EFFECT OF THYMOCYTE AUTOGRAFTS ON POSTULATED REGENERATION OF HEMATOPOIESIS

M. I. Fedotova and Yu. I. Zimin

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The effect of thymocyte autografts ($4 \cdot 10^8$–$8 \cdot 10^3$ cells) on restoration of the cellular composition of the bone marrow and spleen was studied in thymectomized Wistar rats irradiated in a dose of 400 R. Autografting of the thymocytes was bound to have a significant stimulant effect on growth of the erythroid and granulocytic series in the bone marrow and the lymphoid series in the spleen during recovery (5th-7th day) after irradiation. Irradiation of the suspension of thymocytes with γ rays in a dose of 10,000 R did not prevent the effects of the autografts on bone marrow but reduced the stimulant effect. It is suggested that the injected thymocytes interact with the hematopoietic stem cell.

KEY WORDS: autografting; of thymocytes; irradiation with γ rays; restorationof hematopoiesis; thymectomy.

Besides the many investigations into the role of the thymus in immunologic processes its role as a hematopoietic organ has been investigated. However, progress in this field has been limited. In experiments on animals thymectomized at various periods of their lives, changes were found in the lymphoid system, together with various degrees of disturbance of erythroid hematopoiesis [4, 6-9]. However, the results of these investigations do not provide weighty evidence of the direct participation of the thymus in the histogenesis of the blood cells, but they may rather be interpreted as the result of considerable changes in metabolism induced by thymectomy when carried out in the early period of life, with consequent changes in all the systems of the body, including the hematopoietic system.

The study of the role of the thymus in postirradiation repair of hematopoiesis [2, 3] has yielded negative results. The writers' previous investigation [1] showed that the number of lymphoid cells in the bone marrow of thymectomized rats on the 13th-15th day after irradiation by a dose of 400 R is significantly less than in the control.

On the basis of these results it was deceided to continue the study of the role of thymocytes (T-lymphocytes) in postradiation repair of hematopoiesis, and the investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

Altogether 370 male Wistar rats weighing 160-180 g were used. Thymectomy was performed on 300 of the animals under ether anesthesia with aseptic precautions. Immediately after the operation the rats were irradiated with Co^{60} γ rays in a dose of 400 R and at a dose rate of 345 R/min. The irradiated animals were divided into control and experimental groups. The thymus of each experimental rat was suspended in medium No. 199, washed once, centrifuged at 800-1000 rpm, and injected intravenously into the same animal (autografting) immediately after irradiation. The scheme of the experiment is illustrated in Fig. 1. In the course of the next 20 days, at various times, the number of nucleated cells in the spleen and femur was determined in the control and experimental rats. On the basis of myelograms obtained by counting squash preparations stained by Pappenheim's method, the absolute number of cells of the various gener-

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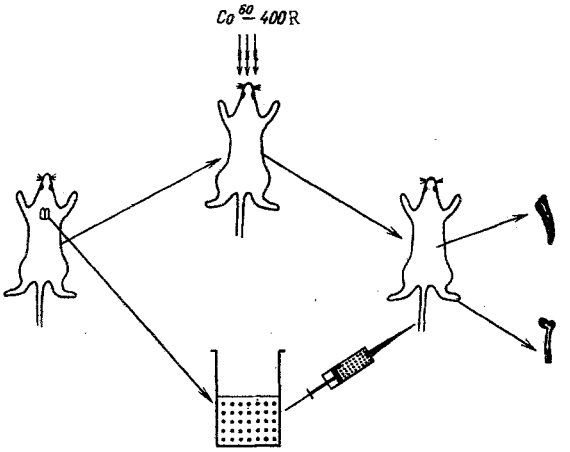

Fig. 1. Scheme of the experiment.

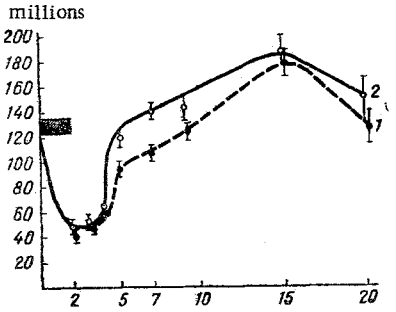

Fig. 2

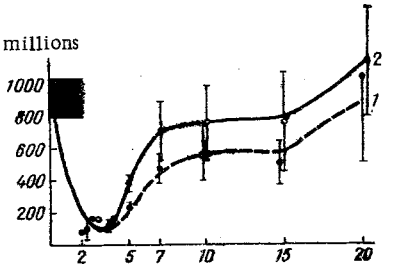

Fig. 3

Fig. 2. Total number of bone-marrow cells after irradiation of thymectomized (1) rats and in rats thymectomized and subsequently autografted with thymocytes (2). Vertical lines show confidence limits. Shaded part gives confidence limits for intact animals. Abscissa, days after irradiation in dose of 400 R; ordinate, number of bone-marrow cells in femur (in millions).

Fig. 3. Total number of cells in spleen after irradiation in thymectomized rats (1) and rats thymectomized and then autografted (2). Ordinate, number of cells in spleen (in millions). Remainder of legend as in Fig. 2.

ations was determined. Statistical analysis of all the results was carried out by the use of Student's criteria.

EXPERIMENTAL RESULTS

The results of determination of the number of bone-marrrow cells (Fig. 2) showed that at the time of maximal destruction of the bone marrow (2nd-4th days) there was no difference between the experimental and control groups of animals. A significant difference appeared during the recovery period. Starting from the 5th day the mean number of bone-marrow cells was significantly higher in the rats receiving an autograft of thymocytes than in the control animals. Examination of the cellular compostion of the bone-marrow (Table 1) reveals that differences existed at different times after irradiation for both erythroid cells (on the 5th day) and granulocytes (in the period after the 7th day). Autografting of thymocytes after irradiation also stimulated recovery of the lymphoid organs. Starting from the 5th day after irradiation the number of cells in the spleen was higher in the experimental rats (Fig. 3) than in the controls. In an attempt to discover whether the viability of the transplanted cells had any bearing on this phenomenon, thymocytes previously irradiated in a dose of 10,000 R on a γ ray source with a dose rate of 3000 R/min were autografted. The results of these experiments (Table 1) showed that irradiation of the cell suspension of thymocytes appreciably reduced the stimulant effect of autografting both in the bone marrow and in the spleen.

These experiments showed reliably that autografting of thymocytes (immediately after irradiation) leads to the more rapid recovery of the bone marrow and spleen. Since this effect was observed in all cell

TABLE 1. Number of Cells in Bone Marrow and Spleen on 5th and 7th Days after Irradiation in Dose of 400 R (M ± m).

Experimental condition	Bone marrow (number of cells in femur)				Spleen	
	erythroid cells • 10^6		neutrophils • 10^6		total number of cells • 10^6	
Thymectomy (control)	5th day	7th day	5th day	7th day	5th day	7th day
Thymectomy + autografting of thymocytes	19,2±1,7	43,5±3,7	50,9±3,0	43,6±3,7	240,3±14,8	481,0±44,0
	39,4±2,8*	46,2±2,6	49,1±2,0	58,6±4,4*	338,0±23,4*	720,0±91,6*
Thymectomy + autografting of thymocytes irradiated in vitro (10,000 R)	33,7±2,3*	41,4±5,48	50,6±2,2	52,7±6,2	280,0±10,0	530,0±70,0

*Differences from control significant at $P \leq 0.05$.

generations (erythroid, granulocytic, and lymphoid), it can be concluded that it is realized at the level of the hematopoietic stem cell and that T-lymphocytes may evidently participate in the histogenesis of the blood cells. This conclusion is confirmed by an investigation [5] in which it was found that injection of syngeneic thymocytes into mice irradiated in a dose of 475 R leads to an increase in the number of endogenous colonies in the spleen compared with the control. The mechanism of this interaction is not yet clear. However, an investigation has been published [10] in which the penetration of the donor's thymocytes was demonstrated into the bone marrow and spleen of a syngeneic receipient.

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