PHENOTYPIC AND FUNCTIONAL CHARACTERISTICS OF A T LYMPHOCYTE REGULATOR SUBPOPULATION INVOLVED IN STIMULATION OF HEMATOPOIESIS DURING STRESS

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The concept that T lymphocytes control processes of proliferation and differentiation of hematopoietic precursor cells in vivo continues to receive further experimental confirmation [1, 4, 5, 13]. Investigations currently in progress are aimed at isolating a subpopulation (or subpopulations) of T-cell regulators of myelopoiesis and at studying their phenotypic and functional characteristics [1, 9, 10]. Previously, on a model of immobilization stress, the writers demonstrated the important role of T lymphocytes in the regulation of hematopoiesis in situ [2, 8]. Under the conditions described, T lymphocytes migrate into the bone marrow and stimulate medullary hematopoiesis at the level of committed precursor cells of erythropoiesis and granulomonocytopoiesis, and of their more highly differentiated progenies [8].

The aim of this investigation was to study phenotypic and functional characteristics of T-cell regulators involved in the stimulation of hematopoiesis during stress.

EXPERIMENTAL METHOD

Experiments were carried out on 200 (CBA × C57BL/6)F_1 hybrid mice weighing 18-20 g (from the "Stolbovaya" nursery). The animals were immobilized for 10 h lying in the supine position. At different times after immobilization the mice were killed by cervical dislocation of the spinal cord. The total number of myelokaryocytes (per femur) was counted. The myelogram was studied in bone marrow films. Colony- and cluster-forming units (CFU_{dc} and $CIFU_{dc}$) were cloned by the diffusion microchamber technique (volume of the chambers 10×10^{-6}-15×10^{-6} liter), which were incubated in the peritoneal cavity of recipient mice, treated beforehand with cyclophosphamide, as described previously [7]. Bone marrow cells were cultured in a culture medium of the following composition: 10% embryonic calf serum (ECS), 40% Leibovitz L-15 medium, 50% intact rabbit plasma in Alsevier's solution, 80 mg/liter of gentamicin. The final concentration of cells (chosen on the basis of a study of dose dependence between the number of cultivated nucleated cells and the number of colonies formed from them) was adjusted to 0.5×10^9/liter of medium. On the 7th day of culture the number of colonies (cellular aggregates containing more than 50 cells) and of clusters (from 5 to 50 cells) formed was counted and their qualitative composition analyzed and a marker reaction performed for hemoglobin [6]. Monoclonal antibodies – anti-Lyt-1 (IgM), anti-Lyt-2 (IgG 2b), and anti-L3T4 (IgG 2b), obtained from the Laboratory of Immunochemistry, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, were used as markers of the individual T-lymphocyte subpopulations. The cytotoxic test was carried out by the method in [3], using trypan blue. The source of complement was a nontoxic guinea pig serum. Anti-Lyt-1 antibodies killed 35%, anti-Lyt-2 – 30%, and anti-L3T4 – 19% of thymocytes from intact mice. During cloning of the hematopoietic cells, supernatants from hybridoma cells were added directly to the culture medium. In this case the bone marrow cells were washed twice with modified RPMI-1640 medium (10% ECS and 90% of medium RPMI-1640), followed by centrifugation (800g for 15-20 min), replacement of the supernatant by medium containing monoclonal antibodies (10% ECS, 30% of medium with monoclonal antibodies, 60% of medium RPMI-1640), adjustment of the cell concentration to 1×10^9-2×10^9/liter, and incubation at 37°C. Complement was added to the incubation medium 1 h later and the mixture was allowed to stand at 37°C for 60 min. After removal of the supernatant by centrifugation the bone marrow cells

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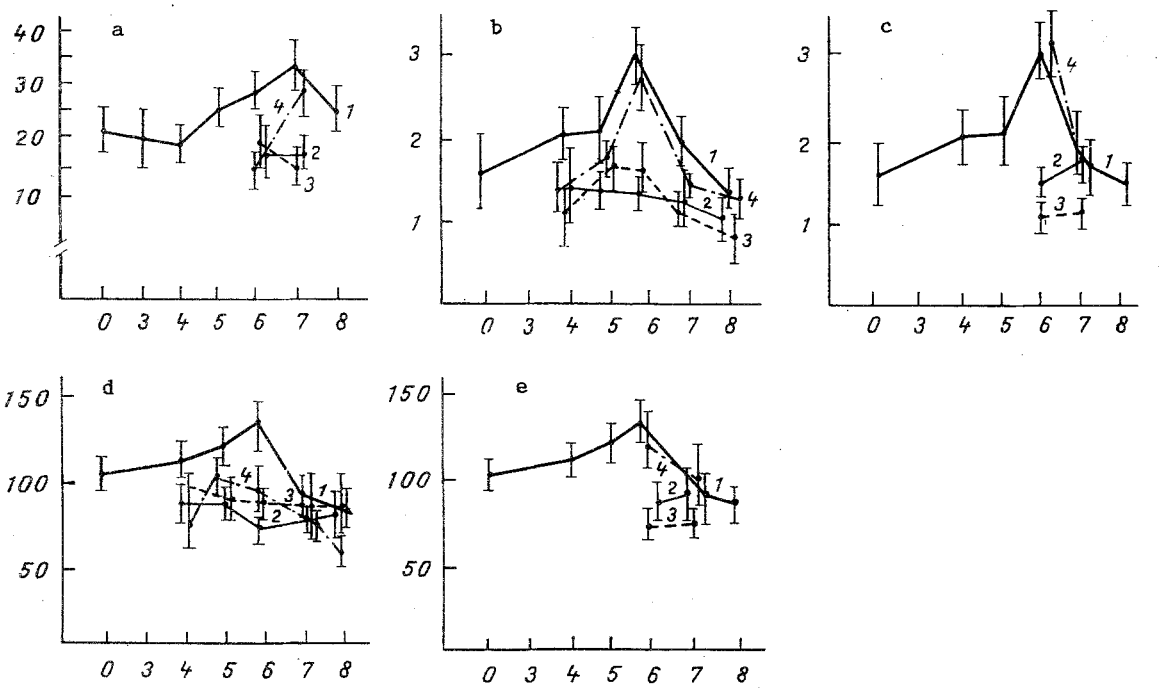

Fig. 1. Dynamics of total number of myelokaryocytes (a), CFU_{dc} (b, c) and $ClFU_{dc}$ (d, e) in bone marrow of mice exposed to stress, preceded by intraperitoneal (a, c, e) injection, or addition to hematopoietic cell culture (b, d), of monoclonal Lyt-1 (2), Lyt-2 (3), and L3T4 (4) antibodies, or culture medium (1). Abscissa, time after immobilization in days); ordinate, total number of myelokaryocytes ($\times 10^6$ cells), CFU_{dc}, and $ClFU_{dc}$ (per diffusion microchamber) in mouse bone marrow.

were transferred into a semisolid culture medium. In a separate experiment supernatants from hybridoma cells producing monoclonal antibodies were injected intraperitoneally (0.5 ml per mouse) on the 3rd, 4th, and 5th days after immobilization. Control animals received culture medium (RPMI-1640, 10% ECS, 1 mM L-glutamine, 10 mM HEPES) at the same time.

EXPERIMENTAL RESULTS

Marked hyperplasia of medullary hematopoiesis, due to stimulation of erythropoiesis and granulocytopoiesis, developed in mice subjected to immobilization, on the 6th-7th day after the beginning of the procedure (Fig. 1). The absolute number of cells of the erythroid and granulocytic series of hematopoiesis increased to 168 and 156% respectively compared with initially. The development of hyperplasia of the hematopoietic tissue was preceded (5th day) by a marked increase in the outflow of CFU_{dc} and $ClFU_{dc}$ from the bone marrow. Moreover, whereas bone marrow cells obtained from intact animals formed only granulocytic and macrophagal types of colonies in the diffusion microchambers, myelokaryocytes taken from mice subjected to immobilization (on the 5th-6th day after the beginning of the procedure) formed aggregates consisting of erythroid cells in plasma culture, giving a positive reaction for hemoglobin (up to 12.0 ± 0.2%).

It was found that the maximum of expression of Lyt-1 antigens on lymphoid cells of bone marrow took place after 4 and 6-7 days, of Lyt-2 after 4 and 8 days, and of L3T4 after 6 days of immobilization (Table 1). Intraperitoneal injection of Lyt-1 and Lyt-2 antibodies into the mice on the 3rd, 4th, and 5th days after immobilization abolished the development of hyperplasia of medullary hematopoiesis, both at the level of morphologically differentiated karocytes (up to 99.1% of the initial value; in the control group of animals exposed to stress this parameter reached 135% of its initial value) and at the level of the committed precursor cells of myelopoiesis (up to 95-97% of the initial number; in the control group of mice this parameter reached 230% of the initial level. A similar picture affecting colony-forming and cluster-forming units of bone marrow obtained from immobilized animals was observed after direct addition of monoclonal Lyt-1 and Lyt-2 (but not L3T4) antibodies directly to the

TABLE 1. Dynamics of Expression of Markers of T-Lymphocyte Subpopulations in Bone marrow of (CBA × C57BL/6)F_1 Mice Immobilized for 10 h (M ± m)

Time of investigation	Index of cytotoxicity of antibodies, %		
	anti-Lyt-1 + C'	anti-Lyt-2 + C'	anti-L3T4 + C'
Before immobilization	2,46±1,55	0,06±0,05	0,05±0,05
After immobilization, days			
3	1,37±1,05	7,79±2,24*	2,80±1,98
4	12,08±2,70*	9,6±1,68*	4,00±1,90
5	5,57±2,70	0,51±0,5	5,1±1,22
6	14,04±3,22*	1,79±1,63	8,08±2,03*
7	10,14±1,06*	3,96±1,51*	2,52±1,22
8	1,66±1,7	6,45±1,9*	1,09±0,48
10	0,6±0,05	1,37±0,41	0,52±0,5

Legend. Asterisk indicates significance of differences at the $p < 0.01$ level.

TABLE 2. Dynamics of Expression of Markers of T-Lymphocyte Subpopulations in Bone Marrow of (CBA × C57BL/6)F_1 Mice Immobilized for 10 h, after Preliminary Intrapeitoneal Injection of Monoclonal Antibodies (M ± m)

Type of antibody	Index of cytotoxicity of antibodies on 6th day after immobilization, %		
	anti-Lyt-1 + C'	anti-Lyt-2 + C'	anti-L3T4+ C'
Anti-Lyt-1	0,06±0,06	0	0,06±0,05
Anti-Lyt-2	5,6±3,24	0,7±0,1	7,09±2,94
Anti-L3T4	1,49±0,91	1,32±0,77	0,29±0,3

Legend. Supernatants of hybridomas (0.5 ml/mouse) were injected on 3rd, 4th, and 5th days after immobilization stress.

tissue culture in vivo (Fig. 1). It must be emphasized that no colonies of erythroid type were found in total preparations from animals of these series of experiments.

The experimental results showing abolition of stimulation of hematopoiesis after intraperitoneal injection of anti-Lyt-1- and anti-Lyt-2-antibodies, and the absence of lymphocytes with Lyt-1^+, Lyt-2^+, and L3T4 antigens in the bone marrow after triple injections of anti-Lyt-1-antibodies (Table 2) suggest that immature Lyt-1^+-, Lyt-2^+-, and L3T4$^-$-lymphocytes initiate proliferation of precursor cells [11]. Elimination of cells carrying the L3T4 antigen under the influence of intraperitoneal injection of anti-Lyt-1-antibodies does not contradict the above conclusion, for inection of anti-L3T4-antibodies did not abolizh development of the phenomenon of stimulation of hematopoiesis. This is evidently a question of one additional subpopulation of T lymphocytes (Lyt-1^+, Lyt-2^-, L3T4$^+$), which migrates into the bone marrow or differentiates from precursors, starting with the 4th day of immobilization (Table 1). The presence of a helper phenotype [10] on these cells was confirmed by experiments with intraperitoneal injection of anti-Lyt-1- and anti-Lyt-2-antibodies into mice. For instance, unlike Lyt-2, Lyt-1-antibodies eliminated L3T4 lymphocytes from the bone marrow. The results of experiments with intraperitoneal injection of L3T4-antibodies confirm the above conclusion (absence of effect in hematopoiesis with a sharp decrease in the content of L3T4$^+$ and Lyt-1^+ cells; see Table 2). These cells (Lyt-1^+, Lyt-2^-, L3T4$^+$) may perhaps regulate the proliferation and differentiation of T lymphocytes suppressing myelopoiesis in the bone marrow, for an increase in the number of L3T4 lymphocytes (6th day) precedes in time the increase (8th day) in the number of cells with a suppressor phenotype (Lyt-1^-, Lyt-2^+, L3T4$^-$) [11] (see Table 1). It must be pointed out that the greatest increase in the number of Lyt-2-lymphocytes was observed after normalization of the absolute number of myelokaryocytes (Fig. 1).

Successive replacement of T-lymphocyte populations was thus demonstrated during the development of bone marrow hyperplasia: Lyt-1$^+$, 2$^+$, L3T4$^-$ → Lyt-1$^+$, 2$^-$, L3T4$^+$ → Lyt-1$^-$, 2$^+$, L3T4$^-$. It can be taken as established that T lymphocytes stimulating hematopoiesis have the Lyt-1$^+$, Lyt-2$^+$, L3T4$^-$ phenotype. The appearance of lymphocytes with a suppressor phenotype on the 8th day of the experiment will be noted. These cells may perhaps inhibit the proliferative capacity of hematopoietic precursors and thus have a normalizing action on hematopoiesis.

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