KINETICS OF HEMATOPOIETIC STEM CELL MIGRATION IN MICE AFTER SEVERE MECHANICAL TRAUMA

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The outcome of severe mechanical trauma is often determined by infectious complications in the late post-traumatic period [1, 2]. Their appearance is connected with a disturbance of humoral and cellular immunity [3], the normal functioning of which is made possible by migration of hematopoietic stem cells into the lymphoid organs, where they differentiate into immunocompetent T and B lymphocytes [5].

In the investigation described below the kinetics of migration of hematopoietic stem cells from the bone marrow was studied after severe mechanical trauma.

EXPERIMENTAL METHOD

Female (CBA × C57B1/6)F_1 mice aged 5-6 months were used. Trauma was inflicted by crushing the soft tissues of both thighs with a force of 1800 g/cm^2 for 4 h. The colony-forming activity of the bone marrow cells was tested by the exogenous cloning in bone marrow [6] and endogenous cloning in the spleen [7] methods. Bone marrow cells from intact and traumatized mice were transplated into lethally irradiated syngeneic recipients in a dose of 1×10^5 in a volume of 0.5 ml medium 199 into the lateral vein of the tail. The spleens 8 days after transplantation were fixed in Bouin's fluid and the number of colonies growing on them (CFU) was counted. To determine the number of endogenous colonies, intact and traumatized mice were irradiated with a sublethal dose (560 R) and the number of colonies on the spleen surface was counted 8 days later. The migration activity of the hematopoietic stem cells in the bone marrow was tested by the method of Petrov and Khaitov [4]. Mice with a screened hind limb (half of the leg) were irradiated with a lethal dose (760 R). The spleen was removed 8 days later and the number of CFU formed by hematopoietic stem cells migrating from the screened area of bone marrow was counted.

EXPERIMENTAL RESULTS

Changes in the stem cell population in the bone marrow of the traumatized mice were phasic in character (Table 1). During the first 4 days after trauma there was a significant decrease in the number of CFU in the bone marrow, but their number was restored by the 8th day.

The time course of the number of endogenous colonies also showed phasic changes (Table 2). During the first 4 days after trauma the number of endogenous colonies on the spleen reached a maximum, but by the 8th day the number of CFU had regained the level in intact mice.

Comparison of the results obtained by exogenous and endogenous cloning of hematopoietic stem cells reveals one special feature. The decrease in the number of CFU in the bone marrow of the traumatized mice was accompanied by a significant increase in the number of endogenous colonies on the spleen and, conversely, the increase in the number of CFU in the bone marrow on the 8th day after trauma was combined with a decrease in the number of endogenous colonies on the spleen. The existence of a relationship of this sort suggests that the decrease in the number of CFU observed in the bone marrow on the first 4 days of the post-traumatic period was due to migration of hematopoietic stem cells. The number of colonies on the spleen increased on account of migration of CFU from the bone marrow.

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TABLE 1. Number of Hematopoietic Stem Cells in Bone Marrow of Mice after Trauma

Time after trauma, days	Number of recipients of bone marrow	Number of DFU counted per $1 \cdot 10^5$ bone marrow cells	Significance of differences compared with control (P)
Control	31	24,5±1,2	—
1	11	17,7±2,8	<0,05
4	10	15,5±3,6	<0,01
8	6	21,8±3,0	>0,05

TABLE 2. Number of Endogenous Hematopoietic Colonies on Spleen of Traumatized and Sublethally Irradiated Mice

Time after trauma, days	Number of mice	Mean number of colonies per spleen	Significance of differences compared with control (P)
—	6	3,6±0,5	<0,01
1	5	10,0±0,75	
—	7	3,8±0,7	<0,01
4	8	14,1±1,5	
—	7	7,4±0,8	>0,05
8	7	7,2±2,3	

TABLE 3. Migration of Hematopoietic Stem Cells from Mouse Bone Marrow after Trauma

Time after trauma, days	Number of mice	Number of CFU migrating from marrow of leg bones, half screened during lethal irradiation	Significance of differences compared with control (P)
—	8	7,1±0,5	<0,01
1	11	18,4±1,3	
—	9	8,5±0,9	<0,01
4	14	19,0±2,1	
—	17	10,1±0,9	<0,01
8	11	4,7±0,5	

This suggestion was confirmed by a study of migration of stem cells from the part of the bone marrow that was screened during lethal irradiation. Migration was sharply intensified during the first 4 days after trauma, but later the intensity of migration weakened, and on the 8th day of migration it was actually weaker than in intact mice (Table 3).

After severe mechanical trauma phasic changes are thus observed in the kinetics of stem cell migration: On the 1st-4th days migration of stem cells from the bone marrow was sharply increased, but by the 8th day migration was reduced. The strengthening of migration was reflected in a decrease in the number of stem cells remaining in the bone marrow and an increase in their number in the spleen. Reduction of migration was accompanied by an increase in the number of CFU in the bone marrow and a decrease in the number of endogenous colonies of the spleen. Immunologic insufficiency in the post-traumatic period is not connected with inhibition of migration of hematopoietic stem cells from the bone marrow.

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