QUANTITATIVE CHARACTERISTICS OF THE RAPID REGENERATION OF HEMATOPOIESIS PHENOMENON FOLLOWING THYMOCYTE TRANSPLANTATION

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Quantitative characteristics of the phenomenon of rapid postirradiation regeneration of the bone marrow following transplantation of syngeneic thymocytes were obtained in experiments on (CBA × C57BL)F_1 hybrid mice. Within the range 10^6–10^8 cells the effect was shown to be independent of the number of transplanted thymocytes. Transplantation in the first 12 h after irradiation was equally effective as regards stimulation of regeneration of the bone marrow. The phenomenon did not appear under the following conditions: a) transplantation of thymocytes 24 h after irradiation; b) irradiation of the recipients in a dose of 600–700 R; c) irradiation of a suspension of thymocytes in vitro in a dose of 10 kR followed by incubation at 37° C for 6–7 h; d) irradiation of the thymocytes in vitro in a dose of 200 kR.

KEY WORDS: irradiation; hematopoiesis; regeneration; stimulation by thymocytes.

Previous investigations by the writers [1, 2] and the work of Lord and Schofield [4] have shown that after transplantation of thymocytes postirradiation regeneration of hematopoiesis takes place much faster. Most probably the thymocytes themselves do not participate directly in bone-marrow hematopoiesis. The suggestion has been made that during regeneration of hematopoiesis cooperation of the T-lymphocytes takes place with the hematopoietic cells, leading to increased proliferative activity of the marrow.

This paper describes a study of the quantitative characteristics of the phenomenon of rapid regeneration of hematopoiesis after transplantation of thymocytes.

EXPERIMENTAL METHOD

(CBA × C57BL)F_1 hybrid mice were used. A suspension of thymus cells was prepared as described in [7]. Recipient mice were irradiated on a γ-ray apparatus (Co^{60}) in a dose of 400 R at a dose rate of 320 R/min. Different numbers of viable (not staining in a 0.1% solution of trypan blue) thymocytes were transplanted intravenously into syngeneic recipients 1, 6, 9, 12, and 24 h after irradiation. The cells were washed once by centrifugation at 800–1000 rpm before transplantation. On the 5th and 7th days after irradiation the mice were decapitated and the number of cells in the femur counted as described previously [2].

EXPERIMENTAL RESULTS

On the 5th and 7th days after irradiation the total number of myelokaryocytes in the femur of mice into which thymocytes were transplanted immediately after irradiation was 25–40% higher than in the control animals. In the next experiments determination of the number of cells in the femur on the 7th day after irradiation was adopted as the sole criterion of the intensity of regenerative processes.

Within a certain range the stimulant effect was independent of the number of cells injected (Fig. 1). A dose of $1 \cdot 10^5$ cells was ineffective. The effect first appeared after injection of $1 \cdot 10^6$ cells, but was unchanged with a further increase in the number of cells to $1 \cdot 10^8$. In the subsequent experiments the same number of cells ($5 \cdot 10^7$) was always injected.

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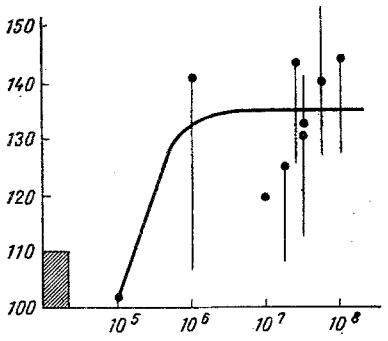

Fig. 1

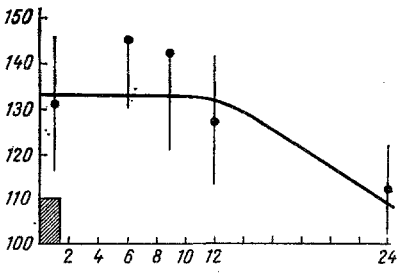

Fig. 2

Fig. 1. Stimulant effect as a function of dose of transplanted cells. Transplantation 1 h after irradiation in dose of 400 R. Abscissa, number of transplanted thymocytes; ordinate, number of cells in femur on 7th day after irradiation (in % of control, irradiated in same dose). Shaded area represents confidence limits of variation of parameter in control; mean values and confidence limits ($P < 0.05$) of parameter for experimental group of animals given.

Fig. 2. Stimulant effect of transplantation as a function of time after irradiation. Transplantation of $5 \cdot 10^7$ thymocytes. Abscissa, time (in h) between irradiation and transplantation; ordinate, number of cells in femur on 7th day after irradiation in a dose of 400 R (in % of irradiated control). Remainder of legend as in Fig. 1.

The time of transplantation played a decisive role in the production of the stimulant effect on postirradiation regeneration of hematopoiesis. Transplantation of thymocytes into syngeneic recipients irradiated in a dose of 400 R in the first 12 h after irradiation was equally effective as regards stimulation of bone marrow-regeneration (Fig. 2). Transplantation of the cells 24 h after irradiation, however, was ineffective. The effectiveness of transplantation of viable thymocytes decreased if the dose of irradiation of the recipients was increased to 600-700 R. Consequently, for the effect to take place, a certain minimal number of cells of the hematopoietic tissue had to remain intact.

The radioresistance of thymocytes, assessed from their ability to stimulate the restoration of hematopoiesis when injected intravenously after irradiation, was fairly high. They could not be completely inactivated by irradiation in vitro in a dose of 10 kR [2]. With this dose of irradiation, the cells began to die after 4-6 h [9]. Interaction between thymocytes and hematopoietic cells evidently takes place while their structural integrity is still preserved. If thymocytes irradiated with γ-rays in a dose of 10 kR were incubated for 6-7 h in medium No. 199 at 37° C or irradiated in a dose causing instantaneous death (200 kR), their ability to stimulate postirradiation regeneration of the bone marrow after syngeneic transplantation was completely lost.

Suspensions of thymocytes were tested by the widely used cloning method of Till and McCulloch [8] to determine whether hematopoietic stem cells were present in them. After transplantation of $5 \cdot 10^7$ thymocytes into lethally (850 R) irradiated recipients, no increase in the growth of colonies in the spleen was found on the 9th day after irradiation (0.92 ± 0.41 colony in the irradiated control, 1 ± 0.23 colony after transplantation of thymocytes). These results are in agreement with those obtained by other workers [5].

After transplantation of thymocytes, among which, as was demonstrated, there were no hematopoietic stem cells, acceleration of postirradiation regeneration of the bone marrow was thus found. The results indicate that this was a manifestation of cellular interaction – one of the important factors in cell proliferation and differentiation. To explain interaction between the T-lymphocyte and the bone-marrow cells it must be assumed that the thymocytes enter the bone marrow after transplantation. That this may occur has been verified experimentally [3, 6].

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