Table 1. CSF levels in serum in mice after injection of carbon particles, and survival of mice after irradiation

Dose of carbon injection	Colonies/10^5 bone marrow cells 3 h	24 h	Survival after 675 rad of X-rays
0	0	0	3/10
0.1 mg	75±4	0	2/10
1.0 mg	97±11	0	8/10
8.0 mg	135±7	0	10/10
Standard CSF	118±4		-

Mice were injected with various doses of carbon particles i.v., and serum was collected 3 and 24 h after injection. Test serum was added to the culture at the final concentration of 10%. Mouse lung- and heart-conditioned medium (15%) was used as standard CSF. 30-day survival of mice after 675 rad of X-irradiation is shown for comparison.

Table 2. Type of colonies produced by CSF in serum from carbon-treated mice

Serum or CSF		Colonies/10^5 cells	Colonies consisted of Granulocyte	Mixed	Macrophage
LPS-serum	5%	96±10	42.2%	43.2%	14.6%
Carbon-serum	5%	35±11	0	0	100
	10%	61± 9	0	0	100
	20%	131±12	0	0	100
GM-CSF	15%	116± 8	21.7	45.5	32.8
GM-CSF+ carbon-serum	20%	127±16	0	0	100

Bone marrow cells were incubated with serum from carbon-treated mice, from LPS-treated mice or with mouse lung- and heart conditioned medium (GM-CSF), and the colonies were cytologically studied after staining with May-Giemsa. LPS-serum was obtained from mice 3 h after i.v. injection of 5 µg LPS.

locyte production[11,12]. Our results show that serum from carbon-treated mice contains not only the CSF that stimulates macrophage production but the inhibitory factor(s) of granulopoiesis. It is, therefore, unlikely that carbon-treatment acts as a direct stimulator of hemopoiesis.

We have already reported that RES-blockade results in the increased survival of the pluripotent stem cells (CFUs), and enhanced recovery of functional blood cells after irradiation[9,13]. Based on the finding that the bone marrow cells, irradiated in vitro, form more colonies in the spleen of the carbon-treated recipient mice than in control mice, we have proposed that RES-blockade offers a favourable microenvironment for the proliferation of stem cells[14]. Seki reported that macrophage layers formed on acetate cellulose membranes, supported granulopoiesis[15]. Carbon-treatment enhances granulocytic colony formation on such layers as well[16].

Together with these observations, present results suggest that carbon-treatment modulates the monocyte-macrophage system in RES, which consequently supports the increased survival and maturation of hemopoietic stem cells.

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Glucan-induced enhancement of hemopoietic recovery in gamma-irradiated mice

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Summary. A single pre- or postirradiation application of β-(1→3)-D-glucan, a potent reticuloendothelial stimulant, enhances hemopoietic recovery in sublethally gamma-irradiated mice. Pretreatment of mice with glucan significantly reduces lethal radiation effects.

Glucan (β-(1→3)-D-glucan), a component isolated from the cell wall of *Saccharomyces cerevisiae,* is a potent reticuloendothelial stimulant as well as a modulator of cellular and humoral immunity[1,2]. The administration of glucan to mice results in increased macrophage and granulocyte production, the enhanced leukopoiesis probably being mediated via an augmented release of colony-stimulating activity from macrophages[3,4]. The increased functional status of RES induced by glucan has been shown to increase nonspecific host resistance to infection[5]. Both hemopoiesis and the anti-infection defence-enhancing effects of glucan could be valuable in prevention and therapy of radiation damage to the organism. The current experiments were undertaken to evaluate these possibilities, and the effects of pre- and postirradiational application of glucan to mice were investigated.

Material and methods. Male mice of the inbred strain C57Bl/10, 12-14 weeks old, weighing 25-30 g, and kept in cages in groups of 20, were used. Standard stock diet and drinking water were given ad libitum. Control and experimental procedures were carried out concurrently in groups of mice from the same cage.

Table 1. Influence of glucan on spleen weight and bone marrow cellularity of mice irradiated with 5.5 Gy

			Days after irradiation 2	4	6	8	12
Spleen weight (mg)	Control	85.0±4.5[a]	35.2±1.9	30.1±0.7	35.4±2.0	31.5±1.1	39.1±2.5
	Glucan 24 h before irradiation		43.0±1.6**	37.8±2.6**	44.8±2.2**	42.0±2.3**	87.1±14.2**
	Glucan 24 h after irradiation		34.7±0.8	31.5±2.0	37.2±2.2	35.5±1.8	64.7±2.0**
Nucleated cells per femur ($\times 10^7$)	Control	1.82±0.18[a]	0.30±0.03	0.37±0.04	1.00±1.13	1.30±0.11	1.61±0.08
	Glucan 24 h before irradiation		0.36±0.04	0.64±0.07**	1.45±0.14*	1.92±0.14**	1.95±0.16*
	Glucan 24 h after irradiation		0.29±0.02	0.35±0.02	0.97±0.06	1.61±0.14	1.67±0.10

[a] Unirradiated, intact mice. Significance, compared to controls, * $p<0.05$; ** $p<0.01$. 6–8 animals per group used.

The mice were irradiated with single whole-body doses from a ^{60}Co gamma-ray source at a dose rate of 0.47 Gy/min. During irradiation the mice were placed individually, without general anesthesia, in chambers in a circular perspex container. Irradiation was performed during the morning. Sublethal and lethal doses were used.

Glucan in water soluble form was isolated by a modification of the alkaline-acid hydrolysis method of Hassid et al.[6] and Suzuki et al.[7]. The mice were injected i.p. with 4 mg of glucan in sterile saline (0.5 ml) either 24 h before or 24 h after irradiation. Control animals were injected with the same volumes of saline. No differences were seen in mice injected with saline before or after irradiation, so the 2 control groups were pooled.

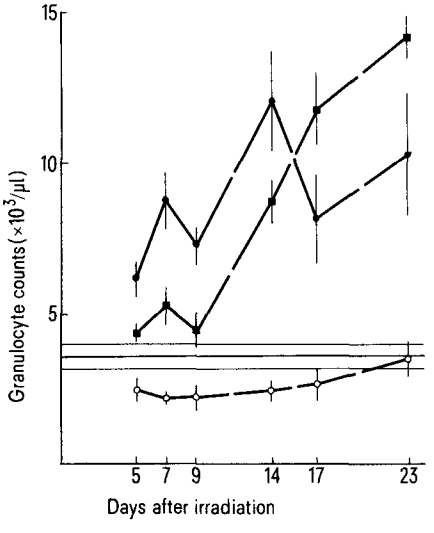
At various intervals after sublethal irradiation blood was collected from the lateral tail vein and erythrocytes as well as leukocytes were counted by means of a Coulter Counter. Granulocytes and lymphocytes were calculated on the basis of blood smear differentiation. In animals killed by decapitation, spleen weight was ascertained, the bone marrow flushed from the femurs, and the number of nucleated cells determined by using a Coulter Counter.

Lethal radiation exposures were used to study the effects of glucan treatment on the survival of animals up to the 30th day after irradiation. The values given in the data represent the mean ±SE. Statistical analysis was carried out using the distribution-free sequential test and the chi-square test.

Results and discussion. As shown in experiments on sublethal irradiation with 5.5 Gy, glucan application induces an increase in postirradiational hemopoietic recovery. The results given in table 1 indicate that in animals treated before irradiation spleen weight was maintained on a higher level at all the intervals studied and reached the normal value on the 12th postirradiational day. Application of glucan after irradiation was less effective in this respect. Similar effects were observed in the recovery of bone marrow cellularity. Results presented in the figure show changes in the granulocyte counts of peripheral blood. Both pre- and postirradiational injections of glucan induce a striking increase in the blood granulocytes with an overshoot of normal values. Preirradiational treatment leads to an earlier enhancement of granulocyte production. No significant differences were observed in erythrocyte counts in the blood of treated and control animals observed up to the 23rd day after irradiation. Similarly, no differences in recovery rates of blood lymphocytes were seen (data on erythrocyte and lymphocyte counts are not given).

The results of experiments using lethal radiation doses are given in table 2. Preirradiational application of glucan reduces the lethality of mice, postirradiational treatment being less effective. The protective effect of glucan in terms of survival is more pronounced after higher radiation doses approaching 100% lethality.

The main mechanism of the radioprotective action of glucan seems to be the intensification of granulocyte production. It is well known that granulocytes play an impor-



Influence of glucan on granulocyte counts in the blood of mice irradiated with 5.5 Gy. ○—○, Control; ●—●, glucan given 24 h before irradiation; ■—■, glucan given 24 h after irradiation; horizontal line - counts in unirradiated, intact mice. 6–8 animals per group used. All the values in glucan treated groups are significant relative to control group at 0.01 level.

Table 2. Influence of glucan on lethality of irradiated mice

Dose of irradiation	Experimental group	Death % 15th day	30th day	No. of animals used
9 Gy	Control	33.3	46.7	30
	Glucan 24 h before irradiation	23.3	33.3	30
9.5 Gy	Control	50.0	81.3	16
	Glucan 24 h before irradiation	8.3	33.3*	12
	Glucan 24 h after irradiation	33.3	66.7	12
10 Gy	Control	87.5	93.8	16
	Glucan 24 h before irradiation	0**	33.3**	12
	Glucan 24 h after irradiation	83.3	91.7	12

Significance, compared to controls, * $p<0.05$; ** $p<0.01$.

tant role in the clearance of bacteria and endotoxin[8]. From this point of view a better manifestation of the protective effectiveness of glucan at higher radiation doses can be understood. At these doses autointoxication and infectious consequences of intestinal damage can be expected to play a leading role in the death of the organism. The decreased efficacy of postirradiational treatment with glucan, as compared with the preirradiational one, may be due to a reduction of available effector cells.

Glucan thus seems to have good radioprotective potencies. However, the proper dosage, time and route of administration have yet to be studied.

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A rapid method of following the spontaneous regression of experimental leukemias

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Summary. Transplantation of virus and chemically induced leukemias from C3H/He-mg × AKR/F_1 hybrid mice into C3H/He-mg males induced leukemias in the latter, which was followed by a spontaneous regression of the disease within a few days. The regression of leukemia could easily be followed by measuring the changes in the pyruvate kinase activity of para-aortic lymph node cells.

Relatively few reports have been published so far on chemically induced leukemia characterized by a spontaneous regression[1-7]. The degree of regression has been studied mainly by laborious morphological methods. It has earlier been observed in our laboratory that transplantation of Gross-virus leukemia and of a chemically induced leukemia from C3H/He-mg × AKR/F_1H-2^k hybrid mice into male C3H/He-mg mice identical at the H-2^k locus induces leukemia in the recipients. It will however, regress spontaneously within a few days. According to Weber, pyruvate kinase is a good marker of malignancy in experimental tumors[8]. The activity of pyruvate kinase also increases in the lymph nodes and in the spleen with both Gross-virus leukemia[9], and chemically induced leukemia[10]. Hence, the changes in pyruvate kinase activity, which can be determined by a simple and rapid method, can serve as a marker of the spontaneous regression of leukemia. In the present communication the changes in pyruvate kinase activity in para-aortic lymph nodes were compared with the results of histological and flow cytometric studies.

Materials and methods. 6-8-week-old male and female AKR and C3H/He-mg inbred mice were purchased from the Laboratory Animal Breeding Center of Hungary (Gödöllö). To obtain hybrid mice, pairs of the 2 strains were cross-bred in our own animal breeding house. Gross-virus leukemia was originally obtained from an AKR mouse, and has been maintained in our laboratory in AKR/Lati × C3H/He-mg/Lati/F_1 hybrid mice by serial passages since 1975. Chemically induced leukemia was originally observed in our laboratory in AKR/Lati × C3H/He-mg/Lati/F_1 hybrid female mice after a simultaneous treatment with urethane and diethylstilboestrol[11]. This form of leukemia has since been maintained in these hybrids by serial passages. Both forms of leukemia were transplanted by i.p. injection of 10^6 viable spleen cells[12]. The animals with Gross-virus leukemia were killed by cervical dislocation 3, 6, 9 or 14 days after injection.

Histological sections, prepared from fragments of different organs fixed in formalin and embedded in paraffin, were stained with haematoxilin-eosin. The activity of pyruvate kinase from para-aortic lymph nodes was measured according to Guttman and Bernt[13]. The size of cells from mesenteric lymph nodes was determined with a Becton-Dickinson FACS-III flow cytometer on the basis of the scattered light intensity of the cells[14,15]. Similar studies were also carried out in the case of chemically induced and transplanted leukemia except that the experiments were performed on the 9th, 14th and 21st (and also, in certain cases, on the 6th, 7th and 28th) days after injection with leukemic spleen cells because of the longer duration of the disease.

Results and discussion. Histological studies indicate that Gross-virus leukemia both in male and in female mice, and also chemically induced leukemia in C3H/He-mg male mice, are accompanied by the gradual formation of diffuse blastic infiltration which destroys the original structure of the lymph nodes spleen and thymus. Then residual proliferating foci are present during regression and finally, only the loose structures indicate the previous leukemic state. The phase of regression is characterized by a considerable increase in macrophage activity.

The size distribution of lymphocytes prepared from the mesenteric lymph nodes of C3H/He-mg and AKR mice is presented in figures 1 and 2, respectively as was determined

Pyruvate kinase activity/mU/mg/in para-aortic lymph node cells

	Gross-virus-induced leukemia	Chemically induced transplantable leukemia
Control	227.4 ± 19.6	
3rd day	351.11 ± 25.4	301.11 ± 24.5
6th day	632.21 ± 22.8[a]	663.90 ± 25.2[a]
9th day	259.22 ± 30.2	494.19 ± 26.8[a]
14th day	222.23 ± 20.0	261.38 ± 29.9
21st day	220.31 ± 18.1	257.12 ± 20.6
28th day	223.22 ± 19.3	285.50 ± 27.3

[a] $p < 0.001$. Each value represents the mean of 12 determinations ± SE.