ENDOGENOUS COLONY FORMATION IN MICE WITH CLOSED HEAD INJURIES

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In the modern view neuroendocrine modulation of the protective functions of the body ensures their optimization, which includes activity of the immune system [5, 6]. Its disturbance in head injuries gives rise to early infectious complications, most frequently pneumonia, which is often the cause of death [4, 12]. The disease develops against a background of activation of the hypothalamo-hypophyseo-adrenocortical system and of marked leukocytosis; the degree of elevation of the blood level of glucocorticoid hormones and the increase in the number of leukocytes depend directly on the severity of the head injury [3, 4, 12, 14].

Activity of the immune system and its potential capacity are largely dependent on the number of precursor cells supplied by the bone marrow.

The bone marrow is known to be the source of hematopoietic stem cells, the differentiated progeny of which function actively in the immune system.

In severe mechanical trauma [7] phasic changes in migration of hematopoietic stem cells are observed: stimulation of migration in the early period is followed by inhibition, immobilization stress intensifies the proliferative activity of hematopoietic stem cells [3], and endogenous colony formation is depressed in burns [10]. The important role of hypothalamic structures, thymus cells, and glucocorticoid hormones in the maintenance of this bone marrow function has been established [1, 8, 13].

The aim of this investigation was to study the effect of closed head injury on function of the bone marrow, as the source of hematopoietic stem cells, assessed on the basis of the intensity of endogenous colony formation in mice.

EXPERIMENTAL METHOD

Experiments were carried out on 772 male (CBA × C57BL)F_1 mice weighing 20-24 g. A closed head injury of measured severity (the model was developed in the Department of General Pathology and Pathological Physiology, Research Institute of Experimental Medicine, Academy of Medical Sciences of the USSR) was inflicted in the occipito-parietal region of the mouse's head by a freely falling weight (25 g), sliding down a guide rod. In the model of a mild head injury the weight fell from a height of 30 cm, and in the model of a severe head injury it fell from a height of 65 cm; the mortality was 1.2 and 17.6%, respectively. Several symptoms characteristic of this type of pathology were observed in experimental head injury. Immediately after trauma, after transient excitation, during the next 1-5 min the animals remained completely immobile, and during this time they did not respond to painful stimulation and their muscle tone was lowered. This period was followed by the development of tonic and chronic convulsions, lasting from a few seconds to 2-3 min.

In some cases trauma was accompanied by bleeding from the nose and mouth, by autonomic disorders, pareses and paralyses, and in some cases the injured animal died during the first 20-25 min after trauma. Motor activity of mice which survived was partially restored after 30-40 min, but even 4-5 h after trauma, these animals still differed considerably (intact mice).

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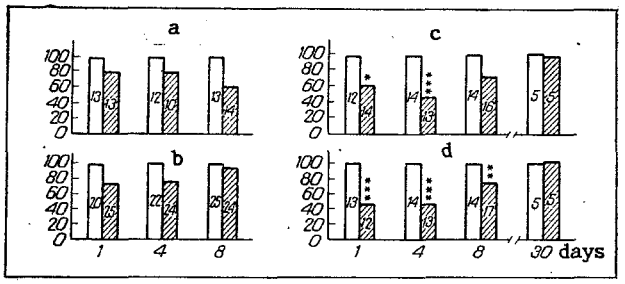

Fig. 1. Changes in endogenous colony formation (a-c) and weight of thymus (d) in mice with closed head injury of varied severity. a) 6.4 Gy, mild head injury; b) 9 Gy + screening of 1/3 of leg, severe head injury; c, d) 6.4 Gy, severe head injury. Abscissa, time after trauma; ordinate, number of macrocolonies (a-c), weight of thymus (d) in percent of control. Number of animals shown on columns. Here and in Fig. 2: unshaded columns – control, shaded – experiment. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Different versions of endogenous and exogenous colony formation were used in the experiments [11, 15]; sublethal total irradiation (6.4 Gy), lethal irradiation with protection of the bone marrow (9 Gy + screening of one-third of the leg), and injection of bone marrow cells into irradiated mice. The mice were irradiated on the RUM-17 apparatus (voltage 200 V, current 15 mA, dose rate 0.47 Gy/min, filters: 1 mm aluminum + 0.5 mm copper, focal distance 50 cm) 1, 4, 8, and 30 days after closed head injury.

To study the effect of head injury on local conditions for splenic colony formation, after 4 days the injured and control animals were given whole-body irradiation (9.2 Gy), followed by intravenous transplantation of 5×10^4 syngeneic bone marrow cells from intact mice. Colony formation was evaluated by counting the number of 8-day macrocolonies in the spleen fixed in Bouin's fluid.

In separate series of experiments, and at the same times, the following parameters were determined in the injured, unirradiated animals: body weight, weight and cell composition of the thymus, cell composition of the bone marrow, and number of reticulocytes in the peripheral blood (in blood films stained with azure II [9]).

The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The experiments showed that the number of endogenous colonies in animals with closed head injury was significantly reduced, and the severity of the effect depended on the severity of trauma (Fig. 1a, b). The clearest depression of colony formation was found in the model with sublethal irradiation: on the 4th day after head injury the number of endogenous colonies was 11.9 ± 2 in the experiment compared with 25.5 ± 1.8 in the control ($p < 0.001$; Fig. 1c). If irradiation was not uniform, a clear tendency was observed for colony formation to be depressed 1 and 4 days after trauma (Fig. 1b).

Meanwhile injection of bone marrow cells into lethally irradiated mice on the 4th day after head injury was not accompanied by any significant changes in the level of endogenous colony formation: the number of splenic colonies was 25.1 ± 0.6 ($n = 14$) in the experiment and 28.2 ± 1.4 ($n = 14$) in the control ($p > 0.05$), against a background of zero endogenous colony formation.

In head injury the local conditions of colony formation in the spleen are evidently not significantly altered, but the main disturbances take place at the bone marrow level, leading to severe inhibition of endogenous colony formation at this particular stage of the investigation.

The results obtained by different methods of cloning indicate depression mainly of proliferative activity of medullary colony-forming cells after head injury, although the possibility of inhibition of their migration under these conditions cannot be ruled out.

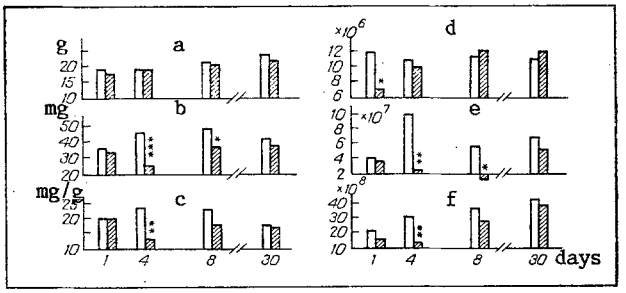

Fig. 2. Time course of changes in body weight, weight of thymus, cell content of bone marrow and thymus, and number of circulating reticulocytes in mice with severe closed head injury. a) Body weight; b) weight of thymus; c) ratio of weight of thymus to body weight; d) cell content of bone marrow; e) cell content of thymus; f) number of reticulocytes in blood; abscissa, time after trauma, seven experimental and seven control animals used at each time; ordinate, numerical value of parameter studied.

In experiments on injured, unirradiated animals changes similar to those in various stress reactions were observed [3]: a decrease in the cell content of the bone marrow on the 1st day, and a decrease in the cell content and weight of the thymus, and so on (Fig. 2).

The clearest changes were discovered in the thymus on the 4th day after severe head injury: the weight of the thymus was reduced by half, its cell content by more than two-thirds, and the ratio of the weight of the thymus to body weight also fell sharply (Fig. 2b, c, e).

A similar decrease in weight of the thymus was observed in sublethally irradiated injured animals compared with the intact control, although in the latter the weight of the thymus also was reduced following the action of ionizing radiation. It is important to emphasize that under these conditions a significant decrease in weight of the thymus was observed from the 1st through the 8th day after head injury (Fig. 1d), i.e., in the period of depression of colony formation.

At the same time, a decrease in the number of circulating reticulocytes was observed in the injured, nonirradiated animals, the decrease in their number being greatest (by 60% compared with the control, $p < 0.01$) 4 days after head injury (Fig. 2f).

The parameters, when studied 30 days after head injury, did not differ significantly from the control animals (compare Figs. 1 and 2).

Thus in the early period after closed head injury inhibition of the hematopoietic function of the bone marrow is observed, as shown by reduction of endogenous colony formation and a decrease in the number of reticulocytes in the blood of the injured animals. Changes in bone marrow function reached a maximum on the 4th day after injury, when the weight and cell content of the thymus were sharply reduced.

Early changes in colony formation after head injury (1st-4th day after trauma) were opposite to the changes in this parameter in immobilization stress [3] or after mechanical trauma associated with crushing of the limb [7], when migration and proliferation of hematopoietic stem cells were stimulated. Similar inhibition of colony formation is observed in thermal burns with marked toxemia and a high corticosteroid level [1] or 8 days after injury to the posterior hypothalamus [8].

The results are in agreement with data on the role of the thymus in the regulation of the colony-forming ability of bone marrow following exposure of the body to destabilizing factors [3, 13].

The changes discovered in bone marrow function may be among the important causes of the reduced resistance of the body to infection in the early period after head injury. The pathophysiological model developed by the writers can be used in the search for ways of correcting the disturbances noted above.

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CHANGES IN AMINO ACID METABOLISM IN DJUNGARIAN HAMSTER FIBROBLAST CELL CULTURE BECOMING RESISTANT TO COLCHICINE

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Multiple drug resistance (MDR) is one of the best studied forms of resistance of tumor cells to cytostatics. The MDR phenomenon is characterized by reduced accumulation of the cytostatic in tumor cells, its active transport from the cell, the presence of P glycoprotein on the cytoplasmic membrane of resistant cells, and abolition of resistance by Ca^{++} antagonists [1, 2]. However, despite our greater knowledge of the mechanisms of MDR, in practice its overcoming is attended by unsolved problems. The use of Ca^{++} antagonists leads to selection of cells with a higher level of resistance [3, 4]. The use of antibodies against P glycoprotein of resistant cells is not accompanied by the destruction of this cell population, for the number of antigenic determinants on the cell falls below the threshold level [5]. Attempts to find differences in metabolism of sensitive and resistant cells have proved unsuccessful [6].

In the present investigation an attempt was made to find differences only in consumption of low-molecular-weight sources of nitrogen (amino acids) from the incubation medium. This approach is preferable, for analysis is restricted to a small class of compounds that are essential for life and activity of the cells.

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