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PROTECTION EXPERIMENTS AGAINST RADIATION INJURY WITH LYMPHOCYTES

By

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PROTECTION EXPERIMENTS AGAINST RADIATION INJURY WITH LYMPHOCYTES

This experiment was undertaken to determine whether the injection of lymphocytes into irradiated animals would offer protection against the effects of radiation. The experiments were so designed as to obtain living lymphocytes in their natural medium and introduce them into genetically compatible hosts. The results were essentially negative and since they will not be published in the open literature they are presented here in some detail.

Methods. Rats of the laboratory's inbred Wistar strain were used. Lymph was obtained by cannulation of the mesenteric duct by the technic of Bollman et al. The lymph was collected in a sterile, heparinized test tube and kept in a refrigerated waterbath at about +7°C. The cannulated rat was a close relative, usually the mother or the father, or a sibling, of the rats to be injected. The recipient rats were irradiated with an approximate LD-50 dose, usually 650 to 750 r, depending on the weight of the rat. Following irradiation, half of the siblings of each litter were injected intraperitoneally with as much lymph as was available, usually about 1 cc; controls were injected with a comparable amount of Tyrode solution. The injections were made 2 to 3 times daily, and were continued for as long as the fistula could be maintained. Thus, the amount injected varied greatly in different experiments, as follows: Exper. 12; 12 to 17 cc, Exper. 13; 9 cc, Exper. 14; 0.9 cc, Exper. 15; 4 cc, Exper 18; 6 cc, and Exper. 19; 8.4 cc. In all experiments listed here siblings were used and injections were made by sterile technic. Other negative experiments in which members of the inbred strain used were not siblings are not analyzed here.

Before injection the white cell count of the lymph was determined and viability of the cells was tested by motility and by the safranine dye technic. The latter test indicated that 95 to 98% of the cells in fresh lymph were viable. In the lymph collected overnight the percentage of viable cells was lower (70 to 85%), and the motility test gave somewhat lower values.

The protective effect was judged by several methods. On the fourth or fifth day and on the eighth or ninth day after irradiation the rats were weighed and white cell counts of the blood were made. Records were kept of the survival time of the rats. The lymph-injected and Tyrode-injected animals were then compared to see if any significant difference could be detected in these parameters.

Table I shows a series of experiments which were conducted under optimal conditions. In these experiments lymph of mothers of an inbred line of rats was tapped and given to half of her babies of the same litter, the other half received the same volume of Tyrode solution. Table I shows that 10 lymph-injected animals died with acute radiation syndrome during the first 3 weeks postirradiation. These animals lived an average of 13.2 days postirradiation. Ten Tyrode-injected animals died with acute radiation syndrome after an average of 12.1 days postirradiation. No animals died of an acute radiation syndrome after the 19th day postirradiation.

No significant differences were found in white cell counts and weights between lymph-injected and control animals.

In all experiments the lymphocyte counts of the blood, at 5 days after irradiation, averaged higher in lymph-injected animals than in the controls even though there was no consistent difference in total white cell counts (Table II). The magnitude of difference in lymphocyte counts seems to be related to two factors: (a) The amount of lymph injected and (b) The length of time between the last injection of lymph and the sampling of blood for the white cell count. The greater the amount injected and the shorter the interval between injection and counting, the more the lymphocyte count of the lymph-injected animals exceeded that of the controls.

A higher lymphocyte count in the lymph-injected animals requires no explanation. However, why there should be fewer polymorphonuclear neutrophils in most of the lymph-injected animal is difficult to understand.

TABLE I

RESULTS OF INJECTION OF LYMPHOCYTES ON MORTALITY OF RATS EXPOSED TO 650 - 750 r

	Days Postirradiation															
Experiment	4	5	6	9	10	12	13	14	15	18	19	23	30	32	36	38
13																2 LK 2 TK
14								1 TD	3 LD 1 TD		1 TD					
15															4 LK 4 TK	
16					1 TD			1 LD						2 LK 1 TK		
17	1 LD		1 TD	1 TD		1 LD 1 TD	1 LD									
18													6 LK 6 TK			
19		1 TD					2 LD 1 TD			1 LD 1 TD		1 TK 1 LK				

LD = Lymph-injected died; LK = Lymph-injected killed

TD = Tyrode-injected died; TK = Tyrode-injected killed

TABLE II

DIFFERENCE IN WBC AND LYMPHOCYTE COUNTS BETWEEN LYMPH AND TYRODE-INJECTED RATS

	Difference in Total White Cell Counts[1]		Difference in Lymphocyte counts[2] (per cu. mm)
Experiment	Lymph injected	Tyrode injected	Lymph injected
12	+ 42		+ 491
13		+ 20	+ 298
14	+ 216		+ 210
15	same		+ 17
18	+ 16		+ 103
19		+ 150	+ 319

These data were obtained on the 4th to 5th postirradiation day in each experiment.

[1] These values were obtained by averaging the white cell counts for the lymph-injected and for the controls in each experiment, and recording the difference. Thus, in Exper. 12, 14, and 18 the lymph injected animals had a higher average cell count than the Tyrode-injected animals.

[2] Differences in lymphocyte counts were obtained in a similar manner as differences in white cell counts. In all experiments the lymph-injected animals had a higher average lymphocyte count than in the controls injected with Tyrode.

On the Fate of Injected Lymphocytes. Large numbers of lymphocytes were injected in rats whose lymphocyte count was depressed with X rays to determine by simple blood counts and differential counts whether the injected lymphocytes would circulate in the blood and if so, how long.

Procedure. The rats were irradiated with 750 r 4, 5, or 6 days previous to injection. Lymphocytes were obtained by cannulation of the mesenteric duct as described. The lymph was injected as obtained or the cells centrifuged and resuspended in a smaller volume. The blood of the rats was sampled and WBC counts made before injection and at various intervals after irradiation. The lymph to be injected was obtained from siblings. The manipulations were carried out under ether anesthesia. The following experiments are detailed as illustrative examples:

Experiment 1. This rat received 2.4 cc of lymph containing 19,450 cells/cu. mm. of which 96% were viable, that is a total of 45,000,000 cells. This rat was estimated to have 9.75 cc of blood and if all lymphocytes had remained in the circulation, the white blood cell count would have been raised by 3700 cells/cu. mm. The findings were as follows:

	Time					
	Before injection	After injection (minutes)				
		4	8	16	32	64
WBC	175	300	200	550	600	500

After injection most cells in the smear were lymphocytes.

Experiment 2. A total of 217,000,000 cells were injected concentrated in 0.5 cc of lymph. This number of cells would raise the white cell count to approximately 21,200 cells/cu. mm. if all cells had remained in circulation. The findings were as follows:

	Time			
	Before injection	After injection		
		6 min.	52 min.	17 1/2 hrs.
WBC	100	1800	950	150

Experiment 3. 138,000,000 cells were injected and this injection should have raised the white blood cell count approximately 13,300 cells/cu. mm. The findings were as follows:

	Time			
	Before injection	After injection		
		4 min.	45 min.	3 1/2 hrs.
WBC	150	5,750	2,950	200

Experiment 4. 0.75 cc of concentrated lymph containing a total of 273,000,000 cells were injected into a rat 5 days after irradiation of the rat. This injection should have raised the WBC of the rat by about 16,700 cells/cu. mm. The findings were as follows:

	Before injection	After injection						
		4 min.	8 min.	16 min.	32 min.	64 min.	128 min.	250 min.
WBC	1050	3350	2200	4950	3100	2050	1050	1150

It is evident that only a relatively small number of the injected lymphocytes circulated in the blood of the irradiated animals shortly after injection, and even most of these left the circulation within 1 to 2 hours.

These results are in agreement with those of several investigators who concluded on the basis of similar findings that the lymphocytes live but a few hours. Nevertheless, under the conditions of these experiments one would expect that at least the introduced lymphoblasts would survive and multiply in the new host and thus exert a protective function. Should the monophyletic view of hemopoiesis be true one would expect restoration of the levels of other types of leukocytes.

These experiments fail to support the view that the lymphocytes exert protection against radiation damage and that the lymphocytes are mother cells of other white cells. Thus, protection by shielding the spleen as described by Jacobson is probably due to a substance related to another cell. Alternate possibilities are that the lymphocytes drained from the body, though motile for some time, are fatally injured or that the lymphocytes drained from the mesenteric duct are different from those in the spleen. *

* This study was made under the guidance of Jacob Furth, Chief of the Section.