MASTER

1. TECHNICAL PROGRESS REPORT

AEC Contract Number: AT(11-1)-1818

Title: Protection of Mice Against Lethal X-Radiation with
a Cell-Free Component of Spleen.

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Period covered by report: 1 September 1968 to 15 May 1969

SUMMARY:

We had previously obtained a cell-free preparation from mouse spleen which, injected into mice following exposure to a lethal dose of x-ray, protected the irradiated mice from death. The protective component had a molecular weight of approximately 70,000 and was derived from the DAL strain of mice obtained from Redwood Game Farms, Salt Lake City, Utah. Similar splenic preparations from CBA, CD-1 and CF-1 strains of mice did not protect against radiation, but the CD-1 and CBA mice could be protected with preparations prepared from spleens of DAL mice. The weight of a spleen from the DAL mice was 225 - 240 mg. In contrast, the spleen weight of the other strains varied between 70 and 120 mg. It was possible to increase the spleen size in these latter strains of mice by the i.p. injection of macerated spleens from DAL mice. Furthermore, the injection of macerated tissue from enlarged spleens of CBA mice induced splenomegaly in normal CBA mice. The splenic hyperplasia was evident 10 to 13 days after the injection of macerated spleen and was believed to be due to an infection. In contrast to the negative results with normal spleens, preparations from the enlarged spleens of CBA and CD-1 mice protected against the lethal effects of total-body irradiation.

REPORT:

This pilot study was funded to verify our earlier claims of the discovery of a cell-free component of spleen which protects mice against radiation lethality. This component is prepared as described in the attached reprint (Proc. Soc. 127:286-9, 1969). Investigators from the AEC suggested the pilot study using mice of other strains and pathogen-free conditions to rule out the interference of a possible chronic infection in our strain. Five related experiments were described in our approved pilot grant proposal; three of them and additional modifications have been executed and are described below. These experiments are numbered as in the approved experimental protocol. Preliminary work on the two experiments using barrier-sustained mice is in progress.

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Mice. Prior to irradiation, mice were housed in groups of 20 to 40 in a clean, well-ventilated, air-conditioned room. All bedding, water bottles and cages were sterilized immediately prior to use. Following irradiation, mice were housed individually to eliminate cross infections. The CD-1 mice were obtained from Charles River Mouse Farms, the CF-1 mice from the Carworth Farms, the CBA mice from the Department of Anatomy, University of Utah College of Medicine and the DAL mice from the Redwood Game Farms, Salt Lake City. The DAL mice were housed in separate animal quarters.

Radiation. Test and control mice of equivalent age, sex and weight were irradiated twelve at a time in a plastic container which rotated at 6 rpm. The radiation factors were 250 KVP; 15 ma; inherent filtration, 0.77 mm cu; target distance, 50 cm; dose rate, 185 r/min; radiation source, Westinghouse Quadrocondex x-ray machine; and total-body dose, 700r.

Treatment. All mice were given 1 ml sc injections immediately following radiation and daily for four additional days. The control animals were injected five times with physiological saline and the test animals with the protective splenic preparation. The mice were examined daily and the 30 day mortality determined.

Experiment 1. Sixty CBA mice were irradiated and divided into two equal groups. One group was given five daily injections of the protective spleen extract prepared from DAL mice. The other group was injected with saline. Twenty spleens with a total wet weight of 4.2 to 4.5 gm were used in the preparation of each batch of extract. Eight mice were injected with a batch of extract. Consequently, four preparations of spleen extract were used in this experiment. The DAL mice were housed in our regular animal quarters and the CBA mice were housed in another animal room which had not previously quartered DAL mice. The thirty-day survival results are recorded in Table 1. Treatment with the splenic fraction from DAL mice increased the survival of CBA mice from 17% to 83%.

TABLE 1

Group	Mouse strain irradiated	Treatment	Survival	
			ratio	%
Control	CBA	PSS	5/30	17%
Treated	CBA	DAL extract (20 spleens)*	25/30	83%

* Number of spleens/extract.

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Experiment 2. A preliminary experiment of the larger one that had been outlined in the original proposal is reported along with a modification of this experiment. When 8 CBA mice were treated with extract prepared from the spleens of 20 CBA, only slight protection was obtained as recorded in Table 2A.

TABLE 2A

Group	Mouse strain irradiated	Treatment	Survival	
			ratio	%
Control	CBA	PSS	0/8	0%
Treated	CBA	CBA extract (20 spleens)	1/8	13%

Since the total weight of the 20 spleens was only 1.7 gm, the experiment was repeated using 50 CBA spleens which had a total weight of 4.5 gms to prepare the extract. This extract also failed to protect as seen in Table 2B.

TABLE 2B

Group	Mouse strain irradiated	Treatment	Survival	
			ratio	%
Control	CBA	PSS	0/8	0%
Treated	CBA	CBA extract (50 spleens)	0/6	0%

An experiment similar to the above was carried out using a spleen extract prepared from the spleens of 41 CF-1 mice. The spleens had a total wet weight of 4.5 gm. The extract from these CF-1 mice also failed to protect CBA mice as recorded in Table 2C.

TABLE 2C

Group	Mouse strain irradiated	Treatment	Survival	
			ratio	%
Control	CBA	PSS	0/8	0%
Treated	CBA	CD-1 extract (41 spleens)	0/8	0%

Experiment 3. The experiments recorded in this section were carried out prior to those in the Experiment 2 section. Forty-six CD-1 mice were exposed to 700r total-body x-irradiation. Half were given daily injections of spleen extract prepared from 20 CD-1 mice and half received injections of saline. The total wet weight of the 20 spleens

used to prepare each of the three batches of extract used in this experiment varied from 1.7 to 3.0 gm. In both treated and control groups the thirty-day mortality was 100% as seen in Table 3A.

TABLE 3A

Group	Mouse strain irradiated	Treatment	Survival	
			ratio	%
Control	CD-1	PSS	0/23	0%
Treated	CD-1	CD-1 extract (20 spleens)	0/23	0%

Since the total spleen weight of the 20 CD-1 spleens was less than that obtained with 20 DAL spleens which would protect against radiation death (Experiment 1), the extracts were prepared from 4.5 gm of spleen. The number of CD-1 spleens required to have a total weight of 4.5 gm varied from 36 to 47 for the 4 batches of extract used in this protection experiment recorded in Table 3B. An interesting observation of this experiment was that 7 of the 8 surviving animals were treated with the batch of extract which was prepared from the smallest number of spleens (36 spleens). Only one animal died with this specific batch of extract. The significance of this will be discussed later.

TABLE 3B

Group	Mouse strain irradiated	Treatment	Survival	
			ratio	%
Control	CD-1	PSS	0/30	0%
Treated	CD-1	CD-1 extract (36-47 spleens)	8/30	20%

Protection of CD-1 mice with splenic extracts from DAL spleens. The work from this point forward were not in the original proposal but were deemed necessary in view of the preceding results. CD-1 mice were treated with DAL splenic extract to determine if protection similar to that of CBA mice (Experiment 1) would take place in CD-1 mice. The DAL splenic extract protected 87% of the CD-1 mice against a lethal dose of x-rays (Table 4).

TABLE 4

Group	Mouse strain irradiated	Treatment	Survival	
			ratio	%
Control	CD-1	PSS	0/30	0%
Treated	CD-1	DAL extract (20 spleens)	26/30	87%

Cumulative results. Unpublished work in which protective splenic extracts were obtained from untreated CF-1, CBA, CD-1 and DAL mice is summarized in Table 5. This includes results recorded in Tables 1 through 5 plus additional unrecorded experiments.

TABLE 5
PROTECTION OF MICE WITH SPLENIC EXTRACTS
FROM DIFFERENT MOUSE STRAINS
(Cumulative Results)

Mouse strain		No. of extracts	Spleens per extract	gm. per extract	Survival	
Irradiated 700r	Extract origin				ratio	per cent
DAL	none	---	---	---	0/52	0%
DAL	DAL	8	20	4.2-4.7	44/49	90%
CD-1	none	---	---	---	0/72	0%
CD-1	CD-1	3	20	1.7-3.0	0/23	0%
CD-1	CD-1	6	36-47	4.5	8/47	17%
CD-1	DAL	4	20	4.5	26/30	87%
CBA	none	---	---	---	5/46	11%
CBA	CBA	1	20	1.7	1/8	13%
CBA	CBA	1	50	4.5	0/6	0%
CBA	CF-1	1	41	4.54	0/8	0%
CBA	DAL	4	20	4.2-4.5	25/30	83%

Increase in spleen size following injection of macerated DAL spleen. Since the DAL spleens were consistently larger than the CBA and CD-1 spleens an attempt was made to increase the size of the CD-1 & CBA spleen. DAL spleens to be macerated were passed through an 18 gauge, a 20 gauge and a 22 gauge hypodermic needle in sequence and suspended in saline to a final volume of 1.0 ml/spleen. Interperitoneal injections of 0.1 ml of the splenic suspensions were injected into recipient mice. As recorded in Table 6, the injection of macerated DAL spleen caused splenomegaly in CBA & CD-1 mice. The injection of normal CBA splenic tissue in normal CBA mice did not cause splenomegaly. In contrast, the injection of CBA spleens enlarged by the previous DAL spleen injection caused splenomegaly in normal CBA animals. These results would be expected if the splenomegaly was caused by an infectious agent from the DAL spleens.

TABLE 6

Strain	Treatment	Average Spleen weight mg	
		Before treatment	After treatment*
DAL	none	225-240	---
CBA	DAL spleen	70-90	200-250
CBA	CBA spleen	70-90	70-90
CBA	Enlarged CBA spleen**	70-90	200-250
CD-1	DAL spleen	95-125	210-250

* 15 days after i.p. injection of macerated spleen.

**Enlarge CBA spleen was obtained from CBA mice injected with macerated DAL spleen.

Protection of mice with splenic extracts prepared from enlarged CBA and CD-1 mouse spleens. Protection experiments similar to those described with normal CBA and CD-1 spleens (Experiment 2 and 3) were carried out with enlarged spleens from these strains of mice. The results of these experiments are recorded on Table 7. In contrast to the negative results obtained with normal CD-1 & CBA spleens, treatment with extracts from enlarged spleens provided significant protection against radiation death.

TABLE 7

Radiated Strain	Extract origin	Spleens/extract	Gram/extract	Survival	
				Ratio	%
CBA	none	---	---	0/20	0%
CBA	CBA (DAL)*	20-22	4.5	23/32	71%
CBA	CBA (CBA)**	20	4.5	6/8	75%
CD-1	none	---	---	0/8	0%
CD-1	CD-1 (DAL)	21	4.7	6/8	75%

* Spleens from strains in parentheses were used to induce splenomegaly in the strains directly above them.

**Those spleens were enlarged by a previous injection of macerated DAL spleen.

Status of the unfinished contracted work. At the present time the experimental procedure such as radiation exposure, animal injections, and maintenance of mice has been successfully carried out in germ-free isolators with conventional mice. The experiments will now be carried out with barrier-sustained C₃H/101 F₁ mice. The splenic extract to be used in the first of these experiments will be of DAL origin.