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COLONY-FORMING ABILITY OF THE BONE MARROW IN MICE AFTER BURN TRAUMA

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UDC 617-001.17-092.9-07:616.419-007.1-07

The dynamics of the number of colony-forming units (CFU) in the bone marrow of CBA mice after receiving third-degree thermal burns covering 15% of the body surface was studied by the exogenous splenic colony method. The number of CFU in the bone marrow of the mice was reduced by 41-52% on the 4th and 16th days after burning. Thymus cells of intact mice, if injected simultaneously with bone marrow of the burned mice, increased the number of exogenous splenic colonies formed in the recipients. The results suggest that not only is the number of CFU reduced in the bone marrow after burns, but also the number of thymus-dependent cells necessary for normal colony formation.

KEY WORDS: colony-forming units; burns; thymus-dependent cells.

The question of the action of thermal burns on hematopoietic stem cells has received insufficient study. Yet it is evident that the principal pathogenetic factors of burns, namely extensive tissue destruction, infection, and toxemia of microbial and nonmicrobial origin, must affect the pool of stem cells. According to data in the literature [3, 6], a decrease in the number of lymphocytes in the thymus and in the peripheral population of T cells, as well as a disturbance of their function, are observed in burns. The T cells are known to play an important role not only in immunity, but also in hematopoiesis [4].

Under these circumstances it was decided to study the number of colony-forming units (CFU) in the bone marrow of mice at different times after burn trauma by the exogenous splenic colony method. The effect of thymocytes on proliferation of the stem cells in the bone marrow of burned mice in the spleen of lethally irradiated recipients also was studied.

EXPERIMENTAL METHOD

Male CBA mice aged 2.5 months were used. The effect of burns on the CFU population was studied by the exogenous splenic colony method [5]. A suspension of bone marrow cells (10^5 cells per mouse) from burned and control (intact) donors was injected intravenously into syngenetic recipients 24 h after they had been irradiated with ^{60}Co γ rays (Gamma-Cell 220 apparatus) in a dose of 900 rad (dose rate 1800 rad/min). A third-degree burn covering 15% of the body surface was obtained by immersing the dorsal region of the anesthetized mouse (0.7% pentobarbital solution, 0.15-0.20 ml intraperitoneally) in hot water (92°C) for 4 sec. In the experiments to study the effect of thymus cells on splenic colony formation by the bone marrow of the burned mice, syngenetic mice of the same age and strain were used as donors of the thymus. Thymus cells (10^7) were injected into the irradiated recipients 40 min before the injection of bone marrow cells. To prevent embolism, the mice were given 50 units heparin by intraperitoneal injection 10-20 min before receiving the injection of thymus cells. On the 9th day the recipients were killed, the spleen was removed and fixed in a mixture of acetic acid and ethanol (1:3), and the number of colonies was counted. The experimental results were analyzed with the aid of Student's criterion.

Research Institute of Medical Radiology, Obninsk. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 12, pp. 1424-1426, December, 1976. Original article submitted May 21, 1976.

TABLE 1. Number of CFU in Bone Marrow of Mice at Different Times after Burn Trauma (M ± m)

Treatment of donor	Time after burning when bone marrow was taken, days	Number of mice	Number of colonies per spleen
Control	—	12	13,6±1,0
Burns	2-nd	12	12,0±0,7
"	4-th	12	6,4±0,5
"	16-th	12	7,4±0,4

Legend. Here and in Table 2, number of colonies given per 10^5 injected bone marrow cells.

TABLE 2. Effect of Thymus Cells on Formation of Exogenous Splenic Colonies by Bone Marrow of Burned Mice (M ± m)

Treatment of donor	Treatment of recipients	Number of mice	Number of colonies per spleen	
Control	Intact bone marrow	34	12,8±0,4	$P<0,01$
Burns	Bone marrow of burned mice	30	7,1±0,4	
Burns	Bone marrow of burned mice + thymus cells	30	9,8±0,6	$P<0,05$
Control	Intact bone marrow + thymus cells	12	12,0±0.7	

EXPERIMENTAL RESULTS

In the experiments of series I the dynamics of the number of CFU in the bone marrow of the donor mice was investigated on the 2nd, 4th, and 16th days after burning. The results of these experiments are given in Table 1. They show that on the 4th and 16th days after thermal burning the number of CFU was 52 and 41% less than in the control, respectively.

In the experiments of series II the effect of thymus cells were studied on the number of exogenous splenic colonies formed from bone marrow taken on the fourth day after burning.

As Table 2 shows, the simultaneous injection of thymus cells and bone marrow cells of the irradiated mice led to an increase in the number of exogenous splenic colonies formed compared with injection of bone marrow cells of the burned mice only. Thymus cells are known not to affect colony formation in the spleen if the bone marrow of intact animals is injected into the recipients [2]. However, thymus cells do considerably increase the number of CFU if the donor's bone marrow is irradiated with small doses of ionizing radiation (180 rad) or if the donor is previously given concanavalin A, which has a specific action on T cells [2, 4].

The results of the experiments described above thus show that 4 days after burning the number of CFU, as tested by exogenous colony formation, in the bone marrow of the injured mice is considerably reduced. They also show that a certain proportion of the disturbances of hematopoiesis is connected with injury to thymus-dependent cells present in the bone marrow and assisting with colony formation. The decrease in the number of stem cells in the bone marrow of burned animals may perhaps take place as a result of an increase in the number of cells destined for differentiation, as a result of destruction of the blood cells, and also because of immune responses caused by the bacteriemia and autoantigens. Elevation of the glucocorticoid level in burn shock may be the cause of the change in the number of thymus-dependent cells.

The results now obtained, indicating that injection of thymocytes improves bone marrow function in burned animals, suggest that substances stimulating thymocyte proliferation or taking over some of their functions may prove effective in the treatment of burns. Evidence has in fact been obtained that thymus extracts improve the healing of burn wounds [1]. Perhaps the course of burns may be alleviated by the use of thymosine or of synthetic double-helical polyribonucleotides capable of stimulating thymocyte proliferation.

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