

Serum Colony Stimulating Factor Levels in Irradiated Germfree and Conventional GFW Mice (36484)¹

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In a recent study of the regulation of granulopoiesis in irradiated mice we observed that there developed a factor (colony-stimulating factor or CSF) which stimulated granulopoiesis *in vitro* (1) as assessed by the agar colony technique (2, 3). The level of CSF correlated in time and magnitude with the neutropenia and the granulopoietic hyperplasia developing after irradiation which suggested that CSF might be a physiological regulator of granulopoiesis. Although neutropenia and marrow destruction seemed to play at least some part in producing the raised level of CSF after irradiation, an unknown question was the role of intestinal damage. The latter is known to follow high doses of irradiation and to lead to bacteremia (4, 5) and endotoxemia (6) at approximately the same time as the various changes we observed in granulopoiesis. Furthermore, injection of bacterial endotoxin into mice produces both high levels of CSF and marked granulopoietic hyperplasia (7). We therefore investigated the role of the bowel flora after irradiation by comparing CSF levels after irradiation in germfree and conventional mice.

Materials and Methods. CFW mice of both sexes 10–14 weeks of age were used. The methods of housing and maintenance have

been previously described (8–10). The source of X-radiation was a 260 kVp clinical therapy X-ray machine operated at 250 kV and 15 mA with filtration of 1.0 mm Al and 0.25 mm Cu (HVL = 1.05 mm Cu) at a rate of approximately 40 R/min as measured in air with a Victorean condenser R-meter. Mice were given a dose of 850 R and killed and bled at various intervals after irradiation in two separate experiments. At least five mice were bled for each time-point in both the germfree and conventional groups. Sera were frozen and put through a 0.45 μ Millipore filter prior to plating. The technique of irradiation of both germfree and conventional mice has been described previously (11).

Serum was obtained and tested for its granulopoiesis-stimulating activity by the soft agar technique as previously described (12). At least three plates were set up for each determination and 0.1 cc of the test serum was added to each in the first experiment. Sera from the second experiment was tested at 0.1 cc and 0.07 cc per plate and assayed on two separate occasions. The results were expressed as the number of colonies stimulated/ 10^5 marrow cells.

Results. Table I shows the results obtained. In germfree mice there was no detectable rise in CSF activity after irradiation whereas in conventional mice there was a clear-cut elevation at variable times after irradiation.

Table II shows the peripheral blood values of CFW conventional and germfree mice, 5 days after 850 R. These data are from Expt. 1. There is obviously a profound depletion of the peripheral lymphocytes and granulocytes in both groups.

¹ Supported in part by Grants HE 7542 and 5600 from the National Heart and Lung Institutes and General Research support Grant FR 5587 Division of Research Facilities and Resources National Institutes of Health to St. Elizabeth's Hospital and USPHS Grant EC-00080 to Lobund Laboratory University of Notre Dame.

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TABLE I. Comparison of CSF Activity in Sera of Germfree and Conventional Mice Irradiated with 850 R.^a

Dose serum/plate	Expt. 1		Expt. 2			
	Germfree 0.1	Conventional 0.1	Germfree		Conventional	
			0.1	0.07	0.1	0.07
Control	0	0	0.2 ± 0.2	0	0	0.2 ± 0.2
Day after irradiation						
1	1 ± 1	1 ± 1	0	0	1.0 ± 0.6	0.7 ± 0.2
2	0	2 ± 1	—	—	—	—
3	0	1 ± 1	0	0	4.5 ± 1.3	2.3 ± 0.7
4	1 ± 1	1 ± 1	0	0	—	—
5	0	0	—	—	—	—
6	1 ± 1	3 ± 1	0	0	24 ± 2.4	25.8 ± 3.1
7	1 ± 1	46 ± 8	0	0	1.5 ± 0.6	4.0 ± 1.8
8	2 ± 1	30 ± 2	0	0	0	13.7 ± 3.3
9	—	—	0	0.3 ± 0.3	1.8 ± 0.3	4.5 ± 2.1
10	—	—	0	0	0.5 ± 0.5	0 ± 0
11						

^a Results are expressed as the mean ± 1 SE of the number of colonies observed/10⁶ marrow cells plated at either 0.1 or 0.07 cc of serum in each Petri dish. On several days in Expt. 2 sufficient serum was not available for testing due to death of mice and technical reasons.

Discussion. The present results suggest that bacteria, presumably intestinal bacteria, are important in the generation of CSF after irradiation. The results of Expt. 2 seem to exclude the possibility that CSF activity develops in germfree animals after irradiation but only does so after a prolonged interval. Although the failure to detect any rise whatsoever of CSF in irradiated germfree mice suggests that bacteria are essential rather than merely important, this is probably not so since other workers have been able to detect CSF in the serum of unirradiated germfree mice of another strain (13).

We have not investigated the means by which the germfree state prevents the rise of CSF after irradiation. It is possible that an increase in a recently described (14) inhibitor of CSF would be involved. It may be that bacteria have no direct role but that germfree animals are somehow unresponsive to the stimulus that produces CSF. In this regard there is some evidence that germfree guinea pigs develop less neutropenia in response to endotoxin (15). However, since injection of endotoxin produces high levels of CSF, perhaps the most likely situation is that in conventional animals irradiation results in damage to the intestine, bacteremia and endotoxinemia, and this does not occur in germ-

free animals. This hypothesis is supported by the time of appearance of serum CSF activity after irradiation, which approximates the time at which one begins to see bacteremia in irradiated mice (4). The neutropenia developing after irradiation might be expected to facilitate the development of bacteremia. Such a situation, showing the possible roles of damage to the marrow and intestine after irradiation, is illustrated in Fig. 1. Damage to the marrow may, however, act not only by facilitating bacteremia but also by decreasing the inactivation of CSF (16).

The evidence that intestinal bacteria play

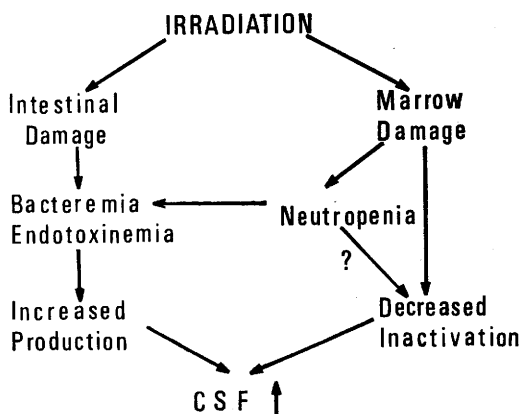


FIG. 1. Possible factors in generation of CSF after irradiation.

TABLE II. Peripheral Blood Values of Germfree and Conventional Mice 5 Days after 850 R From Expt. 1.^a

	Total leukocytes /cumm	Total granulocytes /cumm	Total lymphocytes /cumm	Total monocytes /cumm	Hematocrit %
Germfree					
CFW mice	80 ± 37	8.7 ± 5.4	70.8 ± 37	0.4 ± 0.4	44.2 ± 0.7
Conventional					
CFW mice	20 ± 20	0	20 ± 20	0	40 ± 1.2

^a Data based on values from five mice per group and expressed as the mean ± 1 SE.

a role in the generation of CSF after irradiation and that CSF may be responsible for the granulopoietic hyperplasia after irradiation again raises the question of the role of intestinal bacteria in the physiological regulation of granulopoiesis. A number of investigators have compared various aspects of granulopoiesis in conventional and germfree animals but no clear answer to this question has yet emerged, (17-22). It is of interest that Kivilaakso *et al.* (23) using a different technique found that in rats suppression of the intestinal microflora with neomycin resulted in decreased levels of a granulopoietic stimulator termed antichalone.

Summary. Germfree and conventional CFW mice were evaluated for elevations in serum CSF levels at varying time intervals after 850 R whole body irradiation. Conventional mice showed elevations of CSF levels on Days 6-9 while sera from germfree mice were inactive on Days 1-10. The data suggests that the bacterial flora of the gut play a role in the elevations of serum CSF seen after irradiation.

The technical assistance of Mrs. Mildred Rosenfield is gratefully acknowledged.

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Received Dec. 28, 1971. P.S.E.B.M., 1972, Vol. 140.