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Failure of Endotoxin to Protect C3H/HeJ Mice Against Lethal X-Irradiation

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C3H/HeJ mice are more sensitive to lethal X-irradiation and cannot be protected by pretreatment with endotoxin, compared to the C3HeB/FeJ strain. Lack of a granulopoietic response in C3H/HeJ mice is evident.

Smith and her associates (9, 10) protected mice against the lethal effect of X-irradiation by pretreatment with small doses of bacterial endotoxin. We observed this radioprotective effect by using a *Serratia marcescens* endotoxin detoxified by treatment with potassium methylate (Fig. 1). In both control and treated mice, the number of circulating leukocytes dropped to severe leukopenic levels after X-irradiation, and although control mice began to die soon thereafter, the number of leukocytes in pretreated mice rapidly returned to normal (12). The ability of endotoxin to stimulate granulopoiesis was believed to underlie its ability to protect against X-irradiation death and postirradiation infections.

Another property of endotoxin under extensive investigation is its elicitation of colony-stimulating factor (CSF) (2, 4, 5) from cells of the monocyte-macrophage series (3). Since the production of CSF might be a humoral regulator of granulopoiesis seen in endotoxin-pretreated irradiated mice, it was of interest to determine whether endotoxin protects a strain of mice that has been found unable to produce CSF after endotoxin administration (1). The mice employed in the experiments reported here were C3H/HeJ mice defective in several endotoxin-induced responses (7) and the closely related C3HeB/FeJ strain, which is responsive to endotoxin. As the results show, endotoxin pretreatment under conditions that protect the latter animals failed to protect the C3H/HeJ mice.

Both strains of mice were obtained from Jackson Laboratories, Bar Harbor, Maine, and were maintained on acid water (pH 2.6 to 2.8), as were their mothers, throughout their lives. They were housed in our animal facility and fed sterile food ad libitum. Two weeks after arrival, the pretreated and control mice were subjected to whole-body X-irradiation in groups of 10 in a rotating circular plastic holder by the following schedule: 200 kV; 20 mA; filtration, 0.5 mm of Cu; focus and target distance, 50 cm; and dosage rate, 70 R/min. By varying the X-irradiation phase, it became evident that the C3H/HeJ mice were more sensitive than the C3HeB/FeJ animals. Fifty percent of the former died after exposure to 550 R, and none of the latter succumbed. Even though the C3H/HeJ mice were slightly smaller than the C3HeB/FeJ mice at the time of X-irradiation (18.2 g versus 19.9 g), the cellularity of their spleens and femoral bone marrow was not detectably different.

The endotoxin employed in these experiments was prepared from *Escherichia coli* (0:55) by the Boivin procedure. The 50% lethal dose of this material from the C3HeB/FeJ mice was estimated by the Reed and Muench procedure (6) as 125 μg and for the C3H/HeJ mice as >1,200 μg. Mice were injected intravenously

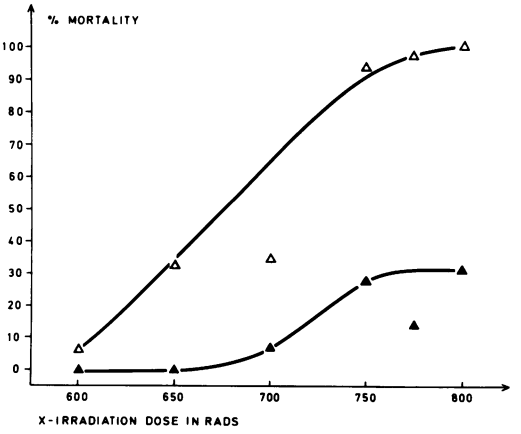

FIG. 1. *Percent survival of 28 or 29 mice of the Brookhaven National Laboratory strain injected 24 h before whole-body X-irradiation with either isotonic sodium chloride solution (△) or 50 μg of S. marcescens endotoxin detoxified by treatment with potassium methylate (▲). These experiments were carried out while one of us (R.U.) was a guest investigator at Brookhaven National Laboratory, Upton, N.Y.*

TABLE 1. *Postirradiation survival rate and leukocyte response of endotoxin-treated C3H/HeJ and C3HeB/FeJ mice*

Strain	Experimental determination			
	% Survival 30 days postirradiation[a] (25 mice per group)	Leukocyte count		
		8 Days postirradiation (5 mice per group)	30 Days postirradiation	2 h Postendotoxin (10 mice per group)
C3H/HeJ				
Endotoxin-treated (1 μg)	20	236 ± 33	1,770 ± 284 (5 survivors)	4,500 ± 845
Control	0	370 ± 29	No survivors	4,310 ± 932
C3HeB/FeJ				
Endotoxin-treated (1 μg)	100	492 ± 156	5,360 ± 1,560 (25 survivors)	1,900 ± 259
Control	32	257 ± 65	1,350 ± 466 (8 survivors)	4,370 ± 1,400

[a] HeJ mice received 575 R, and FeJ mice received 600 R.

with 1 μg of the endotoxin 24 h before X-irradiation.

All control C3H/HeJ mice were dead 30 days after exposure to 575 R, and only 5 of 25 endotoxin pretreated mice survived (Table 1). This contrasts the response of the C3HeB/FeJ mice that were subjected to 600 R. All 25 treated mice survived, but only 8 (32%) of the controls survived. As one might suspect, the number of leukocytes in the treated C3HeB/FeJ mice was almost twice as great 8 days after X-irradiation, compared to those of the controls, but there was no increase in those of the treated C3H/HeJ mice (Table 1). Thirty days after X-irradiation, the leukocytes were at a normal level in the treated C3HeB/FeJ mice, while they were still depressed in the surviving C3H/HeJ animals (Table 1). Two hours after 1 μg of endotoxin is administered—the optimal time of an increased serum CSF level in endotoxin-sensitive mice—leukopenia is present in C3HeB/FeJ mice, although the leukocytes of C3H/HeJ do not respond to endotoxin, as Sultzer (11) showed for peritoneal leukocytes.

These results establish the greater susceptibility of C3H/HeJ mice to X-irradiation death than the closely related C3HeB/FeJ strain and the inability of the former to be protected by endotoxin pretreatment. The failure of the Boivin type of endotoxin to protect the C3H/HeJ mice is reflected in their inability to regenerate leukocytes, even though this material has been reported to stimulate mitogenesis in the B-lymphocytes of this strain (8). Since these mice have also been found to produce no CSF in response to endotoxin, it is consistent with what we know of granulopoiesis to draw inferences about their behavior under these conditions. Moreover, the pluripotent stem cells of the spleens (splenic colony-forming units) of C3H/HeJ mice do not increase in response to endotoxin, as was observed in an endotoxin-sensitive strain with a peak 3 days after injection of endotoxin or detoxified endotoxin (R. Urbaschek, in preparation).

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