The effect of thymic humoral factor (Leucotrofina) upon regeneration of haemopoietic and lymphatic tissues of irradiated mice

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SUMMARY.—Administration of Leucotrofina (a cell-free thymic extract) to whole-body irradiated mice was associated with an increased $LD_{50/30}$ corresponding to a dose reduction factor of 1.21. As indicated by haematological investigations, ^{59}Fe uptake and ESC number, haemopoiesis was significantly stimulated in spleen and bone marrow after Leucotrofina application to irradiated mice. DNA content and the uptake of ^{3}H-thymidine into DNA was significantly enhanced in the thymus and bone marrow of irradiated and Leucotrofina treated mice.

KEY WORDS.—$LD_{50/30}$ values - Recovery of haemopoiesis and lymphopoiesis - ^{59}Fe uptake - ^{3}H-thymidine uptake - ESC number.

INTRODUCTION

Various thymic cell-free extracts have been used in order to obtain informations on the endocrine and immunological role of thymus.[3] Some of these thymic extracts, such as thymosin [2,3] and thymic fraction B [4] which were able to stimulating haemopoiesis, lymphopoiesis and to increasing immunological competence, were also effective radioprotectors.[4,9-12] The aim of the present study was to investigate the radioprotective and therapeutic effect of Leucotrofina, a cell-free thymic extract produced by Ellem (Milano, Italy). It was found that application of Leucotrofina before or after the whole-body irradiation of A2G and DBA mice was correlated with a significant increase of the mean survival time and an enhanced recovery of haemopoietic and lymphopoietic activity.

MATERIALS AND METHODS

1. *Irradiation*

Groups of 20 male A2G or DBA mice weighing 20 to 23 g were whole-body gamma-irradiated by using a ^{60}Co therapeutic unit (Theratron 80) (FSD 80 cm, 174 R/min) or X-irradiated with a therapeutic unit TUR X (180 kV, 10 mA, 1 Cu. FSD 80 cm, 30 R/min). Dose rate was measured with the aid of a Siemens universal dosimeter. Animals received a standard diet.

2. *Leucotrofina application*

Mice were i.p. injected with single or repeated doses of 5 units of Leucotrofina (Ellem, Milano, Italy) a calf thymus cell-free extract. One vial contains 50 units/2 ml of Leucotrofina, corresponding to 500 mg of extract.

3. *Survival experiments*

Groups of 20 A2G mice received daily 5 units of Leucotrofina for the subsequent 6 days following gamma-irradiation with 500 to 900 rad. According to the same schedule the control groups received at 24 hours intervals 0.2 ml of saline.

4. *^{59}Fe uptake in ertyhrocytes*

Groups of 20 A2G mice were whole-body X-irradiated with 100 rad. Animals received 5 units of Leucotrofina at 60 minutes before or after the radiation exposure. At 24 hours after irradiation mice were i.p. injected with

0.2 μCi ^{59}Fe (Fe citrate). The uptake of ^{59}Fe by erythrocytes was measured at 2, 4 and 7 days after labelling, as previously described.[8]

5. *Uptake of ^{59}Fe in spleen and bone marrow*

Groups of 20 DBA mice received 5 units of Leucotrofina daily for the subsequent 6 days after whole-body X-irradiation with 400 or 500 rad.

The control groups were injected at the same time with 0.2 ml of saline. At 9 days after irradiation animals were i.p. injected with 0.5 μCi ^{59}Fe. After 4 hours mice were killed and gamma activity of left femur and spleen and counted by means of a crystal scintillation counter connected to a VA-M-16D VAKUTRONIK counting system. The uptake was expressed as percentage of the activity applied per organ. The endogenous spleen colonies were counted according to Till and McCulloch.[7]

6. *DNA content and ^{3}H-thymidine uptake*

Groups of 10 A2G mice were X-irradiated with 500 rad. The treated group received 5 units of Leucotrofina immediately and for the subsequent 6 days following the radiation exposure, at 24 hours intervals. At 90 minutes before killing mice were i.p. injected with 12 μCi of ^{3}H-thymidine (5 Ci/mM, Amersham, England). DNA content was determined in thymus, spleen and bone marrow by using the diphenylamine colour reaction, as previously described.[6] ^{3}H-DNA radioactivity was measured with the aid of a liquid scintillation spectrometer (Intertechnique ABAC SL_{40}), as previously indicated.[5]

7. *Haematologic investigations*

Groups of 20 DBA mice were whole-body X-irradiated with 400 rad. Animals received 5 units of Leucotrofina daily for the subsequent 6 days after irradiation. According to the same schedule the control group received 0.2 ml saline.

Blood and bone marrow were taken for quantitative and qualitative determinations on the 2nd, 9th, and 16th days from the irradiation. Routine methods were used.

8. *Statistical evaluation*

The results were treated statistically by means of Student t-test.

Results

The survival of control and Leucotrofina treated A2G mice which were irradiated with 500 to 900 rad is shown in Table 1.

Table 1.—*Survival, mean survival time and $LD_{50/30}$ of A2G mice. Animals were whole-body gamma-irradiated with or without the application of Leucotrofina* (see materials and methods).

	Mean survival time (h)	% survival per 30 days	$LD_{50/30}$
Control groups			
500 rad	565	65	570
600 rad	490	45	
700 rad	324	20	
800 rad	190	0	
Treated with Leucotrofina			
500 rad	698	95	690 DRF *=1.21
600 rad	601	75	
700 rad	567	60	
800 rad	301	20	
900 rad	88	0	

*Dose reduction factor.

From the $LD_{50/30}$ data a dose reduction factor of 1.21 was calculated.

The uptake of ^{59}Fe in to the erythrocytes of A2G mice irradiated with 100 rad untreated and treated with Leucotrofina is shown in Table 2.

Table 2.—*Uptake of ^{59}Fe in erythrocytes of A2G mice at different time intervals after the whole-body X-irradiation with 100 rad. Non-irradiated mice* (A), *5 units of Leucotrofina at 1 h before sham irradiation* (B), *irradiated mice* (C), *5 units of Leucotrofina at 1 h before irradiation* (D), *5 units of Leucotrofina at 1 h after the radiation exposure* (E). *For application of Leucotrofina see materials and methods.*

Group	% Uptake of ^{59}Fe		
	3 days	5 days	8 days
A	37.7±3.2	39.3±1.3	41.9±1.9
B	36.3±2.0	39.7±1.5	42.4±1.6
C	10.5±0.7	12.8±1.4	15.1±1.6
D	23.7±2.5 *	25.1±1.7 *	28.7±2.7 *
E	21.7±0.9 *	24.6±0.7 *	25.9±0.2 *

*Statistical significance $p<0.01$.

Table 3.—Uptake of ^{59}Fe in spleen and bone marrow of DBA mice at 9 days after whole-body X-irradiation with 400 or 500 rad. Non-irradiated mice (A), irradiated mice (400 rad) (B), Leucotrofina treated and irradiated mice (400 rad) (C), irradiated mice (500 rad) (D) and Leucotrofina treated and irradiated mice (500 rad) (E). For application of Leucotrofina see materials and methods.

Groups	% Uptake of ^{59}Fe	
	Spleen	Bone marrow
A	7.01±0.6	0.59±0.07
B	9.91±0.8	1.37±0.10
C	13.31±0.3 *	1.84±0.15 **
D	5.35±0.3	0.64±0.06
E	10.30±0.5 **	1.01±0.06 **

Statistical significance:
* $p<0.05$.
** $p<0.01$.

Administration of Leucotrofina before (D) or after irradiation (E) significantly stimulate the recovery of erythropoiesis, increasing the radioiron utilization with 80-100%.

The uptake of ^{55}Fe into the spleen and bone marrow on the 9th day after irradiation in untreated and treated DBA mice is shown in Table 3.

In both experimental groups, irradiated with 400 or 500 rad the recovery of splenic and medullary erythropoiesis are significantly stimulated in Leucotrofina treated mice.

At 9 days after the whole-body irradiation of DBA mice with 500 rad the number of endogenous spleen colonies was of 8.51± 0.57 in the control group and significantly increased to 15.30±0.75 in Leucotrofina treated mice.

The amount of DNA in thymus (Fig. 1) and the uptake of ^{3}H-thymidine into thymic DNA (Fig. 2) were strongly enhanced following irradiation with 500 rad in Leucotrofina treated A2G mice. The recovery effect was also evident in the bone marrow after the application of Leucotrofina (Fig. 3). However, no differences were found in DNA content and ^{3}H-thymidine incorporation into spleen DNA of control and Leucotrofina treated A2G mice (Figs. 1 and 2). The dynamics of haematologic changes in the peripheral blood and in the bone marrow are presented in Table 4. As shown in Table 4 the dynamics of the recovery processes in both compartiments (peripheral blood and

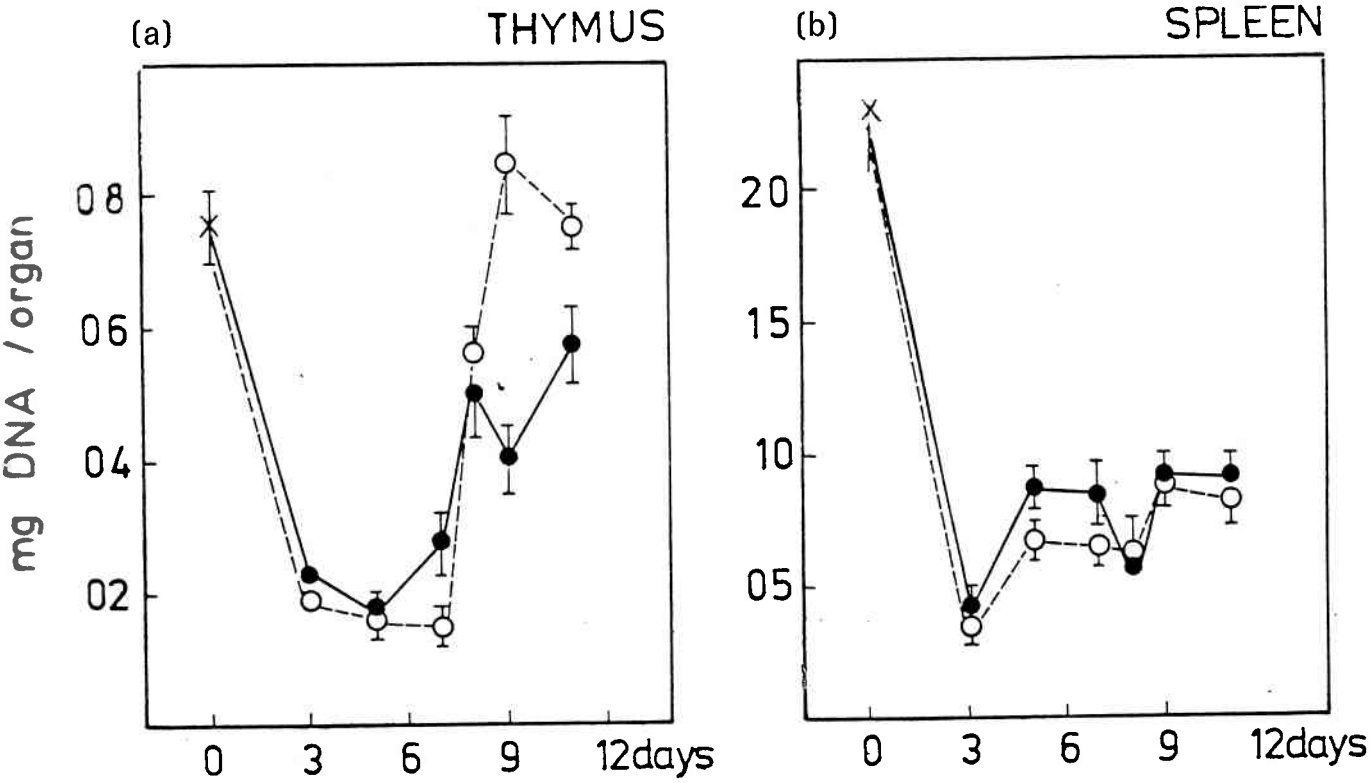

Fig. 1.—Amount of DNA in thymus (a) and spleen (b) of whole-body X-irradiated A2G mice (500 rad), with (—○—) or without (—●—) the application of Leucotrofina (see materials and methods). Non-irradiated control group (×). Statistical significance of $p<0.01$ at 9 days and $p<0.05$ at 11 days for the DNA content in the thymus of irradiated and Leucotrofina treated mice.

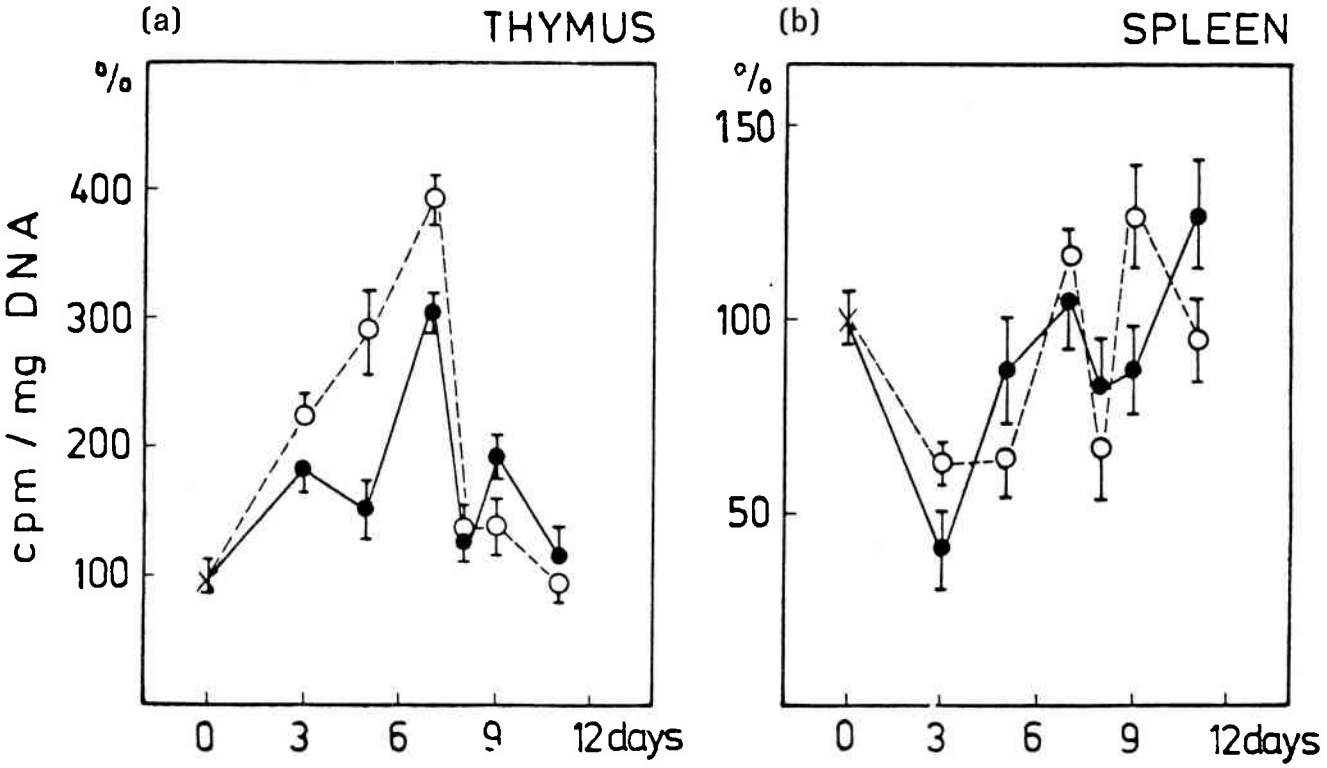

Fig. 2.—Specific activity of DNA in the thymus (a) and spleen (b) of whole-body X-irradiated A2G mice (500 rad) after the administration of 12 μCi of ^{3}H-thymidine at 90 minutes before killing. Mice were irradiated with (—○—) or without (—•—) the application of Leucotrofina (see materials and methods). Statistical significance of $p<0.01$ at 5 days and $p<0.05$ at 7 days of the specific activity of DNA in the thymus of irradiated and Leucotrofina treated mice. Specific activity in unirradiated mice (×) was of 22,450 cpm/mg DNA in thymus and 91,300 cpm/mg DNA in spleen.

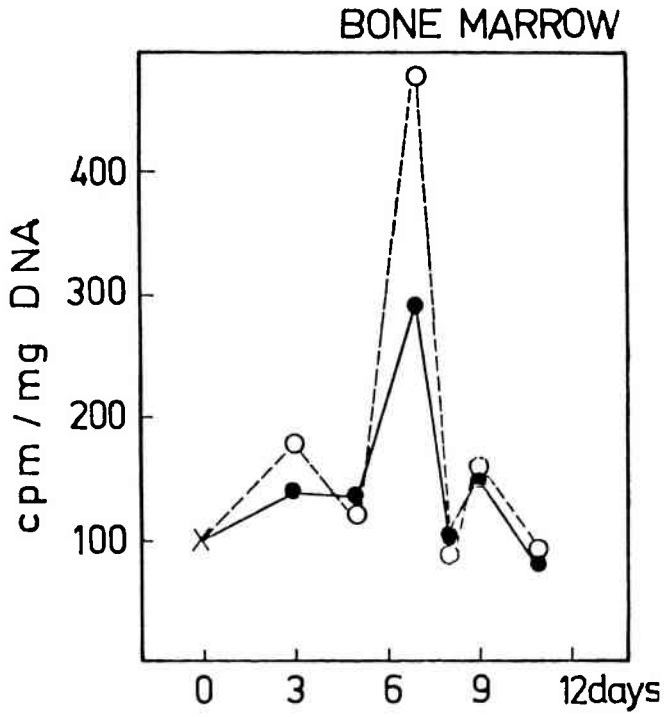

Fig. 3.—Specific activity of DNA in bone marrow. A similar experiment as that described in Fig. 2. Symbols as in Fig. 2. Specific activity in the unirradiated group was 69,300 cpm/mg bone marrow DNA.

TABLE 4.—*Haematologic changes in mice irradiated with 400 rad, untreated and treated with Leucotrofina* (see materials and methods).

Groups	No of elem./mm³ L 10^3	No of elem./mm³ E 10^6	Reticulocytes ‰	CYTOLOGY: Peripheral blood Gran N. %	Peripheral blood lymph. %	Bone marrow: Granulocytic series MBL %	PMC %	MC %	MMC %	Gran N. %	Total %	Erythrocytic series %	Lymphocytic series %
1. Unirradiated controls	4.7	8.6	26	28	72	1.3	2	12	3	47.7	66	B 26	8
2. Irradiated controls at 2 days	1.4	6.1	5	57	43	5.8	2	10	6	72.2	96	B 3	1
3. Irradiated controls at 9 days	1.6	8.0	31	30	70	8	6	—	2	33	49	P 5 B 39	7
4. Irradiated controls at 16 days	5.1	9.1	46	36	64	2	—	8	1	59	70	Pr 22	8
5. Leucotrofina (2×0.2 ml) after irrad. at 2 days	1.5	6.7	6	86	14	2.3	—	8	—	72.2	83	B 13	4
6. Leucotrofina (2×0.2 ml) after irrad. at 9 days	3.8	8.9	63	32	68	3	—	6	—	30	39	Pr 7 B 48	6
7. Leucotrofina (2×0.2 ml) after irrad. at 16 days	5.6	9.1	26	35	65	1	—	2	—	59	63	P 27	10

B=Basophil; P=Polychromatophil; Pr=Proerythroblast.

bone marrow) are more marked in Leucotrofina treated animals.

Discussion

The data presented in this work show that post-irradiation treatment of whole-body irradiated mice with Leucotrofina, a cell-free thymic extract, was associated with an increased survival time (Table 1). A faster haemopoietic recovery was noted by ^{59}Fe uptake into erythrocytes (Table 2), spleen and bone marrow (Table 3) and by haematological investigations (Table 4) of Leucotrofina treated mice. Lymphopoiesis was also enhanced in the thymus (Figs. 1 and 2) and bone marrow (Fig. 3) following application of Leucotrofina to irradiated mice. These data support previous observations concerning the radioprotective and therapeutic effect of different thymic cell-free extracts.[1 4 9-12]

However, the mechanism of action of these thymic extracts is still not clarified. It seems probable that the radioprotective and therapeutic effect of different thymic factors is mainly produced by stimulating the haemopoietic stem cells.

The experimental results presented in this work are in concordance with our clinical trials, where we investigated the effect of Leucotrofina treatment on the recovery of haemopoiesis by patients subjected to antitumoral radiotherapy.[9]

It seems that thymic extracts will be important in reactivating anergic or severely depressed haemopoietic and immune system to increase the resistance to infection and possible to induce endogenous-antitumour activity.[2]

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