ROLE OF LYMPHOCYTES IN REGENERATION OF HEMATOPOIESIS FOLLOWING LOCAL IRRADIATION

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There is evidence in the literature that T lymphocytes play a role in the regulation of proliferation, differentiation, and migration of stem cells [5-10, 12]. Mainly experimental approaches involving the use of an induced deficiency of the thymus-dependent system of lymphocytes or with artificial mixing of bone marrow cells and lymphocytes have been used to study this problem. The results obtained with such experimental models do not give a complete picture of the principles and mechanism of participation of lymphocytes in the regulation of hematopoiesis in the whole organism.

The object of this investigation was to study the role of lymphocytes in the regulation of the hematopoietic stem cell pool using a model of local irradiation of the bone marrow.

EXPERIMENTAL METHOD

Experiments were carried out on 1540 BALB/c mice weighing 18-20 g and four rabbits. Local irradiation of the right hind limb of the mice in a dose of 7.0 Gy)was carried out on the RUM-17 x-ray apparatus (dose rate 0.65 Gy/min). The mice were killed at different times after irradiation by cervical dislocation. The total number of nucleated cells in the thymus, spleen, and bone marrow from irradiated and screened femora was counted in the animals of all groups. The myelogram was determined in bone marrow films. The thymus was removed from some of the mice 1 month before irradiation. To study the effect of thymocytes on postirradiation regeneration of hematopoiesis, $4 \cdot 10^7$ viable thymocytes were injected intravenously into the thymectomized mice 4.5 days after irradiation. In some cases the thymocytes were destroyed by osmotic cytolysis before injection, by suspending them in distilled water. Processes of postirradiation regeneration of hematopoiesis were investigated in the animals after intraperitoneal injection of 0.2 ml of antithymocytic heterologous serum (ATS) obtained by the method in [4], or serum from unimmunized rabbits (ATS titer 1/256) into the animals on the 3rd, 4th, and 5th days after irradiation (three injections). ATS also was used to determine the number of T lymphocytes in various organs of the mice [4]. The hematopoietic stem cell (CFUs) population of the bone marrow was studied by the exogenous cloning method [13] in a lethally irradiated (7.5 Gy) syngeneic recipient. On the 8th day after transplantation of a standard number of cells ($0.5 \cdot 10^5$) colonies were counted in the recipients' spleens. To determine the absolute number of CFUs a coefficient of 0.17 was used [11]. The morphology of the colonies also was studied in histological sections of spleens stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

Irradiation of the limbs of the mice with intact thymus was accompanied by accumulation of lymphocytes in both the irradiated (Fig. 1B) and the screened (up to 190.2% of the background level) bone marrow 24 h after irradiation. Later during the experiment the number of lymphoid cells in the screened bone marrow returned to normal. The number of lymphocytes again rose sharply, however, in the hematopoietic tissue of the irradiated limb on the 5th day of the experiment. The development of lymphocytosis was preceded by a decrease in the cell content of the thymus (to 65.8% of the background level after 12 h and 37.5% on the 4th-

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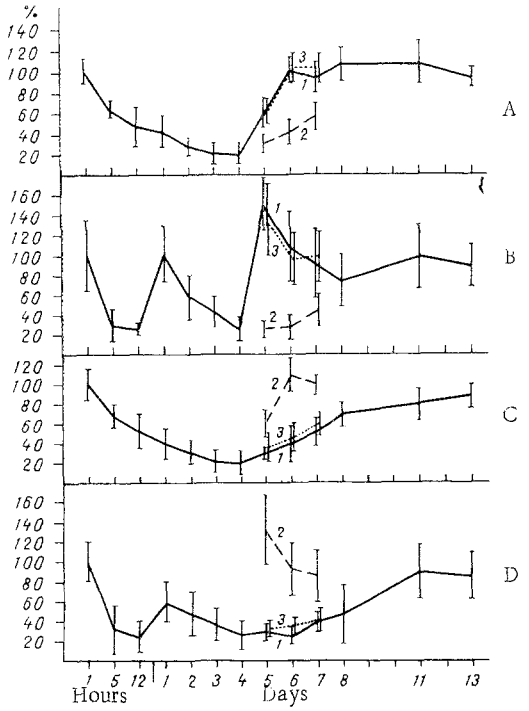

Fig. 1

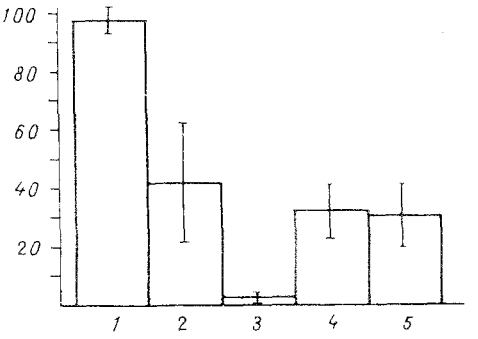

Fig. 2

Fig. 1. Time course of changes in total number of karyocytes (TNK) and lymphocytes in locally irradiated mouse bone marrow. A) TNK, B) number of lymphocytes in animals with intact thymus: 1) without serum, 2) after injection of ATS, 3) after injection of control serum. C) TNK, D) number of lymphocytes in thymectomized mice: 1) without injection of thymocytes, 2) after injection of viable thymocytes, 3) after injection of disintegrated thymocytes. Abscissa, time after irradiation; ordinate, number of cells (in % of control). Confidence intervals at P = 0.05.

Fig. 2. Number of T lymphocytes in various organs of mice. 1) Thymus, 2) spleen, 3) bone marrow (before experiment), 4) bone marrow from irradiated limb on 5th day after local irradiation of mice with intact thymus, 5) the same in thymectomized mice following intravenous injection of viable thymocytes. Ordinate, percentage of cells dying after treatment with ATS. Confidence intervals at P = 0.5.

5th day) and of the spleen (to 70.2% of the background level after 12 h and 54.9% on the 4th day).

To study the role of the thymus in the development of lymphocytosis the number of lymphocytes was analyzed in the bone marrow of thymectomized mice. The lymphocytosis in the irradiated (Fig. 1D) and screened (145.7% of the background level) hematopoietic tissue 24 h after irradiation was less marked in the thymectomized animals. Thymectomy prevented accumulation of lymphocytes in the irradiated bone marrow on the 5th day of the experiment also. Meanwhile a considerable number of T lymphocytes was found in the bone marrow of the mice with an intact thymus at the same period of the investigation (Fig. 2). Injection of ATS into irradiated mice with an intact thymus also prevented the development of lymphocytosis, but transplantation of viable thymocytes into thymectomized mice was followed by accumulation of lymphoid cells in the irradiated hematopoietic tissue (Figs. 1 and 2). The number of lymphocytes in the screened bone marrow at this period, however, was the same in all series of experiments as in intact animals.

The lymphocytosis which developed 24 h after irradiation had no significant effect on the colony-forming activity of the hematopoietic tissue. The number of CFUs in the screened bone marrow was indistinguishable throughout the period of observation in animals of all the experimental groups from that in intact donors, but in the irradiated bone marrow the number of exogenous colonies was slowly restored after an initial period of depression (Table 1). Conversely, lymphocytes which accumulated in the irradiated hematopoietic tissue on the 5th day of the experiment evidently had a stimulating effect on colony-forming activity. For in-

TABLE 1. Changes in Number of CFUs and Ratio of Number of Erythroid Colonies to Number of Myeloid (E/M) in Locally Irradiated Bone Marrow of Mice (M ± m)

Time after irradiation, days	Animals not undergoing operation		Animals undergoing mock thymectomy	No operation + ATS		No operation + control serum	Thymectomized animals		Thymectomy + viable thymocytes		Thymectomy + disintegrated thymocytes
	N	E/M	N	N	E/M	N	N	E/M	N	E/M	N
Before irradiation	15,7±0,74 (38,2)	2,8	14,5±1,25 (35,0)	15,7±0,74 (38,2)	2,8	15,7±0,74 (38,2)	13,9±1,65 (33,0)	2,7	13,9±1,65 (33,0)	2,7	13,9±1,65 (33,0)
1	2,2±0,44 (2,3)	2,4	1,3±0,65 (1,4)	—	—	—	1,2±0,65 (1,1)	2,1	—	—	—
2	3,7±1,52 (2,4)	3,5	3,3±1,11 (2,0)	—	—	—	3,8±0,74 (2,9)	2,6	—	—	—
3	4,7±1,53 (2,4)	3,3	4,9±1,54 (2,6)	—	—	—	5,0±1,35 (2,5)	2,9	—	—	—
4	5,8±2,1 (2,9)	3,1	—	—	—	—	5,9±1,59 (2,8)	3,2	—	—	—
5	6,3±1,4 (9,2)	4,5	6,1±1,01 (8,4)	7,1±1,21 (5,2)	3,1	7,5±1,41 (10,6)	6,0±1,17 4,5	2,3	7,0±1,18 (10,0)	4,3	5,6±0,99 (4,7)
6	11,0±2,05 (27,3)	4,0	12,1±1,90 (28,5)	8,4±1,83 (8,7)	3,2	14,0±1,74 (34,1)	7,8±1,76 (7,5)	2,6	13,0±1,65 (33,8)	3,8	7,2±1,24 (8,1)
7	23,0±1,91 (51,7)	2,8	20,9±1,65 (49,5)	8,7±1,41 (11,7)	2,7	24,2±1,66 (52,7)	7,6±1,31 (9,5)	2,8	25,0±1,49 (59,0)	2,9	8,1±1,41 (11,2)
8	20,1±2,10 (52,0)	3,1	19,5±1,95 (48,3)	—	—	—	8,0±2,41 (13,5)	3,3	—	—	—
11	13,5±1,19 (35,0)	2,5	—	—	—	—	12,6±2,52 (24,6)	2,7	—	—	—
13	15,5±1,5 (34,0)	2,7	—	—	—	—	15,5±1,37 (32,9)	2,9	—	—	—

Legend. Absolute number of CFU per femur (10^3) given in parentheses. N) Number of colonies per spleen.

stance, in the absence of lymphocytosis in the thymectomized animals and in mice receiving ATS the yield of exogenous colonies on the 7th-8th day of the experiment was much lower than in animals with an intact thymus. Meanwhile intraperitoneal injection of ATS had no significant effect on colony-forming activity or the state of bone marrow hematopoiesis in intact mice. On the other hand, injection of viable thymocytes into thymectomized mice restored the colony-forming activity of the irradiated bone marrow. Stimulation of colony formation, it will be noted, was produced only by viable cells; disintegrated thymocytes had no such action.

Morphological analysis of the colonies failed to reveal any significant changes in the direction of differentiation of CFUs in any of the series of experiments. Only a small increase in the relative number of erythroid colonies on the 5th-6th day in the intact and thymectomized mice receiving viable thymocytes could be noted (Table 1).

The use of a model of locally irradiated bone marrow thus showed that two types of lymphocytosis can develop in hematopoietic tissue after exposure to extremal conditions. During the 1st day after irradiation lymphoid cells accumulated in both the irradiated and screened bone marrow. The increase in the number of lymphocytes in bone marrow tissue during the 1st day after exposure to extraordinary stimuli of different nature is one essential component of the stressor reaction [2, 3]. Under the present experimental conditions accumulation of lymphocytes at this period was not accompanied by any increase in the yield of exocolonies or any change in differentiation of CFUs.

In the period preceding active recovery of hematopoiesis a selective accumulation of lymphocytes mainly of thymic origin was observed in the irradiated hematopoietic tissue only. T lymphocytes evidently stimulated the colony-forming activity of the bone marrow and accelerated the postirradiation regeneration of hematopoiesis (Fig. 1a, b), in agreement with data obtained in other experimental models [1, 9]. Lymphocytes not only from the thymus, but also from other lymphoid organs evidently migrate into the bone marrow. However, in the absence of the thymus, no marked accumulation of lymphocytes is observed.

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EXPERIMENTAL MODEL WITH AN ADDITIONAL SOURCE OF ENDOGENOUS SEROTONIN

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The only experimental method of producing a relative selective increase in the serotonin level in the body at present is by administering precursors for its synthesis. An important disadvantage of the method is the short duration of the rise in the serotonin level and the impossibility of differentiating between its central and peripheral effects [9].

In this paper we describe an experimental model with an additional source of synthesis of endogenous serotonin (ASES model). It is based on the ability of grafts of the mouse embryonic gastrointestinal tract to survive when transplanted subcutaneously into adult syngeneic mice, as several workers have discovered [2, 4], and subsequently to develop and, as we ourselves have shown [3], to produce serotonin.

EXPERIMENTAL METHOD

On the 18th-21st day of pregnancy BALB/c mice were decapitated, the fetuses were removed under sterile conditions from the uterus in the cold, and the stomach and adjacent portion of the duodenum, which contain the highest concentration of enterochromaffin (EC) cells, which produce and accumulate serotonin [10], in mice, were isolated. The graft was transplanted subcutaneously into anesthetized adult male mice (aged 2.5-3 months) of the same line. The animals were killed by quick decapitation under standard conditions 30-40 days later, when organ-like formations (cysts) had reached their maximal size.

The serotonin concentration was determined fluorometrically [6] in the blood and also in the brain stem and grafts. The principal metabolite of serotonin, 5-hydroxyindoleacetic acid (5-HIAA), was determined in the brain stem by the same method.

Tissue for morphological investigation was fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. Sections were stained with azure-eosin and the PAS reaction. To identify EC cells and other endocrine cells a combination of staining with Fast Garnet and lead hematoxylin [1] was used. Serotonin in the cells was detected by the Falck-Hillarp fluorescence histochemical method [8].

Male BALB/c mice of the same age (3.5-4 months), both intact and undergoing a mock operation, served as the control for determination of the blood and brain serotonin levels; similar mice at the age of 1-1.5 months were used to investigate the morphology of the stom-

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