mice used in the in vivo CFU assay were B6WCF1 hybrids obtained by mating C57BL/6J-+/+ females with WC/Re-+/+ males at the AFRRI.

Irradiation. Mice were exposed to ^{60}Co whole-body radiation at a dose rate of 150 rads/min to total doses of 600 to 950 rads.

Biological compounds. Lyophilized step III erythropoietin (epo) prepared from plasma of phenylhydrazine treated sheep (Lot 3007-8) was obtained from Connaught Medical Research Laboratories, Ontario, Canada.

TE-CFU assay. B6WCF1 mice of genotypes Sl/+ and +/+ were exposed to 600-800 rads of whole-body radiation and either (1) bled from the orbital sinus (~.5 ml), (2) injected i.p. first with 10 units of epo and 1 unit daily thereafter or (3) injected i.p. with 0.3 ml of heparinized Sl/Sl^d plasma. At intervals thereafter groups of mice were euthanatized and their spleens removed and examined for colonies.

CFU assay. The CFU assay of Till and McCulloch[12] was performed as previously described.[6] In some experiments, the spleens were sectioned (5 μm), H and E stained, and the type of colonies in midline sections determined microscopically.

CFU seeding efficiency. The 2-hour seeding efficiencies "f" of CFU from WCB6F1 mice of genotypes +/+ and Sl/Sl^d were determined according to the method of Siminovitch et al.[11] Briefly, in the case of femoral CFU, 6×10^6 marrow cells from a pool of three donor mice were injected into five intermediate recipients. Two hours later the mice were euthanatized, their spleens removed and 1/16 to 1/12 of a spleen was then injected into 10 secondary recipients. In the case of splenic CFU, 1 to 2×10^7 spleen cells from a pool of three donor mice were injected into five intermediate recipients. Two hours later 1/4 to 1/2 of a spleen was injected into four secondary recipients. The CFU content of the original cell suspensions was determined in primary recipients by the in vivo CFU assay.

Hydroxyurea (OHU) killing. Each donor mouse was injected i.v. with 900 mg/kg of body weight of freshly prepared OHU in isotonic saline.[10] After 2 hours the mice were euthanatized and a femur cell suspension derived from three mice was prepared. The CFU content of the femurs was then determined and compared with the CFU content of the femurs of control mice which had received no treatment.

RESULTS

Femur cellularity and CFU numbers. Presented in Table 1 are the number and colony forming potential of nucleated cells from the femurs of WCB6F1-Sl/Sl^d and +/+ mice. As reported earlier[6] the average number of nucleated cells per femur is reduced in Sl/Sl^d mice to approximately 40 percent of control values. Although the remaining cells have a lower colony forming capacity than do cells from +/+ mice, this appears to be a consequence of a lower seeding efficiency "f". Thus, on a per cellular basis the concentration of CFU in the

femurs of Sl/Sl^d mice appears to be normal, but on an organ basis the number of CFU parallels the number of nucleated cells resulting in an overall deficiency of Sl/Sl^d femur CFU of approximately 50 percent.

Table 1. Colony Forming Ability of Marrow Cells

	+/+	Sl/Sl^d
Colonies/10^5 cells	23.52 ± 1.84* [10]†	13.52 ± 1.59 [11]
Nucleated Cells (x 10^7)	2.55 ± .24 [10]	1.55 ± .13 [11]
"f" (%)	15.10 ± .14 [4]	9.37 ± .72 [4]
CFU/10^5 cells ǂ	155	144
CFU/Femur ‡	39,800	19,500

*Mean ± S. E.

†Figures in brackets refer to number of separate determinations.

ǂCalculated from data for "f" and colonies/10^5 cells.

‡Calculated from CFU/10^5 cells and average number of cells per donor femur.

Splenic cellularity and CFU numbers. Like marrow cells, Sl/Sl^d splenic hematopoietic cells have a lower colony forming potential (due to a lower "f") than do +/+ splenic cells. However, the nucleated cell count of the Sl/Sl^d spleen is normal and, therefore, the size of the splenic CFU population in Sl/Sl^d mice appears to be normal on both a per cell and organ basis (Table 2).

Colony morphology. The ratios of erythroid colonies to granuloid colonies (E/G ratio) observed in midline section of spleens from primary and secondary recipients (mean ± S. E. M. of two determinations) receiving Sl/Sl^d marrow cells were 1.02 ± .34 and 1.03 ± .83, respectively. While for +/+ marrow cells, the values were 1.69 ± .31 and 1.65 ± .63, respectively. These

E/G ratios suggest that the lower "f" value characterizing CFU of Sl/Sl^d origin probably is not a result of an impaired spleen localization potential of erythrocytic committed or committable CFU subpopulation from Sl/Sl^d mice as compared to +/+ mice.

Table 2. Colony Forming Ability of Spleen Cells

	+/+	Sl/Sl^d
Colonies/10^5 cells	2.89 ± .59* [5]†	0.87 ± .33 [5]
Nucleated Cells/Spleen ($\times 10^{-8}$)	1.75 ± .30 [5]	2.20 ± .17 [5]
"f" (%)	13.8 [1]	5.7 ± 1.6 [2]
CFU/10^5 cells ǂ	20.8	15.3
CFU/Spleen ‡	36,000	33,900

*Mean ± S. E.

†Figures in brackets refer to number of separate determinations.

ǂCalculated from data for "f" and colonies/10^5 cells.

‡Calculated from CFU/10^5 cells and average number of cells per donor spleen.

Marrow CFU proliferation. In previously reported work, Lahiri et al.[5] suggested that a relationship might exist between the state of CFU cycling and the numerical "f" value. Apparently, rapidly proliferating CFU are characterized by a lower "f" value than quiescent CFU. Experiments undertaken with OHU suggest that this relationship also holds for CFU of Sl/Sl^d origin. This S-phase specific cytotoxic drug, within 2 hours after i.v. injection, reduced the size of the marrow Sl/Sl^d CFU population to 40 percent of control values (Table 3) while having no effect on the size of the marrow CFU population in normal +/+ mice.

Table 3. The Effect of OHU on Femur CFU Population Size in Sl/Sl^d Mice and Their Normal +/+ Congenic Littermates

Type	OHU	Cells/Femur	Colonies/10^5	"f"	CFU/10^5	CFU/Femur
+/+	-	2.7×10^7	24.0	12.0	200	54,000
+/+	+	2.0×10^7	19.3	7.2	268	52,000
Sl/Sl^d	-	1.5×10^7	15.4	9.0	270	40,050
Sl/Sl^d	+	1.0×10^7	21.2	8.3	254	25,400

- Control group recieving no treatment.

+ Experimental group receiving OHU.

Apparent TE-CFU population sizes. While marrow CFU are characterized by their high self-renewal capacity, splenic TE-CFU are characterized by their low self-renewal capacity.[2] Because the population size of femoral, but not splenic, CFU is restricted in Sl/Sl^d mice, it was of interest to determine what, if any, effect the Steel stromal defect might have on the population size of low self-renewal splenic TE-CFU. Therefore, groups of B6WCF1-Sl/+ mice and their normal (+/+) congenic littermates were exposed to 600 rads whole-body radiation, bled immediately afterwards and groups of three animals euthanatized at intervals thereafter for spleen colony counts. As seen in Figure 1, fourfold fewer transient endogenous colonies appeared in the spleens of Sl/+ mice than in the spleens of +/+ mice. Examination of midline sections of the spleens removed at 5 days indicated that in both +/+ and Sl/+ mice over 80 percent of the colonies were erythrocytic. Other erythrocytic stimulants and radiation doses were employed (Table 4). However, it was not possible to demonstrate an equivalence of TE-CFU in +/+ and Sl/+ mice under any of these conditions.

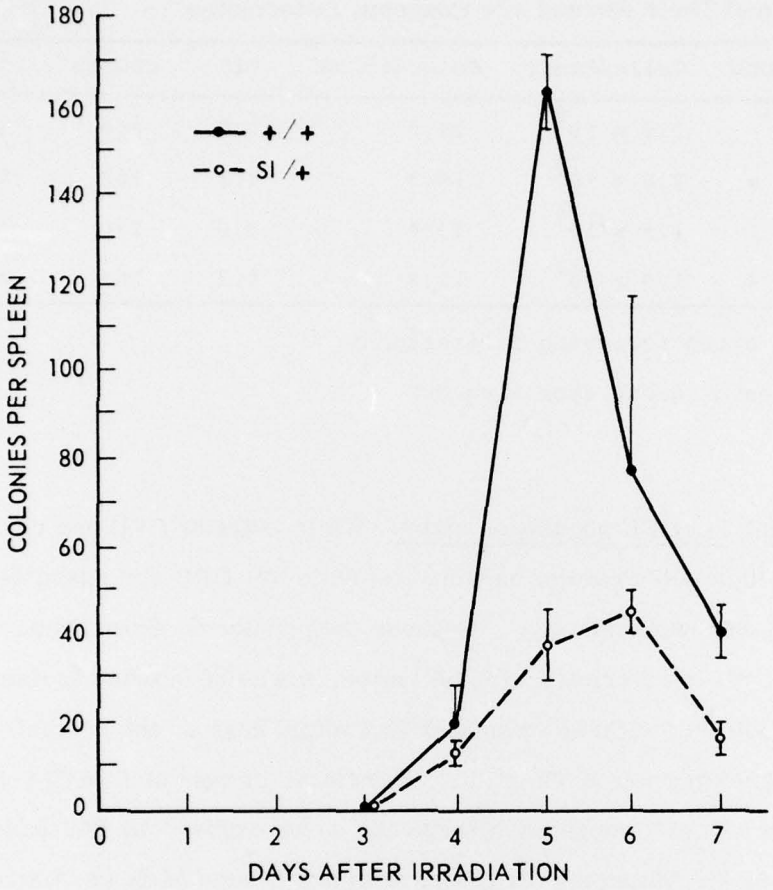

Figure 1. A comparison of transient endogenous colony development in bled B6WCF1 mice of genotypes Sl/+ and +/+. Means and standard errors are plotted for one experiment.

DISCUSSION

One of several hypotheses concerning the effect of Steel loci mutations on erythropoiesis is that the defective stromal tissue in Sl/Sl^d mice fails to support normal commitment of hematopoietic stem cells to the erythrocytic cellular line of differentiation. Indeed, it has already been reported from this laboratory that Sl/Sl^d mice suffer a deficiency of those CFU characterized as having a low self-renewal potential, i.e., peripheral blood CFU.[7,8] In the present work, it was

Table 4. Number of 5-Day Transient Spleen Colonies Appearing in Sl/+ and +/+ Mice Following Various Irradiation Doses and Erythropoietic Treatments

Sex	Genotype	Rads	Postirradiation Treatment day 0; day 0 to sacrifice		Colonies/Spleen (no. of mice)
♂	+/+	600	bled	none	163.3 ± 8.5 (3)
♂	Sl/+	600	bled	none	37.0 ± 8.1 (3)
♂	+/+	600	none	none	23.5 ± 4.5 (2)
♂	Sl/+	600	none	none	5.0 ± 1.0 (2)
♀	+/+	650	bled	none	24.3 ± 8.7 (3)
♀	Sl/+	650	bled	none	5.0 ± 2.5 (3)
♀	+/+	650	none	none	2.6 ± 1.7 (3)
♂	+/+	750	Sl/Sl^d plasma	none	13.7 ± 4.2 (3)
♂	Sl/+	750	Sl/Sl^d plasma	none	2.0 ± 1.5 (3)
♂	+/+	750	none	none	.6 ± .6 (5)
♂	Sl/+	750	none	none	0 (1)
♀	+/+	800	10 units epo	1 unit epo	13.8 ± 8.2 (4)
♀	Sl/+	800	10 units epo	1 unit epo	.8 ± .9 (4)

also observed that Sl/+ mice suffer a deficiency of TE-CFU. These stem cells like peripheral blood CFU are characterized by their low self-renewal potential.[2] Therefore, if the genetic "block" is between the high self-renewal (multipotent?) and low self-renewal (committed?) CFU compartments, then one might expect a normal high self-renewal CFU population size in Sl/Sl^d mice. As such, an extensive amount of effort has been directed towards measuring and comparing CFU population sizes in +/+ and Sl/Sl^d mice.

In contrast to a previous study reported from this laboratory,[6] it was found in the present study that the population size of splenic CFU in Sl/Sl^d mice was

the same as that in +/+ littermates. This was a result primarily of a lower "f" characterizing Sl/Sl^d splenic CFU as compared to +/+ splenic CFU. However, in agreement with the previous study, a gross deficiency of femoral CFU in Sl/Sl^d mice was observed even though in the present study differences in "f" were taken into consideration when calculating total femoral CFU population sizes. This anomaly of a normal size splenic CFU population detected in the presence of a subnormal size femoral CFU population might argue against the Steel defect specifically "blocking" commitment of multipotent CFU to the erythrocytic cellular line of differentiation for marrow CFU are believed to have a higher self-renewal potential than splenic CFU.[1]

However, the present characterization of CFU in Sl/Sl^d mice would indicate that CFU in these mice are responding to a strong stimulus for differentiation. This observation is in agreement with the work of Harrison *et al.*[3] who reported that the erythropoiesis in Sl/Sl^d mice is essentially stress erythropoiesis. Because stress erythropoiesis is characterized by substantial increases in extramedullary but not medullary CFU population sizes,[4,9] it is uncertain whether the size of various CFU population in Steel mice should be considered in the light of (1) the size of comparable CFU populations in normal +/+ congenic littermates as was done in the present study or (2) in terms of adaptive long-term erythrostatic processes most likely initiated and perpetuated by the chronic macrocytic anemia suffered by these mice. Therefore, a comparison of CFU population sizes in polycythemic Sl/Sl^d mice and their +/+ congenic littermates is now being attempted. In light of the present work, this comparison should allow a more meaningful conclusion to be drawn concerning the interaction of the Steel stromal element with multipotent-committed CFU populations and, in general, the erythrocytic cellular line of differentiation.

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