Production of Humoral Factors by Bone Marrow Cells Subjected to Different Extreme Conditions

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Maintenance of tissue homeostasis under normal and stressful living conditions is determined in many respects by complicated co-dependent interactions between various regulatory systems vital to life. It is universally acknowledged that the compensatory-adaptive processes in specific organs, including the bone marrow, are realized via activation of local regulatory units able to translate and transform distant signals into a form accessible for executive target cells. The current concept of the hemopoiesis-inducing microenvironment (HIM) is one of the most fruitful for interpreting the local mechanisms of hemopoiesis regulation. The numerous experimental data on this problem are documented in recent monographs [3,5,7,9]. However, the data reported do not reflect consensus on the regularities and mechanisms of the control of hemopoiesis under various conditions of existece, because there nas been no systemic approach and there are no unified techniques for parallel testing of the functional state of individual regulatory units or for examining their cooperative interaction.

The aim of the present investigation was thus to study the capacity of bone marrow cells for producing humoral regulators of hemopoiesis under stress factors of different genesis.

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MATERIALS AND METHODS

Experiments were carried out in the fall-winter season in the morning hours on male F1 (CBA× ×C57Bl/6) and CBA mice weighing 18-20 g. The hybrid mice were subjected to a 10-h immobilization on their backs, and the CBA mice were exposed to irradiation, infection, or cytostatic treatment. Irradiation was performed using RUM-17 apparatus in a dose of 2 Gy (voltage 200 kV, current strength 15 mA, filters: Cu 0.4 mm + Al 1 mm, focal distance 45 cm, dose rate 0.062 Gy/min). Inflammation was studied on a model of peritonitis simulated by i.p. injection of 1/2 LD_{50} *E. coli* strain ATSS 25922 in 0.3 ml saline. Adriamycin or cyclophosphamide was administered i.p. in the maximum permissible dose (MPD, 6 and 250 mg/kg respectively),and 5-fluorouracil in a dose of 114 mg/kg (1/2 MPD). The ganglioblocker pentamine (6 mg/kg), the α-adrenergic blocker dihydroergotamine (3.9 mg/kg), or the β-adrenergic blocker propranolol (5 mg/kg) was injected s.c. in combination with 5-fluorouracil (3-5 min before and 5 h after the cytostatic). Control animals were injected with an equal volume of saline (0.2 ml).

The mice were killed by dislocation of the neck at different times (1-10 days) after treatment. Hemopoietic islets were isolated using a method

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TABLE 1. Cytokine Levels in Supernatant of Bone Marrow Cells of Mice Subjected to Different Extreme Factors

Cytokine	Bone marrow fraction	Character of stimulation	Initial level		% of initial level				
			F_1 (CBA× ×C57Bl/6)	CBA	immobilization	inflammation	cytostatics adriamycin	cyclophosphamide	radiation
IL-1	Adhesive	LPS	0.44-1.25	1.24-2.57	270-1290 (1,4)	316 (1,4,7)	222-496 (1-2,5)	291-483 (3,5)	–
IL-1	Adhesive	–	0.44-0.50	–	950-1307 (1,4)	–	–	–	–
IL-3	Nonadhesive	ConA	0.3-1.82	0.78-1.3	395-633 (1-2,4,7)	–	8153 (2,5)	723 (1-2,5,10)	–
IL-3	Nonadhesive	–	1.46	0.8	286 (4-5)	–	–	2375 (1,7)	–
EPA	Adhesive	LPS	0.06	0.2-0.8	3333 (2,6)	275 (2,5)	220 (1,5)	–	276 (6,7)
EPA	Nonadhesive	ConA	0.06	0.05-0.8	883 (3,7)	350 (1.5)	1740 (1,4,6)	–	426 (1,6)
CSA	Adhesive	LPS	0.75-5.0	0.8-3.33	667-1100 (1,3,6)	375 (3,6)	375 (2,5)	316 (3,7)	1019 (2,8)
CSA	Adhesive	–	1.25-2.4	0.5	233-360 (4,6)	–	–	724 (1)	–
CSA	Nonadhesive	ConA	0.75-10	0.5-3.33	533-550 (2-3,5-6)	267 (1,5)	573 (3,6)	424 (1,3,7)	476 (1,5,10)
CSA	Nonadhesive	ConA	0.5	0.75	900 (2,5)	–	–	200 (4)	–

Note. Peaks of activity (days) are in parentheses.

described previously [11] in a modification reported elsewhere [7]. The levels of erythropoietic activity (EPA), colony-stimulating activity (CSA), and interleukin-1 (IL-1) and interleukin-3 (IL-3) activity in conditioned media obtained by culturing of mitogen-stimulated or nonstimulated adhesive myelokaryocytes and nonadhesive myelokaryocytes were assayed after Gol'dberg *et al.* [2]. Stimulation of adhesive and nonadhesive bone marrow cells from the femur was achieved by the addition of 10 mg/μl lipopolysaccharide (LPS) of *E. coli* strain 0.III B4 (Sigma, USA) or 5 μg/ml concanavalin A (ConA) (Sigma, USA) to the culture medium, respectively. EPA levels were expressed in international units (IU/ml); CSA was estimated as the ratio of the number of granulocyte-macrophage colonies to 105 nonadhesive myelokaryocytes. A unit of activity of IL-1 or IL-3 was taken as the logarithm of the dilution of the conditioned medium under twofold augmentation of ^{3}H-thymidine incorporation in target cells [2]. The data were processed statistically using the Student *t* test.

RESULTS

Hemopoietic tissue responds to the action of extreme factors of different nature and biological properties by a uniform enhancement of the functional activity of HIM cells detected via the production of sertain humoral substances (Table 1) using unified methodological techniques. High levels of cytokines in the supernatants of adhesive and nonadhesive myelokaryocytes are evident as early as the first day after stimulation (immobilization stress, inflammation) and suppression (cytostatics, irradiation) of hemopoiesis. More specifically, adriamycin or cyclophosphamide injection resulted in a more than 81-fold elevation of IL-3 activity in nonadhesive bone marrow cell-conditioned medium (the ConA-stimulated variant) and a 23-fold rise in nonstimulated cells. An analogous augmentation of the spontaneous (by 186% from the initial level) and the mitogen-induced (4-6-fold) production of IL-3 is also obtained in immobilization stress. The levels of IL-1 were markedly increased in all series. An enhancement of IL-1 and IL-3 secretion seems to be the first reaction of HIM cells under extreme conditions, no matter what the nature of the agent. IL-3 stimulates the processes of proliferation and differentiation of the polypotent hemopoietic stem cell and of the committed precursors of erythrocytic, granulomonocytic, and megakaryocytic clones [13], and boosts the expression of the CSF and erythropoietin receptors on precursor cells [10,15], thereby modulating their sensitivity to growth factors. In its turn, IL-1 stimulates CSF production by HIM cells and induces proliferation of hemopoietic stem cells [14]. The increased content of EPA and CSA in both adhesive and nonadhesive fractions of myelokaryocytes after 1-2 days of investigation obtained in the colony-formation test is probably related to the described properties of immunoactive lympho- and monokine. The high levels of EPA and CSA production by HIM cells after 3-7 days promote, as we noted ealier [5,12], the development of hyperplasia of erythrocyte and granulocyte clones under hemopoiesis-disturbing influences (im-

TABLE 2. Dynamics of Content of Hemopoietic Islets in Bone Marrow of CBA Mice under Conditions of Combined Administration of 5-Fluorouracil and SNS Antagonists ($M \pm m$)

Time after treatment, days	Total number of islets, $\times 10^3$/femur			
	5-fluorouracil	5-fluorouracil + + ganglioblocker	5-fluorouracil + + α-adrenoblocker	5-fluorouracil + + β-adrenoblocker
Before treatment	30.88±2.36	30.88±2.36	30.88±2.36	30.88±2.36
4	2.00±0.48*	3.83±1.13*	4.50±1.45*	2.13±0.64*
5	2.50±0.81*	2.33±0.64*	3.34±0.65*	1.50±0.64*
6	2.00±0.62*	1.20±0.38*	3.84±1.29*	0.67±0.31*
7	3.33±0.80*	1.80±0.58*	2.20±0.77*	0.80±0.38*,**
8	6.00±2.42*	4.01±1.14*	8.34±1.61*	4.16±0.96*
9	17.67±1.77*	11.16±1.94*,**	9.40±2.09*,**	12.80±1.73*
10	12.51±1.73*	10.32±1.45*	6.40±2.11*	7.33±1.12*,**

Note. Asterisks denote the significance of differences: one – in comparison with intact (before treatment) mice; two – in comparison with animals treated with cytostatic only.

mobilization stress, inflammation) and their regeneration under conditions of myelotoxic effects (cytostatics, irradiation).

The findings testify that the common feature in the reaction to the different extreme conditions is the markedly enhanced functional activity of HIM cells in the regulation of the proliferation and differentiation of hemopoietic precursors and morphologically identified elements via the production of short-range humoral factors. The levels and periods of increased secretory activity of adhesive and nonadhesive karyocytes of the bone marrow are comparable in the case of both myelopoiesis-stimulative and suppressive effects (Table 1).

It is known that the reactions of the blood system subjected to the extraordinary irritants exhibiting no myelosuppressive effect develop via universal stress-realizing systems of the macroorganism (namely, the autonomic nervous system, the hypophyseal-adrenal system, the system of opioid peptides and so on) [4,12]. The regulatory effects of the latter are mainly mediated by elements of HIM, which transform and specifically enhance the instructions delivered from the neuroendocrine apparatus to the hemopoietic precursors [1,6,12]. An inhibitory effect of adrenergic antagonists, administered together with 5-fluorouracil, on the dynamics of postcytostatic repair of erythro- and granulocytopoiesis was established previously [8] and was found to be analogous to the cancelation of the phenomenon of hemopoiesis hyperplasia related to the use of adrenoblockers under conditions of immobilization stress [1,6]. Repair processes are controlled by transmitters of the sympathetic nervous system (SNS) via regulation of HIM cell activity. This is attested to by the fact that the stress limiting drugs (ganglioblocker, α - or β-adrenoblocker) induced damage to the formation of hemopoietic islets in the hemopoietic tissue regenerating after treatment with 5-fluorouracil (Table 1), islets which form the structural and functional organization of the bone marrow and promote the proliferative and differentiative potencies of the hemopoietic cells [11]. In other words, an enhancement of the functional properties of HIM elements is an obligatory component of the nonspecific adaptive reactions that, regardless of the type of irritant (but depending on the intensity of its action), link the central and local levels of hemopoiesis regulation together in a unified, comlicated system that serves to provide for optimal functioning of the blood system in extreme situations.

REFERENCES

1. E. D. Gol'dberg, A. M. Dygai, I. A. Khlusov, *et al., Pat. Fiziol.*, № 3, 14-17 (1991).
2. E. D. Gol'dberg, A. M. Dygai, and V. P. Shakhov, *Methods of Tissue Culturing in Hematology* [in Russian], Tomsk (1992).
3. V. A. Lishchuk, B. Lord, V. Pavlovich-Kentera, *et al.* (eds.), *Homeostasis and Regulation of Physiological Systems of the Organism* [in Russian], Novosibirsk (1992).
4. P. D. Gorizontov, O. I. Belousova, and M. I. Fedotova, *Stress and the Blood System* [in Russian], Moscow (1983).
5. A. M. Dygai, and V. P. Shakhov, *Role of Cell-Cell Interactions in Hemopoiesis Regulation* [in Russian], Tomsk (1989).
6. A. M. Dygai, I. A. Khlusov, V. P. Shakhov, *et al., Pat. Fiziol.*, № 3, 17-20 (1991).
7. A. M. Dygai and N. A. Klimenko, *Inflammation and Hemopoiesis* [in Russian], Tomsk (1992).
8. I. A. Khlusov, S. G. Aksinenko, and A. M. Dygai, *Byull. Tomsk. Nauch. Tsentra Ross. Akad. Med. Nauk.*, № 1, 13-23 (1992).
9. A. P. Yastrebov, B. G. Yushkov, and V. N. Bol'shakov, *Regulation of Hemopoiesis under the Influence of Extreme Factors on the Organism* [in Russian], Sverdlovsk (1988)
10. B. Chen and C. Clark, *J. Immunol.*, **137**, 563 (1986).
11. P. Crocker and S. Gordon., *J. Exp. Med.*, **162**, 993-1014 (1985).
12. E. D. Gol'dberg and A. M. Dygai, in: *Hematology Reviews*, Ed. O.K. Gavrilov, Harwood (1992), pp.11-67.
13. J. Ihle, J. Keller and S. Orozzlan, *J. Immunol.*, **131**, 282-289 (1983).
14. M. A. S. Moore, *Immunol.*, **8**, 165-175 (1989).
15. Ch. Tsao, T. Arinobu, and F. Hiromi, *J. Immunol.*, **140**, 89-93 (1988).