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Different Responses of Two Mouse Strains to 650 Rads and Protection by Surgical Stress[1] (38695)

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Irradiation particularly affects fast-proliferating tissue of the hemopoietic system such as bone marrow. Animal mortality depends on the dose rate as well as the total dose and type of radiation (1, 2). The extent of proliferation of hemopoietic cells remaining after irradiation is a measure of repair capacity (1) or radioresistance (3), and this may be related to the size of cell cycle phase of the bone marrow stem cell pool remaining (4, 5), or to the capacity of this remaining pool to recover from radiation insult (6).

The bone marrow stem cell pool can be partly measured by counting the number of endogenous stem cells in the spleen which survive sublethal irradiation and proliferate to form colonies (7), or by exogenously repopulating lethally irradiated syngeneic recipients with bone marrow cells (8). These hemopoietic spleen colonies are clonal (9) and pluripotent (10, 11) in origin. The same stem cell can develop into either a colony of committed granuloid cells or committed erythroid cells, depending on the microenvironment in which it happens to settle (12–14).

Stress, whether intrinsic or extrinsic, affects physiological tissue function. The increase in radiation survival produced by such diverse treatments before irradiation as bleeding (15, 16), cysteamine-S phosphate (17), endotoxin (15, 18), estradiol (19), foreign plasma (15), phenylhydrazine (20), phytohemagglutinin (21), polycythemia (22, 23), vinblastine (24), and viral infection (25, 26) may suggest a common physiological mechanism of stress. We observed a profound difference in mortality to 650 rads between sham-operated and untreated C57Bl/6J mice when the irradiation dose was given one week after mice were sham-operated, and investigated the possible hematologic factors involved in both C57Bl/6J and DBA/1J mice. We report here for the first time that delaying irradiation as long as three weeks after stress can confer significant hematological protection, thus emphasizing the importance of using sham controls if the duration of experiments is at least three weeks and involves irradiation.

Materials and Methods. Figure 1 shows the experimental protocol. C57Bl/6J and DBA/1J female mice (Jackson Memorial Laboratories), 38–47 days old, were divided into five groups of ten mice each. Stressed groups were sham-operated by a routine sham thymectomy procedure of teasing the submaxillary gland up and cutting the sternum after anesthesia with Nembutal (27). There was no apparent bleeding. Mice in three groups received 650 rads gamma rays from a ^{137}Cs source in a Model M Gammator at a dose rate of 110 R/0.1 min. Each animal was separately irradiated upright in a perforated plexiglas cylinder while rotating at 4.5 rpm. Mice were checked daily for mortality.

Control groups included non-stressed irradiated mice and stressed unirradiated mice as well as untreated mice. In one group the elapsed time between stress and irradiation was increased from 1 to 3 wk in order to evaluate temporal decrease in protection by stress. Ten days after irradiation, hematocrits and total and differential white blood cell (WBC) counts were determined on blood taken from the periorbital sinus in capillary tubes. Endogenous (7) and exogenous (8) colony forming units (CFU) were determined by the method of Till and McCulloch. A 23-gauge needle mounted on a 1 ml syringe containing RPMI 1640 medium was inserted into snipped ends of the right femur and the marrow was extruded by forcing the

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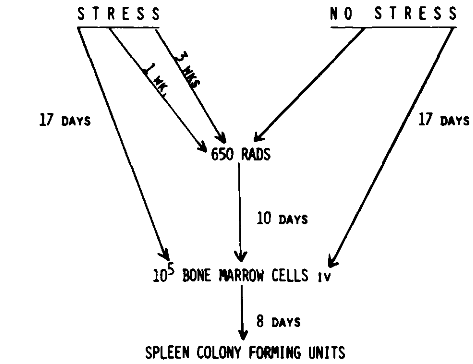

FIG. 1. Experimental Protocol.

TABLE I. PERCENT SURVIVAL OF C57Bl/6J AND DBA/1J MICE FOLLOWING 650 RADS[a]

	Sham-operated + 650 rads		650 Rads	
Strain	Number of mice surviving/ total mice	Percent	Number of mice surviving/ total mice	Percent
C57Bl/6J	27/41	66	1/59	1.7
DBA/1J	22/36	61	21/41	51

[a] Most mice died within 30 days.

medium through the marrow cavity. After cell dispersion by successive passages through 25 and 27 gauge needles, nucleated bone marrow cells suspened in 2 ml cold medium were counted in a hemacytometer.

Within 2 hr lethally irradiated (880 rads) syngeneic age-matched mice received 1×10^5 bone marrow cells iv from each donor. Spleens were removed from donors for endogenous CFU and from recipients for exogenous CFU 8 days later and fixed in Bouin's fluid for 24 hr before counting the colonies under a dissecting microscope.

Data are presented as the mean of ten mice per group ± standard error.

Results. Results are presented in Tables I–II and Fig. 2–4. There was a significant difference in the survival of C57Bl/6J mice after 650 rads with (66%) and without (1.7%) stress 1 wk prior to irradiation (Table I). In contrast, similarly treated DBA/1J mice did not show a significant difference.

Hematocrit and peripheral leukocyte counts. Stress alone significantly depressed the hematocrit in C57Bl/6J mice (Table II). Stress followed by irradiation elevated the hematocrit over that of unstressed irradiated mice. Stress 3 wk before irradiation increased the hematocrit in C57Bl/6J mice, although not significantly so. The erythrogenic compartment of C57Bl/6J mice was more strongly influenced by stress than that of DBA/1J mice. C57Bl/6J and DBA/1J mice show identical patterns of leukocyte levels following the various treatments (Fig. 2). Stress alone depressed the WBC count, but stress one or 3 wk before irradiation significantly elevated the WBC count over that after irradiation alone. The absolute lymphocyte numbers generally followed the same pattern (Table II).

Endogenous CFU. Endogenous CFUs are a measure of the repopulating capacity of the pluripotent hemopoietic stem cells in the spleen which survive irradiation. Irradiation after stress increased the number of endogenous CFUs, especially in DBA/1J mice (Table II). Significantly greater numbers of endogenous CFUs were found after irradiation alone in C57Bl/6J mice as compared to DBA/1J mice, indicating either a more radioresistant, a larger stem cell pool, or a more rapid rate of proliferation of CFUs in C57Bl/6J mice. Stress caused a larger increase in the endogenous CFU pool in DBA/1J mice, which increase persisted for at least 3 wk with over-compensation at that time.

Exogenous CFU. Exogenous CFUs are a measure of the repopulating capacity per number of transplanted bone marrow cells. However, the fraction of potential CFU which lodge in the spleen and form colonies can change with treatment of the donor animal and complicate interpretation of the data. Stress tends to lower the CFU repopulating potential of C57Bl/6J bone marrow cells (Table II). Normal C57Bl/6J mice had 43% of the number of CFUs per 10^5 bone marrow cells that normal DBA/1J mice had, indicating that there was a lower proportion of stem cells in the C57Bl/6J bone marrow compartment or that a lower fraction of CFUs lodged in the spleen or proliferated to form colonies. At 10 days after irradiation, 33% of the normal C57Bl/6J CFU repopulating potential remained vs 42% of the normal DBA/1J CFU repopulating potential. C57Bl/6J mice thus

TABLE II. SELECTED HEMATOLOGIC VALUES FOR C57Bl/6J MICE.

Treatment	Hematocrit	Lymphocytes/mm^3[a]	Endogenous CFU/spleen	Exogenous CFU/10^5 BMC
Stress (1 wk) + 650 rads	41.9 ± 1.2	117	4.7 ± 2.9	1.6 ± 1.6
650 rads	36.9 ± 0.7	32	3.5 ± 0.4	5.0 ± 2.0
Stress (3 wk) + 650 rads	38.1 ± 1.1	100	4.4 ± 1.8	2.8 ± 0.7
Stress	49.3 ± 1.4	6,109 ± 527	0.5 ± 0.3	4.5 ± 3.5
None	54.2 ± 0.6	9,820 ± 1,504	0.5 ± 0.2	15.25 ± 1.6
Selected hematologic values for DBA/1J mice				
Stress (1 wk) + 650 rads	47.1 ± 1.2	227	2.9 ± 1.1	9.6 ± 2.2
650 rads	45.3 ± 0.8	30	0.4 ± 0.2	14.8 ± 6.0
Stress (3 wk) + 650 rads	41.0 ± 1.2	31	18.1 ± 2.9	16.0 ± 2.7
Stress	50.9 ± 0.9	4,680 ± 940	0.3 ± 0.2	38.9 ± 3.9
None	51.6 ± 0.7	8,052 ± 686	0.5 ± 0.2	35.2 ± 3.5

[a] The lymphocytes in three groups were pooled as not enough WBCs could be found per smear.

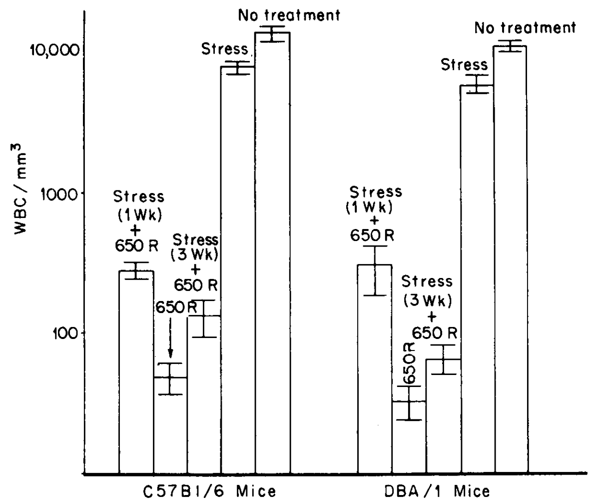

FIG. 2. Peripheral white blood cell counts of experimental groups of mice. Results were plotted logarithmically as the mean of ten mice per group ± SE.

had a lower CFU repopulating potential per bone marrow cell inoculum than DBA/1J mice at 10 days after irradiation.

Bone marrow cells/femur. Ten days after irradiation 4.1% of C57Bl/6J and 1.46% of DBA/1J nucleated bone marrow cells remained per femur. Normal C57Bl/6J mice had 1.7 times more nucleated cells per femur than normal DBA/1J mice. Stress alone significantly lowered the number of nucleated cells in the bone marrow compartment of both C57Bl/6J and DBA/1J mice (Fig. 3). Stress one week before irradiation significantly increased the number of nucleated cells over that seen after irradiation alone. This sparing effect was still apparent in DBA/1J mice three weeks after stress with an overcompensation.

CFU/femur. Stress depressed the total CFU/femur compartment in irradiated C57Bl/6J mice, but spared irradiated DBA/1J mice. This effect of stress lasted at least 3 wk, producing an overcompensation in DBA/1J mice (Fig. 4).

Discussion. We first observed a profound difference in survival between sham-operated and untreated C57Bl/6J mice after 650 rads. This study was initiated to seek an explanation for that observation. The results revealed marked strain differences in CFU

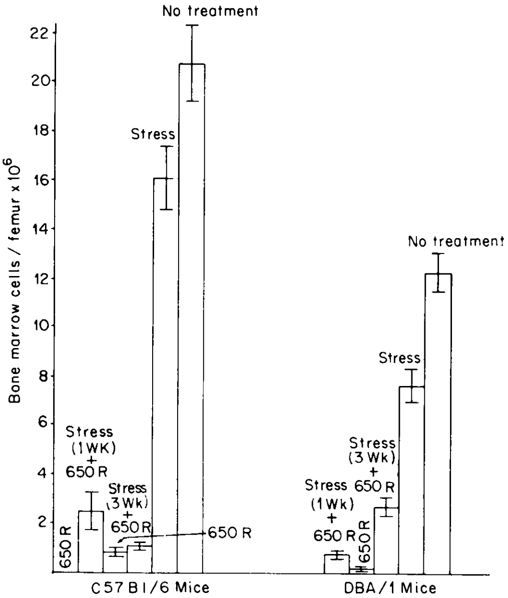

FIG. 3. The number of nucleated bone marrow cells per femur $\times$ 10^6 for experimental groups of mice. Results were plotted linearly as the mean of ten mice per group $\pm$ SE.

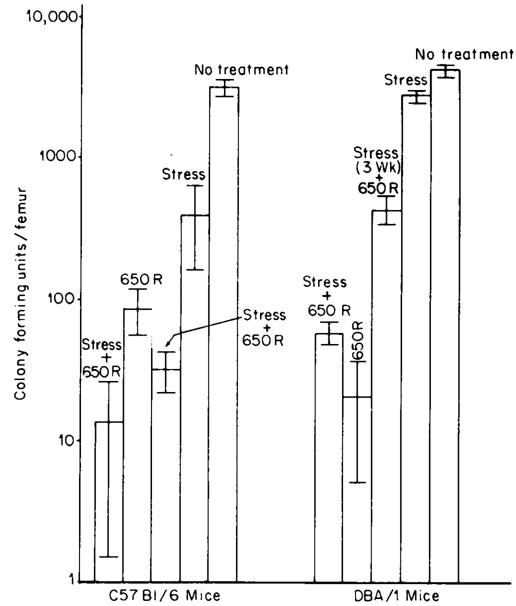

FIG. 4. The number of exogenous CFU per femur for experimental groups of mice. Results were plotted logarithmically as the mean of ten mice per group $\pm$SE.

behavior. The high mortality of C57Bl/6J mice has also been observed by McCulloch and Till (28), and by Sugahara and Tanaka (3), who found bleeding to increase radiation resistance at 670 rads, with 66% survival, identical to our observation.

The results of our study show that the surgical trauma inflicted affected the stem cell compartment for a longer period of time (3 wk) than do other radioprotective forms of stress reported, none of which lasted more than a few days before irradiation. The number of stem cells in a mouse is in a balanced steady state reflecting the rate of self renewal or turnover time of the cells, and the rate of removal or loss by differentiation, death, or migration. Stress may influence the differentiation of the granuloid cell line remaining after irradiation by altering the microenvironment to either increase the rate of differentiation or to increase the proportion of granuloid cells differentiating. Explanations for the larger increase in endogenous CFUs of DBA/1J mice after stress include: (a) a more rapid rate of proliferation of stem cells, (b) a decrease in the rate of differentiation, (c) an increase in radioresistance of stem cells, (d) a net migration of CFUs from the bone marrow to the spleen prior to irradiation.

Many studies evaluating the relationship between CFU behavior and survival of mice have been published with controversial results. Several authors have assumed that endogenous and exogenous CFUs have measured the same stem cell pool. Conflicting claims have been made that survival of the animal is related to the size of the stem cell pool (4, 5, 25, 29), and that survival does not parallel stem cell pool size (18, 20, 22–24). Survival of the animal has often been correlated with an increase in endogenous CFUs after irradiation (6, 15–17, 19, 21, 30–33), but usually has not been correlated with an increase in exogenous bone marrow CFUs.

In an attempt to explain these discrepancies, Boggs postulated that endogenous and exogenous CFUs were distinguished by existing in different stages of the cell cycle (34, 35). According to Chaffey and Hellman cells are most radiation resistant in the S phase with the majority of the cells producing transplantable CFUs being in the resting (Go) phase in normal mouse bone marrow (36). Boggs suggested that more stem cells might be in the S phase as a result of certain experimental treatments. Thus, endogenous CFUs might be selected for by their ability to survive irradiation. The decrease in exogenous CFUs could reflect decreased transplantability of S phase cells so that exogenous CFU cells were mostly in the nonreplicating Go phase (34, 35). Stress may cause stem cells to differentiate or it may recruit cells from Go into S phase. If all forms of stress increase endogenous CFUs and expand the proportion of stem cells in the S phase, then the stem cell compartment is easily influenced. Our data are compatible with Boggs' explanation of the kinetics of stem cell recovery from irradiation: an increase in endogenous CFUs with a decrease in exogenous CFUs. Unstressed C57Bl/6J mice had 43% of the proportion of exogenous CFUs of unstressed DBA/1J mice and this proportion decreased to 11.6% with stress. DBA/1J mice may have more stem cells in the Go phase than C57Bl/6J mice at any time. C57Bl/6J mice may have an abnormal pool of stem cells predominantly in the S phase which is compensated for by a greater number of nucleated cells per femur. Since irradiation at 650 rads killed all but 4.1% of the nucleated bone marrow cells in C57Bl/6J mice and 1.46% in DBA/1J mice, the main difference between the C57Bl/6J and DBA/1J strains must be in the rate of restoration of the bone marrow nucleated cell count, which was higher in DBA/1J mice than in C57Bl/6J mice by a factor of 1.4 times at 1 wk after stress and 12.7 times at 3 wk after stress. Comparison of the number of nucleated cells and endogenous CFUs with the depressed hematocrit in DBA/1J mice stressed 3 wk before irradiation suggest that competitive proliferative demands were made on stem cells of this strain; that is, a relative lack of erythroid cells in favor of proliferating CFUs. This has been reported for other strains of mice as well (10, 31).

C57Bl/6J mice are an autoimmune strain, producing antinuclear antibodies during old age (37). We (38), and Yuhas (39), found an increase in exogenous CFUs with age up to 11 mo in C57Bl/6J mice. The genetic defect responsible for autoimmunity might involve a delayed or insufficient control mechanism of the hemopoietic stem cell compartment. There is a wide range of genetic defects in hemopoietically deficient mice (40, 41). The stem cell compartment of autoimmune strains of mice should be studied as well as B-cells or T-cells in order to locate their genetic defect.

Whatever the mechanism, surgical stress significantly protected C57Bl/6J and DBA/1J mice from the effects of irradiation, and spared C57Bl/6J mice from death after 650 rads. Previous studies on radiation survival or hematological parameters which lacked sham-operated controls may need to be re-evaluated when treatment was less than three weeks before irradiation (33, 42–44).

Summary. Radiation survival of C57Bl/6J mice after 650 rads improved from 1.7% to 66% when mice were stressed surgically one week before irradiation. Surgical stress one or three weeks before irradiation increased endogenous CFUs and decreased exogenous CFUs in C57Bl/6J and DBA/1J mice. Survival may be related to the proportion of stem cells in different phases of the cell cycle, which was abnormal in C57Bl/6J mice and compensated for by a greater number of nucleated cells per femur.

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