lated after acetylation of endotoxin exhibits grossly reduced pyrogenicity and acute toxicity but retains the original potency in stimulating non-specific host resistance to infection.

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Suppression of the Foreign Bone Marrow Reaction by Preirradiation of Donor Mice. (26767)

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The bone marrow of normal mice is composed of a mixed population of cells differing in morphology and function. On transplantation of viable marrow cells into lethally irradiated homologous recipients, various blood-forming elements repopulate the marrow, the red pulp of the spleen [see review article of Smith and Congdon(1)] and the antibody-forming lymphoid tissues of the host(2,3). Probably only a small percentage of the marrow cells or their precursors are antibody-forming, since as few as 1×10^6 transplanted marrow cells protect against radiation lethality(4,5), whereas as many as 12×10^6 transplanted marrow cells do not confer on lethally irradiated recipients the capacity to synthesize antibodies(6). The radiation dose required to reduce cell survival by a factor of 0.37 is estimated at about 115 rads of Co^{60} rays for blood-forming cells(7) and 130 r of 250 kvp X-rays for spleen cells producing humoral antibodies(8). It seemed possible, therefore—the radiosensitivity of the 2 cell types being comparable—that the antibody-forming cells in mouse bone marrow could be largely eliminated by exposing the animal to an X-ray dose lethal to all but 2-6% of its total marrow population. Marrow thus deprived of immunologically active cells and transplanted into a lethally irradiated homologous recipient would presumably repopulate the recipient's hemopoietic sites but lack usual graft-versus-host immunological reactivity. Data presented here do, in fact, indicate that preirradiation of marrow donors reduces the incidence and severity of secondary disease in irradiated homologous recipients.

Materials and methods. Adult hybrid mice (5-8 months old) of the following strains were used as recipients and donors: (101/Cum × C3H/Anf Cum)F_1 (C57BL/Cum × 101/Cum)F_1, (C57L/Cum × A/He Cum)F_1, (C57BL/6 Cum × DBA/2 Cum)F_1, and (BALB/c Cum × A/He Cum) F_1. These strains are hereafter designated as $1C3F_1$, $B1F_1$, LAF_1, $B6D2F_1$, and CAF_1, respectively. In every case, donor and recipient were of the same sex. Recipients were exposed to 950 r whole-body X radiation; irradiated donors were exposed to 400 r or 500 r 30 minutes before sacrifice. In some instances, the donor mice were given a radioprotective compound, S, β-aminoethylisothiuronium · Br · HBr (AET), 20-30 minutes before irradiation. Nine milligrams of AET in 0.2 M phosphate buffer (pH = 7.4) were injected intraperitoneally per mouse. The X-ray dose rate was approximately 85 r/min; TSD, 93.5 cm; 300 kvp; 20 mA; 4.78 mm of Be inherent filtration and 3 mm of Al added filtration, and hvl, 0.55 mm of Cu.

* Operated by Union Carbide Corp. for U. S. Atomic Energy Comm.

TABLE I. Survival of Lethally Irradiated Mice Following Implantation of Homologous Marrow (30 Mice per Group).

Recipient strain	Donor		No. surviving 21 days	No. surviving 90 days		Incidence secondary morbidity and mortality (%)
	Strain	Treatment		Secondary disease present	Secondary disease absent	
$B1F_1$	CAF_1	None	29	0	0	100
		400 r	22	6	9	59
$1C3F_1$	$B6D2F_1$	None	30	2	0	100
		400 r	28	4	13	53
$1C3F_1$	LAF_1	None*	27	0	0	100
		"	30	6	0	100
		AET	30	3	0	100
		400 r	24	0	15	37
		AET + 400 r	30	6	18	40
		500 r	20	2	18	10
		AET + 500 r	28	0	28	0

* 1.5×10^6 nucleated cells inj. instead of 60×10^6.

Marrow cells were flushed from the femur with Tyrode's solution using a syringe with a 24-gauge needle. The nucleated cells were counted in a hemocytometer. Recipients were inoculated in a tail vein with 60×10^6 nucleated cells within 3 hours after irradiation, unless otherwise stated. The hemoglobin type in the peripheral blood of marrow recipients was characterized(9) at 60 and 90 days after marrow infusion to ascertain whether the donor cells had transplanted. Mortality was recorded for 90 days after treatment; the terms "early death" and "delayed death" refer to death during the first 21 days and during the 21- to 90-day-period after irradiation, respectively. The incidence of secondary disease was estimated by including the dead and clinically affected mice surviving more than 21 days after irradiation.

Results. The experimental results are presented in Table I. In all host-donor combinations, the unirradiated marrow protected against early radiation lethality. Secondary mortality, however, occurred during the second and third months. Variations in severity and incidence of secondary disease were observed in different host-donor strain combinations, but not in relation to number of marrow cells transferred. Preirradiation of donor mice with 400-500 r usually reduced the ability of 60×10^6 nucleated marrow cells to protect irradiated recipients against early radiation lethality; however, such animals surviving 21 days exhibited symptoms of secondary disease less frequently than comparable recipients of unirradiated marrow. Secondary disease, recognized by weight loss, diarrhea, and delayed hair-graying was almost invariably fatal to mice protected with unirradiated marrow. However, the disease was mild and transient in recipients of irradiated marrow. The latter mice survived and their hair grayed 40 to 90 days after irradiation. When LAF_1 donor mice were given AET before exposure to 400-500 r, the marrow was just as effective in preventing early radiation death as marrow from unirradiated donors, indicating that a significant number of hemopoietic donor cells were protected against radiation damage by the AET. In the recipients given marrow from irradiated donors either treated with AET or not, incidence and severity of secondary disease was equally low, which suggests that the compound did not protect antibody-forming cells in the marrow. Chemical treatment of unirradiated donors had no detectable effect on the occurrence of secondary disease. All the $1C3F_1$ recipients of LAF_1 or $B6D2F_1$ marrow that survived more than 90 days after treatment, had donor-type hemoglobin (90-100% of total hemoglobin) in the peripheral blood, irrespective of the treatment given the donor. All the $B1F_1$ recipients of irradiated CAF_1 marrow had donor-type hemoglobin (90-100% of total) 60 days after treatment,

but by 90 days the hemoglobin had reverted to host type in 10 out of 15 mice. Probably the number of irradiated viable CAF_1 blood-forming cells inoculated was suboptimal, since the CAF_1 mouse is more radiosensitive than the other 2 donor strains employed(10). Regression of the grafted marrow in homologous chimeras occurs when small numbers of donor cells are inoculated into the irradiated recipients(11).

Discussion. The incidence and severity of secondary disease in homologous bone marrow chimeras was reduced by pre-exposure of the donor mice to 400-500 r of total-body X radiation, which strongly indicates that immunologically competent cells or their precursors in the donor marrow did not survive irradiation in large numbers. Conversely, 90-100% of the hemoglobin in the blood of the chimeras was of donor type, indicating that erythropoietic cells of the irradiated marrow survived in large enough numbers to repopulate the host, although 400-500 r were estimated to have killed 94-98% of all donor hemopoietic cells(7). Transferring a small number of unirradiated bone marrow cells, the number being comparable to that estimated to survive 400 r, does not prevent secondary disease in homologous chimeras. Hence, a nonselective reduction of the overall population *per se* does not reduce the incidence of secondary disease and is not the mechanism by which preirradiation of the marrow donors suppressed secondary disease in homologous chimeras.

Simonsen(12) and Popp(13) suggested that immunologically competent cells transplanted at different stages of maturation may behave differently in homologous recipients, the more mature cells reacting against host-type transplantation antigens, the immature ones being unresponsive when they mature. It may be postulated, therefore, that irradiation of donor mice eliminated mainly the mature antibody-forming cells and that the immature ones surviving may have become specifically unresponsive to the host antigens. Preliminary tests, *i.e.*, retransplantation of chimeric spleen cells, indicate that the immune machinery in the surviving recipients of irradiated marrow is of donor type (Cudkowicz, unpublished data).

Administration of a radioprotective compound to the donor mice to be irradiated apparently had a selective effect in that the hemopoietic cells survived irradiation treatment better than the cells reacting immunologically against transplantation antigens. This conclusion is inferred from the fact that donor hemopoietic cells were functional at time of transplantation and were able to proliferate and to repopulate the host. Nevertheless, the chimeras failed to develop immunological disorders (secondary disease), implying that the irradiated immunologically competent cells were not protected by AET. That AET was able to protect hemopoietic elements of marrow against radiation injury agrees with results obtained by Smith(14) and by Doherty(15). That the drug *did not protect* immunologically competent cells against radiation injury to the same extent is supported by other direct(16) and indirect evidence(17).

Summary. Preirradiation of donor mice with 400-500 r of X-rays resulted in a marked reduction of secondary disease in homologous radiation bone marrow chimeras. The results are tentatively attributed to elimination of immunologically competent cells from donor marrow by X radiation to an extent adequate to inhibit the development of clinically significant graft-versus-host immune reactions. This effect was not detectably inhibited by administration of a radioprotective compound (AET) 20-30 minutes before irradiation of donor mice, implying that AET did not protect immunologically competent cells to the same extent as hemopoietic cells against radiation injury.

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Modification of the Friend Virus Disease by Splenectomy.* (26768)

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Friend Virus infection in Ha/ICR Swiss mice produces a syndrome characterized by 2 distinct responses. The first, a malignant disorder, involves uncontrolled proliferation of reticulum cells with massive involvement of spleen and liver. The second response involves the hematopoietic system and manifests itself by an increased production of erythroblasts, erythrocytes and leukocytes. Previous studies by Mirand, *et al.*(1) demonstrated selective uptake of Fe^{59} in the spleen of infected animals and supported the hypothesis that the increased erythropoiesis was largely due to increased erythropoietic activity in the spleen. Further studies of the relationship of the spleen to the hematopoietic response were done and constitute the basis of this report.

Method. Mice employed in this study were Ha/ICR Swiss male mice 6-8 weeks old, obtained from our own breeding colonies and maintained on a diet of Derwood-Morris pellets.

Friend Virus(2) was obtained through the courtesy of Dr. Charlotte Friend, Sloan-Kettering Institute, New York City and was in its 12th serial passage in our laboratory at the beginning of this study. A pool of the virus was obtained from this passage by homogenization of 10% infected spleen mince suspension in cold Locke-Ringer solution, clarification by centrifugation for 10 minutes at 5000 G at 5°C and subsequent filtration of the supernate through a HA-grade Millipore filter with an *E. coli* marker. This filtrate was stored in sealed ampoules in a dry ice chest until used. The standard inoculum for each mouse was 0.2 ml intraperitoneally. This inoculum produced typical disease in about 85-90% of our mice. A minimum of 15 mice was used for each point in the various experimental groups.

Hematocrits were determined with Drummond microhematocrit capillary tubes. Leukocyte and erythrocyte counts were done in standard human diluting pipettes and a hemocytometer. Blood films were stained with Wright-Giemsa stain.

In the groups having splenectomies, the procedure was performed 3 days prior and 4, 19, 21 and 27 days after inoculation of the virus.

Results. Table I illustrates the characteristic splenomegaly which occurs in Ha/ICR Swiss mice following infection with Friend Virus. The rapidity with which splenic enlargement occurs is related largely to the size of the original inoculum. When a dilution of 10^{-1} was inoculated there was roughly

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