5. H. M. Vriesendorp et al., Tissue Antigens, 3, 145 (1973).
6. C. M. van der Weerdt et al., Vox Sang. (Basel), 8, 513 (1963).
7. L. E. Young et al., Am. J. Vet. Res., 13, 207 (1952).

CHARACTER OF THE STIMULATING ACTION OF ANTILYMPHOCYTIC SERUM IN THE EARLY STAGES OF RESTORATION OF HEMATOPOIESIS IN THE SPLEEN OF RADIATION CHIMERAS

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The object of this investigation, conducted on 236 BALB/c mice, was to study the effect of antilymphocytic serum (ALS) on the early stages of restoration of hematopoiesis in the spleen of radiation chimeras, having regard to the fact that ALS increases the number of macroscopic hematopoietic colonies. The results showed that this increase is connected with intensification of the first stage of restoration of hematopoiesis, i.e., with acceleration and intensification of the activation process of the reticular cells of the recipient's spleen.

KEY WORDS: radiation chimeras; spleen; antilymphocytic serum; hematopoietic colonies.

Restoration of the ability to form morphologically identifiable hematopoietic cells, when disturbed by irradiation, in the spleen of mouse radiation chimeras takes place in three stages: First, the reticular cells are activated, then microcolonies of hematologically undifferentiated blast cells are formed, and this is followed by the appearance of differentiated hematopoietic cells [1].

Antilymphocytic serum (ALS), obtained 24 h before irradiation and subsequent bone marrow transplantation, approximately doubles the number of hematopoietic colonies arising in the spleen after 8 days [2].

The object of this investigation was to study the effect of ALS on restoration of hematopoiesis in radiation chimeras.

EXPERIMENTAL METHOD

Experiments were carried out on 236 male BALB/c mice. The animals of group 1 (control) were irradiated once with γ rays on a cobalt (^{60}Co) apparatus in a dose of 750 rad with a dose rate of 80 rad/min. Each animal received an intravenous injection of 10^5 syngenetic bone marrow cells 24 h after irradiation. The mice of group 2 (experimental) were irradiated in the same dose and received an injection of the same number of myelokaryocytes. These mice also received a subcutaneous injection of rabbit antimouse ALS 24 h before the injection of bone marrow cells in a dose of 0.25 ml per mouse.

The mice of both groups were decapitated 2 h and 1, 2, 3, 4, 5, and 6 days after the transplantation of bone marrow cells and a morphological analysis was made of the cell composition of the red pulp of the spleen and the number of colony-forming units (CFU) in the spleen was counted [1].

EXPERIMENTAL RESULTS

Subcutaneous injection of ALS into the mice caused rapid death of the lymphocytes in the white pulp of the spleen, and this evidently delayed their postradiation recovery. By 48 h after injection of the ALS only the

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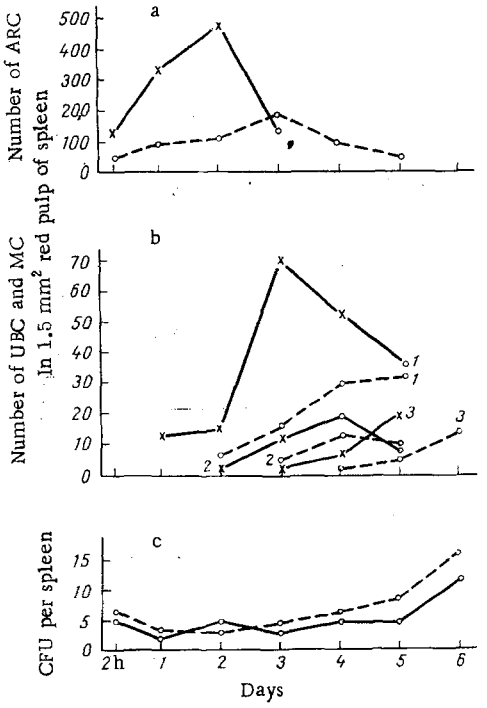

Fig. 1

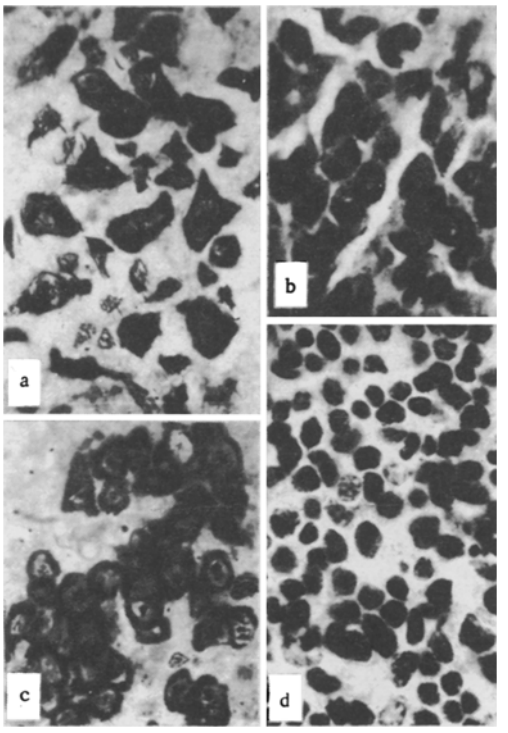

Fig. 2

Fig. 1. Quantitative characteristics of activation of reticular cells (a), hematopoietic differentiation (b), and restoration of number of CFU (c) in spleen of BALB/c mice after injection of ALS, irradiation, and transplantation of 10^5 syngenetic myelokaryocytes. 1) Undifferentiated blast cells (UBC); 2) microscopic colonies (MC) from UBC; 3) MC from hematologically differentiated cells (HDC). ARC) Activated reticular cells. Broken line indicates without ALS (group 1), continuous line with ALS (group 2). x) Differences between means are statistically significant.

Fig. 2. Mouse spleen after injection of ALS, irradiation, and bone marrow transplantation: a) many activated reticular cells in red pulp, 2 days; b) microscopic colony of UBC, third day; c) microscopic granulocytic colony, third day; d) microscopic erythrocytic colony, fifth day. Azure II–eosin, 1000×.

reticular and plasma cells remained undamaged in the white pulp, where they were scattered among cell debris formed from dying lymphocytes. Lymphoid hypoplasia continued in the spleen throughout the 6 days of observation. In the control animals, on the other hand, on the fifth to sixth day some lymphoid follicles were large and contained many small and medium lymphocytes.

Intensive activation of the reticular cells of the red pulp was observed in the spleen of the mice stimulated with ALS (Figs. 1a and 2a). In the first 2 days after transplantation of bone marrow the number of activated reticular cells (large cells with basophilic, frequently branching cytoplasm and with a large and deeply stained nucleus) was much greater than in the animals not receiving ALS. Mitotic figures were observed in some reticular cells. The number of activated reticular cells reached a maximum on the second day, but not until the third day in the control group of mice.

Solitary diffusely scattered undifferentiated blast cells with a basophilic cytoplasm and large nucleus containing one or two nucleoli appeared in the red pulp of the spleen as early as 24 h after bone marrow transplantation in group 2 and 48 h after transplantation in group 1. Between the second and third days, during a period of a sharp decrease in the number of activated reticular cells (Fig. 1a), the number of single undifferentiated blast cells increased to reach a maximum on the third day (Fig. 1b). The number of single undifferentiated blast cells increased gradually in the red pulp of the animals not stimulated by ALS, and this process was quantitatively less marked.

TABLE 1. Stimulating Action of ALS on Formation of Exogenous Hematopoietic Colonies in Irradiated BALB/c Mice

ALS	Number of mice	Number of cells injected	Diameter of colonies, mm			Mean number of colonies per spleen	P
			0,5—1	1—1,5	1,5		
—	8	5×10^4	9,0±0,2	—	—	9,0±0,2	—
+	10	"	12,0±0,9	3,0±0,6	5,1±0,4	20,1±0,8	<0,001

On the second day after transplantation of bone marrow small foci of undifferentiated blast cells, or microcolonies, were detected in the spleens of the animals of group 2. By the third day they had increased in number (Fig. 1b) and size (Fig. 2b), and by the fourth day the number of microcolonies of undifferentiated blast cells had reached its maximum, with a sharp decline to the fifth day.

In the control animals microcolonies of this type were found a day later and they were smaller. However, just as in the experimental mice, their number reached a maximum on the fourth day after transplantation of the bone marrow and fell sharply on the fifth day. No statistically significant differences were present in the animals of either group (Fig. 1). Microscopic colonies in mice stimulated by ALS simply appeared larger.

Hematologically differentiated cells in microscopic colonies in the animals of group 2 appeared on the third day (Fig. 1b), but in the control mice not until the fourth day after bone marrow transplantation. In the animals stimulated with ALS, to begin with only colonies consisting of cells of the granulocytic series appeared (Fig. 2c) and it was not until the fourth to fifth day that colonies of erythroid (Fig. 2d) and megakaryocytic types appeared. In the control mice erythroid microscopic colonies first appeared on the fourth to fifth day, and granulocytic colonies were not found until the sixth day. Under the influence of ALS differentiation of the colonies of erythroid type evidently changes to myeloid, as a result of which the ratio between the number of erythroid and myeloid microcolonies (E/M) was 3.2 in the control mice, but only 0.37 in the mice stimulated with ALS; i.e., 10 times smaller. On the fourth to sixth day after bone marrow transplantation the number (Fig. 1b) and size of the microcolonies of hematologically differentiated cells were increased in the mice of both groups, and in group 2 the increase in the number of microcolonies took place 24 h earlier.

The number of macroscopically detectable hematopoietic colonies on the eighth day in the spleens of mice stimulated with ALS was twice that observed in the mice of the control group, and some of the colonies were larger (Table 1). Differences in the number of CFU in the spleens of the animals were not statistically significant (Fig. 1c). Similar results were obtained previously by other workers using the same model [2], nor did they find any increase in the proliferative activity of the CFU under the influence of ALS by the use of the "thymidine suicide" method.

It can accordingly be concluded that the stimulating effect of ALS on colony formation takes place through the more rapid and intensive activation of the reticular cells of the red pulp of the spleen. As a result, the first stage of restoration of hematopoiesis in the spleen of mice stimulated with ALS took place within a shorter time, so that the second stage (formation of microcolonies from undifferentiated blast cells), and also the subsequent third stage (formation of hematologically differentiated cells) of hematopoiesis began earlier. All this led to the more rapid appearance of macroscopically visible hematopoietic foci and, consequently, an increase in the number of colonies recorded, on the surface of the spleens of these mice.

LITERATURE CITED

1. M. M. Nezdatnyi and K. K. Zaitseva, Dokl. Akad. Nauk SSSR, 225, 981 (1975).
2. I. L. Chertkov, L. N. Lemeneva, and O. A. Mendelevich, Probl. Gematol., No. 1, 3 (1972).