PROLIFERATION OF COMMITTED PRECURSOR CELLS FOR ERYTHROPOIESIS AND GRANULOMONOCYTOPOIESIS BY T LYMPHOCYTES DURING STRESS

A. M. Dygai, V. P. Shakhov, and E. D. Gol'dberg

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Processes of adaptation of hematopoietic tissue to the action of extremal factors are realized primarily through a change in the proliferative and differential status of committed precursor cells of myelopoiesis [2, 6]. An important role in this process is evidently played by elements constituting the hematopoiesis-inducing microenvironment (HIM) [2, 6]. It is considered that the less differnetiated pool of ancestral blood cells does not respond to regulatory stimuli, but follows the path of committedness in accordance with stochastic laws [1, 2, 6]. Under the influence of extremal stimuli of varied genesis, T lymphocytes appear in the bone marrow tissue [1, 2, 4]. It is suggested that it is these cells which play an important role in the activation of HIM, which leads to stimulation of proliferation and differentiation of myeloid precursors. Nevertheless, concrete proof of this hypothesis has not yet been obtained.

The aim of this investigation was to study the role of T lymphocytes in regulation of proliferation and differentiation of precursor cells of erythropoiesis and granulomonocytopoiesis during stress.

EXPERIMENTAL METHOD

Experiments were carried out on 340 (CBA × C57BL/6)F_1 hybrid mice, male and female, weighing 18-21 g, obtained from the "Stolbovaya" nursery. The animals were immobilized for 10 h in recumbency in the supine position. On the 2nd, 3rd, and 4th days after immobilization some of the mice were given an intraperitoneal injection of 0.5 ml of antithymocytic serum (ATS), corresponding to the quantity of serum from intact rabbits (control serum – CS) or physiological saline. ATS was obtained by the method in [3] and its titer was 1:256. The erythroid precursor population was studied by culturing nonadherent bone marrow cells in methylcellulose medium with the addition of erythropoietin (AP) [8] in a CO_2 incubator (GPI-1, USSR) at 100% humidity, in an atmosphere with 5% CO_2, at 37°C for 3 days. The composition of the culture medium was as follows: 20% fetal calf serum (FTC), from "Flow Laboratories" (England), 79% of medium D1-MEM, from "Sigma," USA), 1% methylcellulose ("Sigma"), 200 mg/liter L-glutamine ("Sigma"), 40 mg/liter gentamicin ("Serva," West Germany), and 0.5 U/ml of erythropoietin ("Serva"). The optimal cell concentration in the culture was chosen in a preliminary series of experiments to study the dose dependence between the number of viable karyocytes subcultured into the medium and the number of erythroid colonies growing from them, and was found to be $2 \cdot 10^5$/ml. The term erythroid colonies was taken to mean cell aggregates containing more than 40 cells. The erythroid nature of these foci of hematopoiesis was established by a marker test for hemoglobin with benzidine (Austria) [5]. Granulocytic-macrophagal precursors were grown in nutrient medium of the following composition: 10% FCS, 10% healthy human placental serum, 79% McCoy's medium 5A ("Flow"), $5 \cdot 10^{-5}$ M 2-mercaptoethanol ("Sigma"), 40 mg/liter gentamicin, 1% methylcellulose, and 280 mg/liter L-glutamine. The optimal concentration of the nonadherent viable medullary myelokaryocytes (after preliminary treatment as indicated above) was $1 \cdot 10^5$/ml. The cells were cultured in an atmosphere with 5% CO_2 and 100% humidity at 37°C for 7 days [7]. After incubation, individual colonies (aggregates containing more than 50 karyocytes) were removed, washed twice or 3 times with medium RPMI-1640 ("Flow") with 1% bovine serum albumin ("Sigma") by centrifugation at 150 g for 5-10 min, then transferred to a slide, and a cytological preparation was obtained by means of a cell centrifuge, and then stained with azure II and eosin, or by

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TABLE 1. Dynamics of Content of Medullary E-CFU_c and Fraction of E-CFU_c in the S-Period of the Cell Cycle of (CBA × C57Bl/6)F_1 Mice, after Receiving Injection of Physiological Saline, CS, or ATS, after Immobilization for 10 h (X ± m, p)

Time after immobilization, days	Physiological saline		CS		ATS	
	total number of E-CFU_c, $\times 10^5$	number of E-CFU_c in the S-phase	total number of E-CFU_c, $\times 10^5$	number of E-CFU_c in the S-phase	total number of E-CFU_c, $\times 10^5$	number of E-CFU_c in the S-phase, %
Before immobilization	8,5±0,34	41,18±4,0	7,4±0,21	40,54±3,33	7,4±0,21	40,54±3,3
1	10,5±0,53	31,72±5,05	—	—		
2	10,5±0,29	49,24±2,76*	—	—		
3	17,5±0,58*	85,71±3,31*	—	—		
4	27,7±0,4*	96,16±1,65*	22,0±0,38*	78,81±3,3*	9,0±0,48	42,85±3,78
5	19,7±0,7	69,5±3,45*	16,0±0,19*	58,31±4,73*	7,0±0,29	38,15±3,32
6	7,13±0,43	64,65±6,03*	4,67±0,28	57,17±0,28*	7,0±0,8	44,5±2,43
7	8,0±0,24	43,75±3,0	5,67±0,38	53,9±2,63*	6,0±0,38	30,77±2,83
8	7,0±1,17	42,86±1,67	5,0±0,68	10,0±3,57	6,0±0,48	44,5±0,97

Note. Here and in Table 2, *p < 0.05.

the cytochemical reaction for myeloperoxidase [5]. Proliferative activity of precursor cells of erythropoiesis and granulomonocytopoiesis was determined with aid of hydroxyurea ("Sigma") which was injected into the animals intraperitoneally in a dose of 900 mg/kg 2 h before sacrifice. The level of "cell suicide" was calculated by the usual method [6]. The animals were killed by cervical dislocation. All experiments were conducted in the morning, during the fall and winter.

The numerical results were subjected to statistical analysis by a nonparametric test, and the level of significance was calculated by the method of Wilcoxon, Mann, and Whitney.

EXPERIMENTAL RESULTS

The experiments showed that immobilizaiton stress induces significant changes in the content of erythroid and granulocytic-macrophagal colony-forming units (E-CFU_c, GM-CFU_c) during development of adaptive changes in medullary hematopoiesis. For instance, a significant increase in the number of E-CFU_c was observed on the 3rd day after the beginning of immobilization and of GN-CFU_c from the 4th through the 6th days of the experiment (Tables 1 and 2). It is important to note that proliferative activity of these categories of myeloid precursors increased sooner (from the 2nd day of the experiment) and remained significantly raised for a long period of time – to the 6th day (E-CFU_c) and to the 7th day (GM-CFU_c) after the beginning of immobilization, respectively (Tables 1 and 2). Thus one of the first reactions of cells to the action of regulatory factors is a change in the pool of actively proliferating precursors, followed by quantitative changes in their content. On the other hand, even after normalization of the numerical composition of E-CFU_c and GM-CFU_c their proliferative activity remained quite high (Tables 1 and 2).

The writers showed previously that, starting with the 2nd day after the beginning of exposure to stress, T lymphocytes, regulators of myelopoiesis, carrying Lyt-1^+,2^+-antigens on their surface, migrate into the bone marrow tissue [2]. One manifestation of the effector action of T lymphocytes on hematopoiesis is evidently activation of proliferation and differentiation of myeloid precursors. The creation of a T-cell deficiency with the aid of ATS leads not only to abolition of the increase in the number of committed precursor cells of

TABLE 2. Dynamics of Content of Medullary GM-CFU_c and Function of GM-CFU_c in the S-Period of the Cell Cycle of (CBA × C57Bl/6) Lines of Mice, after Receiving an Injection of Physiological Saline and CS or after Immobilization for 10 h (X ± m, p)

Time after immobilization, days	Physiological saline		CS		ATS	
	total number GM-CFU_c, $\times 10^5$	number of GM-CFU_c in the S-phase, %	total number of GM-CFU_c, $\times 10^5$	number of GM-CFU_c in the S-phase %	total number of GM-CFU_c, $\times 10^5$	number of GM-CFU_c, in the S-phase, %
Before immobilization	7,0±0,39	33,29±5,57	6,4±0,28	34,37±2,81	6,4±0,28	34,37±2,81
1	7,17±0,49	39,17±2,92	—	—	—	—
2	6,5±0,34	46,15±5,2*	—	—	—	—
3	7,13±0,44	64,96±6,17*	—	—	—	—
4	18,4±0,34*	75,49±1,79*	12, ±0,38*	56,14±2,4*	6,0±0,19	33,33±1,91
5	15,0±0,3*	64,47±1,6*	15,33±0,48*	65,23±6,1*	6,0±0,38	34,5±3,85
6	9,93±0,84*	66,47±3,42*	6,33±0,28	42,02±3,67	9,67±0,48	39,11±2,31
7	6,17±0,15	59,48±2,43*	4,33±0,19	38,33±5,2	5,0±0,38	40,0±0,91
8	6,5±0,29	43,54±4,46	4,67±0,28	28,69±3,5	5,0±0,19	26,6±0,46*

erythropoiesis and granulomonocytopoiesis observed during stress, but also to changes characteristic of the general adaptation syndrome, observed in their proliferative status. Consequently, one manifestation of regulatory influences of lymphocytes of thymic origin on hematopoiesis is triggering of E-CFU_c and GM-CFU_c for proliferation, followed by an increase in their number, the basis for development of the phenomenon of hyperplasia of medullary hematopoiesis in stress.

LITERATURE CITED

1. E. D. Gol'dberg, A. M. Dygai, and G. V. Karpova, Rose of Lympocytes in the Regulation of Hematopoiesis [in Russian], Tomsk (1983).
2. A. M. Dygai and V. P. Shakhov, Role of Intercellular Interaction in the Regulation of Hematopoiesis [in Russian], Tomsk (1989).
3. N. A. Kraskina, V. M. Man'ko, and M. S. Blyakher, Progress in Immunology [in Russian], Moscow (1977), pp. 103-108.
4. R. V. Petrov, R. M. Khaitov, V. M. Man'ko, and A. A. Mikhailova, Control and Regulation of the Immune Response [in Russian], Moscow (1981).
5. F. G. J. Hayhoe and D. Quaglino, Hematological Cytochemistry [Russian translation], Moscow (1983).
6. I. L. Chertkov and O. A. Gurevich, The Hematopoietic Stem Cell and Its Microenvironment [in Russian], Moscow (1984).
7. D. Metcalf, Haemopoietic Colonies, Berlin (1977).

ACTION OF HELIUM–NEON LASER RADIATION ON THE TRACHEAL AND BRONCHIAL MUCOSA: AN EXPERIMENTAL STUDY

E. Ya. Gatkin, A. L. Vladimirtseva, E. K. Balandina, and A. P. Éttinger

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Published reports on the use of low-intensity laser radiation in pulmonology [2, 4, 6] have described properties of coherent light such as ability to intensify metabolic processes, to accelerate wound healing, to relieve the pain syndrome, and to enhance immunity [1, 3, 7, 9]. Some publications have described the biosynthetic capacity of laser radiation in lung tissue culture [5, 8]. No experimental research into the action of energy of a helium–neon laser (HNL) on the bronchial and tracheal mucusa could be found in the accessible literature. The aim of the present investigation was to study the effect of exposure to coherent red light on the mucosa of the tracheobronchial tree and to choose optimal parameters of irradiation for clinical use.

EXPERIMENTAL METHOD

The LG-75 laser system with the following parameters was used: power at the output of the emitter 20 mW, power at the output of the light guide 10 mW, thickness of the quartz thread of the light-conducting cable 400 μm, distance from the end of the light guide to the object 1 cm. The emission energy was calculated by the equation: E (in J) = P (in mW) × T (in sec). The dose was calculated by the equation: W (J/cm^2) = E (J)/S (cm^2). In acute experiments (61) on 14 gray rabbits (weighing 2-5 kg) the mucosa of the trachea and bronchi was irradiated after preliminary tracheotomy and bronchotomy. Group 1 comprised seven animals, whose tracheal mucosa was irradiated. Under pentobarbital anesthesia (40 mg/kg body weight, intravenously) the trachea was exposed. The anterior wall of the trachea was removed 2 cm

Department of Surgery of the Lungs, Moscow Research Institute of Pediatrics and Child Surgery, Ministry of Health of the RSFSR. Department of Pathological Anatomy, Patrice Lumumba Peoples' Friendship University. Department of Experimental Survery, N. I. Pirogov Second Moscow Medical Institute.

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