ROLE OF HUMORAL FACTORS IN REGULATION OF HEMATOPOIESIS DURING STRESS

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It has been conclusively demonstrated in recent years that proliferation and differentiation of hematopoietic precursor cells are regulated by elements forming the hemopoiesin-inducing microenvironment (HIM). Besides bone marrow cells, an important role in the regulation of hematopoiesis is played by T lymphocytes migrating into the bone marrow in extremal states [5, 9]. These T lymphocytes exert their regulatory influences on hematopoiesis in cooperation with mononuclear phagocytes [3]. Incidentally, these cells produce biologically active molecules which perform mediator functions not only in the immune system but also outside it. For instance, interleukin-3 (IL-3), produced by activated T lymphocytes, exerts regulatory action on different stages of lympho- and hematopoiesis [8]. Interleukin-1 (IL-1) induces many reactions in different organs and tissues and, in particular, it stimulates the production of an HIM of colony-stimulating factors (CSF) [13] by cells of the HIM. In addition, endogenous and exogenous colony formation may be controlled by factors produced by precursors of T lymphocytes in the bone marrow and thymus [6].

It was accordingly decided to investigate the role of IL-1, IL-3, and CSF in the regulation of hematopoiesis during stress.

EXPERIMENTAL METHOD

Experiments were carried out on 200 hybrid (CBA × C57BL/6)F_1 mice weighing 18-20 g ("Rasvet" nursery, Tomsk), immobilized for 10 h in recumbency in the supine position. At different times after the beginning of immobilization the mice were killed by cervical dislocation. The total number of myelokaryocytes (TNMK) in the femur was determined, Islets of hematopoiesis were isolated by the method of Crocker and Gordon [10]. Granulocytic-macrophagal colony-forming units ($GM\text{-}CFU_c$) were cloned in vitro in methylcellulose. Cells were flushed out of the femoral cavity with 1 ml of medium RPMI 1640, containing 10% fetal calf serum (FCS). Adherent bone marrow cells were removed on plastic Petri dishes at 37°C for 45 min in an atmosphere with 5% CO_2. The final concentration of viable nonadherent nuclears was adjusted to $0.1 \cdot 10^9$ cells/liter by means of a semiviscous culture medium of the following composition: 80% medium RPMI-1640 ("Serva," Germany), 9% FCS ("Flow," Great Britain), 1% methylcellulose ("Sigma," USA), 10% healthy human placental serum, 280 mg/liter L-glutamine ("Sigma," USA), 50 mg/liter gentamycin ("Serva," Germany), and $0.4 \cdot 10^{-5}$ M 2-mercaptoethanol ("Sigma," USA). The suspension thus prepared was poured in volumes of 2 ml into 35-mm plastic Petri dishes and incubated at 37°C, with 100% humidity and 5% CO_2 for 7 days. After culture, the colonies (aggregates containing more than 50 cells) were counted. To obtain adherent bone marrow cells of hybrid (CBA × C57BL/6)F_1 mice, myelokaryocytes ($10 \cdot 10^6$/ml) were incubated on plastic Petri dishes in medium RPMI-1640 containing 10% FCS, at 37°C and for 1 h in an atmosphere of 5% CO_2. Nonadherent cells were removed, and adherent cells were incubated for 24 h in culture medium. The level of activity of IL-1 was estimated by Mizel's method [12]. A suspension of C3H mouse thymocytes ($5 \cdot 10^5$ cells per well) was cultured in medium of the above composition in the presence of a submitogenic dose of PHA (2 μg/ml) ("Sigma," USA) and supernatants from adherent mouse bone marrow cells in a concentration of between 50 and 12.5% for

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72 h at 37°C in an atmosphere of 5% CO_2. During the 16 h before the end of incubation, 1 μCi of ^{3}H-thymidine was added to each well. The cells were then transferred to glass-fiber filters Radioactivity was estimated on a Mark III scintillation counter (USA). The level of synthesis of IL-1 activity was estimated in conventional units. The unit of activity was taken to be that dilution of supernatant at which incorporation of ^{3}H-thymidine by thymocytes was doubled. The level of IL-3 activity of nonadherent mouse bone marrow cells ($2 \cdot 10^6$/ml), incubated for 24 h in medium of the same composition, was estimated. At the end of incubation the supernatants were collected and kept for not more than 1 month at –20°C. IL-3 activity was estimated by the use of an IL-3-dependent cell line, obtained by Schrader's method [12] in our own modification. Spleen cells of CBA mice ($2 \cdot 10^6$/ml) were cultured for 21 days in medium RPMI-1640 with the addition of 10% fetal serum, 2 mM L-glutamine, 10 mM HEPES, $2.5 \cdot 10^{-5}$ 2-mercaptoethanol, 40 μg/ml gentamycin, and 50% conditioned medium of spleen cells incubated with Con A (5 μg/ml) for 24 h. At the end of culture 85% of the viable cells were mast cells. The cells thus obtained proliferated on the addition of the conditioned medium from WEHI-3 cells but did not proliferate in the presence of recombinant IL-2. Cells ($2 \cdot 10^5$ per well) sensitive to IL-3 were incubated with 50, 25, and 12.5% conditioned medium from nonadherent mouse bone marrow cells, either stimulated by Con A or not stimulated, for 48 h at 37°C in an atmosphere containing 5% CO_2. To each well 1 μCi of ^{3}H-thymidine was added 16 h before incubation. The cells were then transferred to glass-fiber filters Radioactivity was estimated on a Mark III scintillation counter (USA). IL-3 activity was estimated in conventional units. The unit of activity was taken to be that dilution of supernatant at which incorporation of ^{3}H-thymine by target cells was doubled. The colony-stimulating activity (CSA) of the conditioned medium of nonadherent mouse bone marrow cells was tested by using myelokaryocytes of intact CBA mice weighing 20-23 g as the target cells. The cells were flushed out of the femoral cavity with medium RPMI-1640 containing 5% fetal serum, and washed twice by centrifugation at 100 g for 5-10 min. Adherent bone marrow cells were removed on plastic in the course of 45 min at 37°C The final concentration was adjusted to $0.5 \cdot 10^9$ viable nuclears per liter culture medium of the following composition: 65% medium RPMI-1640, 20% fetal serum, 15% tested conditioned medium, 0.9% methylcellulose, 280 mg/liter L-glutamine, 50 μg/ml gentamycin, and $4 \cdot 10^{-4}$ M 2-mercaptoethanol The suspension was poured into 24-well "Costar" dishes (USA) in a volume of 0.5 ml per well and incubated at 37°C in 100% humidity and with 5% CO_2 for 7-8 days. After culture the colonies (aggregates containing more than 50 cells) were counted under the MBS-9 microscope (magnification 30-40). The MAF level was recorded by the method described previously [7].

EXPERIMENTAL RESULTS

In the immobilized mice marked stimulation of medullary hematopoiesis was observed. For instance, in particular, the number of GM-CFU_c was significantly increased on the 1st, 4th, and 5th days after the beginning of immobilization. Under these circumstances the increase in the number of precursor cells of myelopoiesis in the bone marrow tissue, accompanied by a decrease in the number of hematopoietic islets on the 2nd day, preceded the development of marked hyperplasia of medullary hematopoiesis and an increase in the number of hematopoietic islets on the 5th-6th day after the beginning of exposure to stress (Fig. 1a).

Changes in IL-1 synthesis by adherent bone marrow cells of (CBA × C57BL/6)F_1 mice exposed to stress, was fluctuating in character. A sharp increase (tenfold) in the spontaneous IL-1 production was observed as early as 24 h after exposure. The level of IL-1 activity in supernatants from adherent bone marrow cells then decreased toward the 3rd day to 1.5 ± 0.6 conventional unit. On the 4th day after exposure to stress, IL-1 production again was increased, but later the ability of the conditioned medium to stimulate thymocyte proliferation fell sharply.

During investigation of the trend of IL-3-like activity in supernatants obtained from nonadherent medullary nuclears, elevation of the level of IL-3 activity was observed from the 1st day of the experiment and reached peak values on the 4th-5th days after exposure. The ability of the test supernantants to stimulate mast cell proliferation fell, but still remained significantly higher (by 1.8 times) than the initial level.

We found no macrophage-activating activity in supernatants from nonadherent, nonstimulated myelokaryocytes at any time of the investigation. Meanwhile, on incubation of nonadherent bone marrow cells with Con A, on the 3rd-4th day after exposure to stress the test supernatants potentiated the cytotoxic action of peritoneal macrophages against P-815 cells.

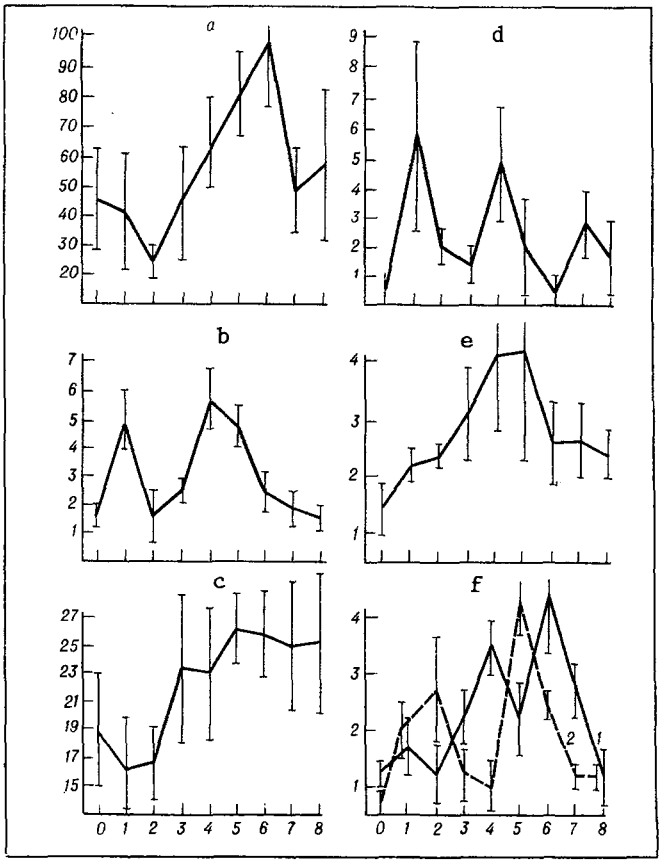

Fig. 1. Dynamics of number of hematopoietic islets (a), GM-CFU$_c$ (b), total number of myelokaryocytes (c) in bone marrow, and production of IL-1 activity by adherent cells (d), activity of IL-3 of nonadherent cells (e), and CSA of adherent cells (f, 1) and nonadherent (f, 2) bone marrow cells from (CBA × C57BL/6)F$_1$ hybrid mice exposed to immobilization for 10 h. Abscissa, time after immobilization (in days); ordinate: a) number of hematopoietic islets ($\times 10^3$ per femur), b) number of GM-CFU$_c$ ($\times 10^5$), c) total number of myelokaryocytes ($\times 10^6$ per femur), d) IL-1 activity (in conventional units), e) IL-3 activity (in conventional units), f) CSA level (number of colonies $\times 10^5$). Confidence intervals at $p = 0.05$.

The question arises, with which cells of the hemopoiesin-inducing microenvironment are the successive changes we found in synthesis of biologically active macromolecules connected. As was shown previously, during development of the stress reaction (starting with the 3rd day after the beginning of immobilization) T-lymphocytes migrate into the bone marrow, and stimulate proliferation and differentiation of the precursor cells of the erythroid and granulomonocytic branches of hematopoiesis and their more highly differentiated progenies, T Lymphocytes with the Lyt-1$^+$2$^+$ phenotype initiate proliferation of precursor cells [1]. Meanwhile, the regulating action of T lymphocytes is realized in cooperation with macrophages and is connected with activation of bone marrow stromal cells [2]. Probably the increase in synthesis of IL-3 and macrophage-acting factor on the 3rd day after the beginning of exposure to stress is connected with this lymphocyte population. Activated macrophages begin to produce IL-1, and in turn they stimulate production of CSA by bone-marrow nuclears.

When the changes in synthesis of regulatory molecules on the 1st-2nd days after exposure to stress are interpreted, it can be tentatively suggested that IL-1 activates the function of T lymphocytes, which accumulate in the bone marrow during the first few hours of development of the stress reaction [4]. Under these circumstances migrating T cells produce IL-3, which initiates processes of hematopoietic stem cell proliferation.

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COMPARISON OF THE EFFECT OF ADAPTATION TO STRESS AND TO HIGH ALTITUDE HYPOXIA ON RESISTANCE OF THE HEART TO REPERFUSION INJURY AFTER TOTAL ISCHEMIA

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During repeated exposure to stress or hypoxic situations, adaptation develops, not only increasing the body's resistance to severe stress or acute hypoxia, but also giving rise to a broad spectrum of protective cross-effects, i.e., it protects the body against direct ischemic [4], chemical [14], and cold [11] injuries. So far as the heart is concerned it has been shown that animals adapted to short-term stress or to periodic exposure to high-altitude hypoxia acquire additional resistance to ischemic and reperfusion arrhythmias, reproduced in the whole organism [2, 3]. A common feature of the cardioprotective effects of the two forms of adaptation is that activation of stress-limiting systems plays a role in them: GABA-ergic [4], opioidergic [9], antioxidative [2], prostaglandin [4], etc. The difference between these effects is very clearly exhibited at the heart level, where adaptation to hypoxia has a primary antiischemic effect, i.e., due to adaptive growth of

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