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MIGRATION OF HEMATOPOIETIC STEM CELLS AFTER BURNS

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Experiments on (CBA × C57BL)F_1 mice showed that during the period of a sharp rise in the blood endogenous glucocorticoid level 30 min-6 h after burns the number of circulating colony-forming units (CFU) falls by 50-60%. At the same time migration of CFU from an area of bone marrow screened during irradiation (850 R) was inhibited. On the 3rd-4th day after burns, migration of CFU was intensified.

KEY WORDS: burns; hematopoietic stem cells; endogenous glucocorticoids; migration of CFU.

Migration and circulation of hematopoietic stem cells *in vivo* are essential conditions for the normal functioning of the hematopoietic and immune systems. The writers showed previously that when the blood endogenous glucocorticoid level is considerably elevated, migration of colony-forming units (CFU) from the bone marrow is inhibited. After burns, periods of a sharp rise in the blood corticosteroid concentration are observed [5]. There is no information in the literature on the state of migration of hematopoietic stem cells after thermal injury. The only data given relate to a fall in the CFU content in the bone marrow of burned mice [3].

The object of the present investigation was to study the migration and circulation of CFU after burns.

EXPERIMENTAL METHOD

Male (CBA × C57BL)F_1 mice were obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR. Third degree burns were inflicted on an epilated area of the animals' back (about 10% of the total body surface) by exposure for 35 sec to an IK-500 electric lamp. The temperature under the skin was 55-60°C. At different times after infliction of the burns the mice were decapitated and the number of CFU in their whole blood determined by the exogenous colony formation method. For this purpose, at each time blood was taken from 5-7 burned donor mice and injected intravenously in a volume of 0.2 ml into lethally irradiated syngeneic recipients. The latter were killed eight days after transplantation and the number

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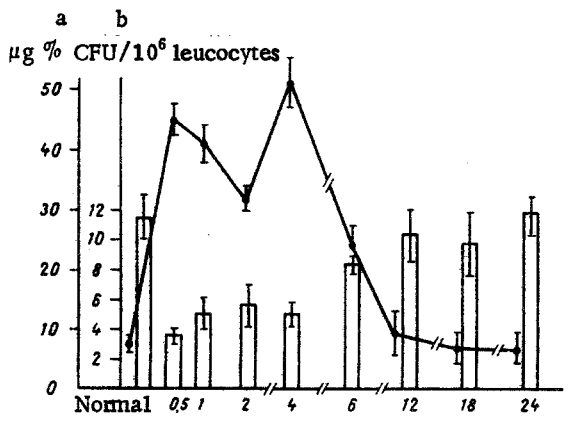

Fig. 1. Changes in 11-HCS concentration and number of CFU in blood after burn trauma. Abscissa, time after burning (in h); ordinate: a) 11-HCS concentration (in μg %) in blood plasma (curve), b) number of CFU per 10^6 peripheral blood leukocytes (columns).

of colonies on the surface of their spleens was counted. Knowing the number of leukocytes in the donors' peripheral blood, the number of CFU per million leukocytes was calculated. The concentration of 11-hydroxycorticosteroids (11-HCS) in the blood plasma was studied in parallel experiments. To assess the effect of burns on the migrating ability of the CFU, the mice were irradiated with x rays (850 R) after infliction of the burn, with the hind limb screened to the midcalf level [6]. The number of endogenous splenic colonies recorded on the ninth day after irradiation reflected the level of CFU migration from the screened bone marrow into the spleen. The results were subjected to statistical analysis by means of Student's t-test.

EXPERIMENTAL RESULTS

As Fig. 1 shows, during the first 6 h after infliction of the burn the 11-HCS concentration in the blood plasma was sharply increased but the number of CFU circulating in the blood stream was reduced by 50-60%. Later during the investigation, after normalization of the blood corticoid level, the number of circulating stem cells also returned to its initial level. In the next series of experiments the state of migration of CFU was investigated immediately after burning. As Table 1 shows, in the early period after burning the intensity of migration of stem cells fell significantly: in three repeated experiments the number of endogeneous colonies formed from CFU migrating into the spleen was 33-67% less than in the control. In some experiments the dynamics of the blood 11-HCS concentration was studied in burned animals subsequently irradiated in a dose of 850 R. The results showed that after a combination of these two extremal stimuli, acting at an interval of not more than 1 min, changes in the 11-HCS concentration during the first 24 hours were practically identical as after burning alone (Fig. 1). The writers showed previously that when the blood glucocorticoid level rises by a certain amount (injection of ACTH), the number of circulating CFU falls regularly, and if this increase lasts not less than 5-8 h, migration of stem cells from the bone marrow is inhibited [2]. Since after burns a high blood 11-HCS concentration was observed at least for 6 h, it must be concluded that the reduction in the number of circulating CFU and in their migrating ability was the result of the hypercorticoid state caused by burn trauma. However, the decrease in the number of colonies formed on account of migrating CFU could also have been the result of changes in the hematopoietic microenvironment in the spleen under the influence of the hemodynamic and metabolic disturbances observed in burns [4]. To test this hypothesis experiments were carried out in which recipient mice were first subjected to whole-body lethal irradiation (850 R), 20 h later they were burned, and after 1 h they received an injection of 5×10^4 bone marrow cells from intact syngeneic donors. In this case the exogenous bone-marrow CFU recirculated under the same conditions as the endogenous stem cells migrating from the area of bone marrow protected during irradiation. Determination of the number of colonies in the spleen 8 days after transplantation showed that in the mice with burns the number was 12.8 ± 0.6 compared with 12.1 ± 0.6 colonies in the control mice. Consequently, under these experimental conditions in the early phase of burns changes

TABLE 1. Number of Migrating CFU in Animals Burned Immediately before Irradiation (850 R) with Part of Bone Marrow Screened

Expt. No.	Number of endogenous colonies in spleen (M ± m)		Error (P)
	850 R + screening up to midcalf level (control)	burn + 850 R + screening up to midcalf level	
1	11,0±2,0 (8)	3,8±1,5 (5)	<0,02
2	15,7±1,4 (12)	10,5±0,8 (18)	<0,01
3	12,1±1,5 (7)	6,7±0,8 (7)	<0,01

Legend. 1) Number of endogenous colonies in totally irradiated control in experiment No. 1 was 0.1, in No. 2 0.5, and in No. 3 0.2 colony. 2) Here and in Table 2 number of mice shown in parentheses.

TABLE 2. Number of CFU Migrating from Screened Bone Marrow at Different Times after Burning

Expt. No.	Interval bet. burning and irrad., days	Number of endogenous colonies in spleen (M ± m)		Error (P)
		850 R + screening up to mid-calf level (control)	burn + 850 R + screening up to mid-calf level	
1	1	14,6±2,0 (10)	17,3±0,8 (9)	>0,05
	2		16,9±2,8 (7)	>0,05
	3		23,9±2,7 (8)	<0,02
2	3	15,3±1,8 (10)	24,0±1,8 (11)	<0,01
	4		21,2±1,5 (9)	<0,05

Endogenous background: 0.4 in experiment No. 1 and 0.5 colony in No. 2.

in the microenvironmental factors in the spleen that would appreciably affect the settling and fixation of stem cells in the spleen and their subsequent cloning did not take place.

The blood 11-HCS concentration 2-4 days after burning was increased but not significant: between 13.1 ± 0.7 and 15.3 ± 1.3 μg % (8.7 ± 1.1 μg % in the control). The results of investigation of the state of migration of CFU at this period are given in Table 2. They show that 1-2 days after burning the intensity of migration of stem cells did not differ significantly from that in the control, and migration of CFU was intensified after 3 and 4 days. Proteolytic enzymes and endotoxins are known to have the power of substantially increasing the number of CFU in the circulating blood [8]. An increase in the activity of proteolytic enzymes and the phenomena of bacteriemia, such as are observed during burns [4], may probably be the reason for the stimulation of migration of stem cells observed in these experiments. The reduction in the number of CFU in the bone marrow observed 4 days after burning [3] may have been due to some extent to increased migration of stem cells from the bone marrow at that time.

The high blood glucocorticoid level in burns thus inhibits migration and circulation of stem cells. The pathogenetic role of this phenomenon will evidently be determined by the degree and duration of the hypercorticism, which themselves depend on the size and severity of burn trauma.

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ORGAN CULTURES OF RAT EMBRYONIC BRAIN TISSUE (HIPPOCAMPAL REGION) IN THE STUDY OF THE TRANSPLACENTAL ACTION OF NITROSOETHYLUREA

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The transplacental action of nitrosoethylurea (NEU) was studied in organ cultures of embryonic rat brain tissue (from the region of the hippocampus). Brain tissue is distinguished by high sensitivity to NEU. This was manifested as a higher rate of survival of the experimental cultures compared with the control and as the appearance of foci of proliferation of the epithelium of the vascular plexus, in some cases resembling adenomas. The morphological changes observed in the experiments depended quantitatively on the dose of carcinogens.

KEY WORDS: transplacental carcinogenesis; organ cultures; nitrosoethylurea; hippocampus.

Ever-increasing attention is being paid to the study of transplacental carcinogenesis. On the one hand, it offers a method of studying fundamental problems in oncology, and on the other hand it may be of direct clinical importance, as observations starting in 1971 have shown [6]. In the writers' laboratory investigations into the nature of transplacental carcinogenesis both *in vivo* and *in vitro* in organ cultures have been in progress for many years [4].

The object of this paper is to describe the results of organ culture of embryonic rat brain tissue from the hippocampal region; it is in this part of the brain that tumors are found most frequently after transplacental exposure of the progeny to nitroso-compounds and, in particular, to nitrosoethylurea (NEU) [7].

EXPERIMENTAL METHOD

NEU was injected intravenously in doses of 30 and 60 mg/kg into noninbred female albino rats during the last week of pregnancy. The compound was synthesized by Candidate of Chemical Sciences O. A. Pan'shin, working in the writers' department. The animals were killed on the 20th day of pregnancy and the brain of the embryos, from the hippocampal region, was used for organ culture. The method of culture suggested by Chen [5] and modified by Adil'gireeva [1] was used. The nutrient medium consisted of two parts medium No. 199, one part calf serum, and one part chick embryonic extract, and contained 0.3% glucose. This concentration of glucose has the most favorable effect on nerve tissue cultures [8, 9]. The duration of culture in the present experiments was 18-20 days. Every 3-4 days some of the cultures were removed for fixation (in Bouin's fluid and 10% neutral formalin), and after further histological treatment serial sections of the explants were stained with hematoxylin-eosin and by Van Gieson's and the Bielschowsky–Gros methods. Altogether 196 control and 252 experimental explants were studied.

EXPERIMENTAL RESULTS

The morphological structure of the organ in control explants of embryonic brain remained throughout the period of culture characteristic of the original embryonic hippocampus and

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