GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Role of Thy 1,2$^+$ Cells in the Regulation of Hemopoiesis during Experimental Neuroses

E. G. Skurikhin, O. V. Pershina, N. I. Suslov, A. M. Dygai, and E. D. Gol'dberg

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 6, pp. 608-612, June, 2005
Original article submitted February 16, 2005

We studied the direct and stromal cell-mediated effects of bone marrow Thy 1,2$^+$ cells on the growth of granulocyte-macrophage and erythroid colonies from the bone marrow of CBA/CaLac mice with experimental neuroses (conflict situation and paradoxical sleep deprivation). Proliferation and differentiation of hemopoietic precursors during neuroses are controlled by regulatory T cells. In conflict situation Thy 1,2$^+$ cells stimulate the growth of hemopoietic precursors, which is associated with their direct effect and interaction with adherent cells of the hemopoiesis-inducing microenvironment. The interaction of Thy 1,2$^+$ cells with adherent bone marrow cells during paradoxical sleep deprivation stimulates the formation of only granulocyte-macrophage colonies.

Key Words: *Thy 1,2$^+$ cells; hemopoiesis; hemopoiesis-inducing microenvironment; regulation; neuroses*

Proliferation and maturation of erythroid and granulocytic precursors during experimental neuroses are regulated by the hemopoiesis-inducing microenvironment (HIM) [4]. The regulation of hemopoiesis by HIM cells (macrophages and stromal mechanocytes) is mediated by production of cytokines and interaction with hemopoietic cells [1-3]. Under conditions of acute inflammation, immobilization stress, and cytostatic treatment the increase in functional activity of resident macrophages and stromal mechanocytes, cooperative interaction between HIM cells, and production of humoral hemopoiesis regulators are impossible without active participation of regulatory T cells, whose phenotype includes Thy 1,2$^+$ antigen [2,3]. Our previous experiments revealed a correlation between activity of the system for local regulation of hemopoiesis and number of bone marrow lymphocytes. We showed that secretory activity of nonadherent myelokaryocytes changes during paradoxical sleep deprivation [4]. Among a variety of nonadherent karyocytes, Thy 1,2$^+$ cells are most potent in producing cytokines [2,6]. These data suggest that T cells are involved in plastic reconstruction of the blood system during experimental neuroses.

Here we studied the interaction of Thy 1,2$^+$ cells with adherent HIM cells and hemopoietic precursors during experimental neuroses.

MATERIALS AND METHODS

Experiments were performed on 160 CBA/CaLac mice (class I conventional mouse strain) aging 2.0-2.5 months and obtained from the collection of the Laboratory for Experimental Biological Modeling (Institute of Pharmacology, Tomsk Research Center).

Conflict situation (10 min) [8] and paradoxical sleep deprivation (PSD, 48 h) [15] served as the mo-

Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences. ***Address for correspondence:*** edg@pharm.tsu.ru. E. D. Gol'dberg

0007-4888/05/1396-0643

dels of experimental neurosis. The animals were anesthetized with ether and euthanized by cervical dislocation on days 1, 2, 3, 4, 5, 6, and 7 after neurosis modeling. The number of regulatory T cells in the bone marrow tissue was estimated in the complement-dependent test with monoclonal antibodies [7]. The minimum effective titer of monoclonal anti-Thy 1,2 antibodies (clone 5A-8 CL 8600A, Cedarlane) in the cytostatic reaction (1:100) and complement cytotoxicity were evaluated in previous experiments with thymocytes from intact CBA/CaLac mice. We studied the regulatory role of Thy 1,2$^+$ cells in colony formation. Suspension of bone marrow cells from experimental animals was purified from erythrocytes and separated to adherent and nonadherent fraction (adhesion to plastic). The number of nonadherent karyocytes was brought to 2 million cells per 1 ml RPMI 1640 medium containing 10 mM HEPES. Monoclonal anti-Thy 1,2 antibodies were added to a half the volume (10 μl anti-

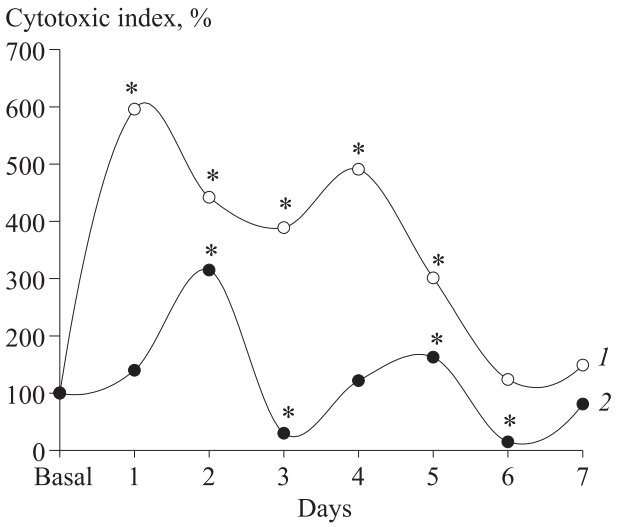

Fig. 1. Number of Thy 1,2$^+$ cells in the bone marrow of CBA/CaLac mice in conflict test (*1*) and during paradoxical sleep deprivation (*2*). *p<0.05 compared to the baseline level.

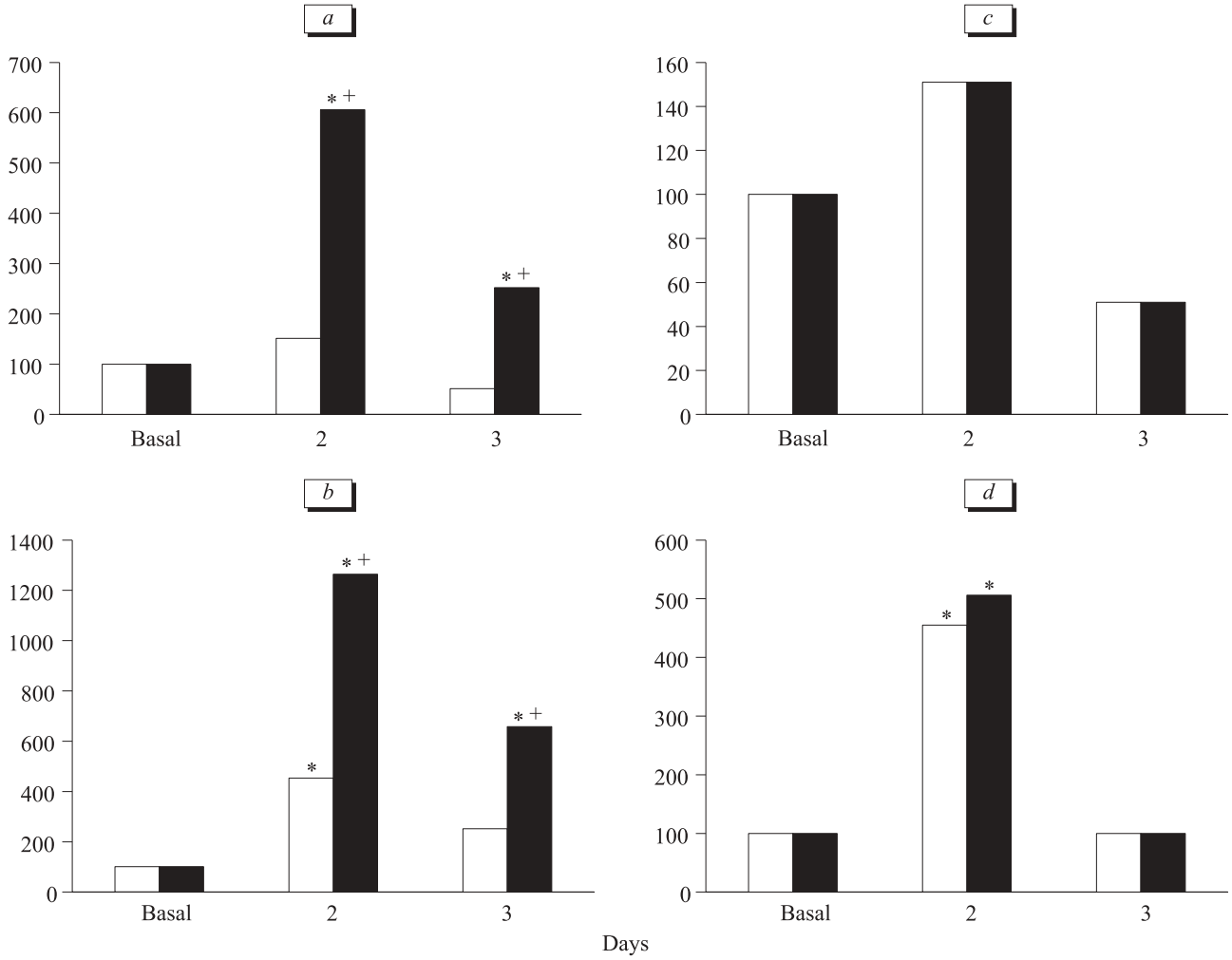

Fig. 2. Number of erythroid colonies from bone marrow cells of CBA/CaLac mice nonadhering (*a*, *c*) or adhering to the sublayer (*b*, *d*): conflict situation (*a*, *b*) and paradoxical sleep deprivation (*c*, *d*). Here and in Fig. 3: suspension of nonadherent myelokaryocytes depleted of Thy 1,2$^+$ cells (light bars) or treated with the complement (dark bars). Ordinate: colony-forming activity of the bone marrow (per 10^5 nonadherent myelokaryocytes). p<0.05: *compared to the baseline level; $^+$compared to Thy 1,2$^+$ cell-depleted suspension.

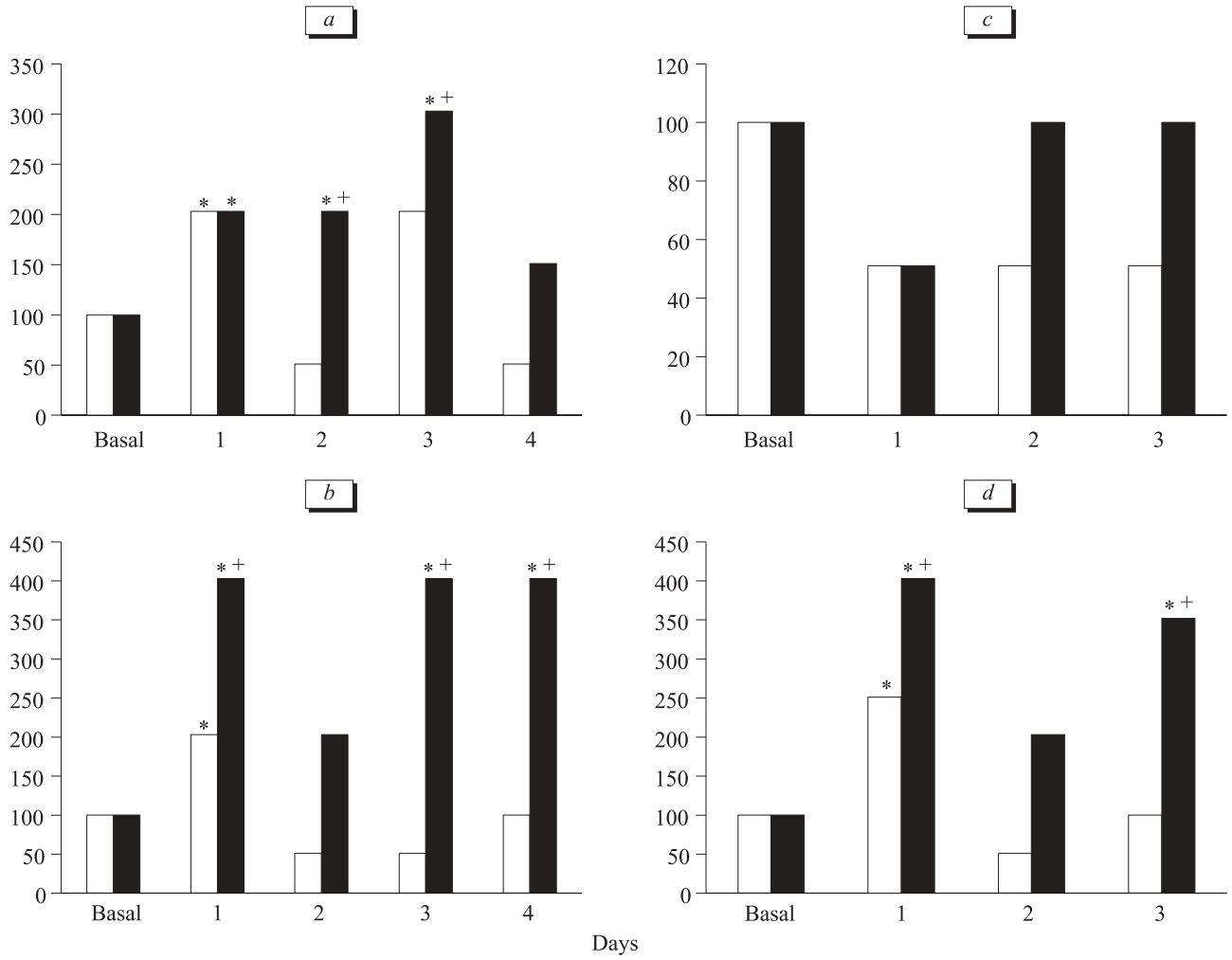

Fig. 3. Number of granulocyte-macrophage colonies from bone marrow cells of CBA/CaLac mice nonadhering (*a*, *c*) or adhering to the sublayer (*b*, *d*): conflict situation (*a*, *b*) and paradoxical sleep deprivation (*c*, *d*).

bodies per 1 ml cell suspension). The control and experimental suspensions were incubated at 4°C for 45 min. Both samples were mixed 10:1 with rabbit serum (source of complement) and kept in a thermostat at 37°C. After 45-min incubation the control and experimental cells were washed 2 times by centrifugation. Cell concentration was brought to 2×10^5 karyocytes per 1 ml culture medium. The culture medium contained 79% RPMI 1640, 1% methylcellulose, 20% fetal bovine serum, 280 mg/ml L-glutamine, 4 μM 2-mercaptoethanol, and 50 mg/liter gentamicin (Sigma). An aliquot of semisolid cell suspension was placed in 24-well plates (0.5 ml). It was layered on the washed layer containing 5×10^5 adherent myelokaryocytes from mice of the corresponding group. Another aliquot was mixed with 1 ng/ml recombinant mouse granulocyte-macrophage colony-stimulating factor (Sigma) or 2 U/ml recombinant erythropoietin (Sigma) and placed in 24-well plates (0.5 ml). Depending on the type of the culture system, incubation was performed in a CO_2 incubator at 37°C, 5% CO_2, and 100% humidity for 7 or 3 days. Granulocyte-macrophage or erythroid colonies were counted by the end of incubation [5].

The results were analyzed by standard methods of variational statistics. The significance of differences was evaluated by parametric Student's *t* test and nonparametric Wilcoxon—Mann—Whitney *U* test.

RESULTS

The number of Thy $1,2^+$ cells progressively increased over a long period after conflict testing (up to 596%, days 1-5, Fig. 1). We revealed an increase in the ability of karyocytes possessing these phenotypic characteristics to stimulate the formation of erythroid and granulocyte-macrophage colonies in a methylcellulose medium (days 2 and 3, Figs. 2, *a*, 3, *a*). Feeder activity of T cells in relation to erythroid (CFU-E; days 2 and 3) and granulocyte-macrophage colony forming units

(CFU-GM; days 1, 3, and 4) was most pronounced upon interaction with adherent bone marrow cells (Figs. 2, *b*, 3, *b*).

The number of bone marrow Thy $1,2^+$ cells increased on days 2 and 5 (up to 315 and 163%, respectively), but decreased on days 3 and 6 after PSD (up to 30 and 15%, respectively, Fig. 1). Removal of T cells had no effect on erythroid colony formation by bone marrow cells at the stages of accumulation (day 2) and decrease in cell count (day 3, Fig. 2, *c*, *d*). Under conditions of experimental Thy $1,2^+$ cell deficiency, proliferation and differentiation of granulocyte-macrophage precursors in the culture of nonadherent myelokaryocytes did not differ from those in intact animals (Fig. 3, *c*). However, the interaction of Thy $1,2^+$ cells with adherent HIM cells contributed to a significant increase in the growth of CFU-GM on days 1 and 3 (Fig. 3, *d*).

The function of hemopoietic precursors during experimental neuroses depends on activity of bone marrow T lymphocytes that express surface Thy $1,2^+$ antigen. Regulatory T cells stimulated the growth of hemopoietic precursors in conflict situation. It was associated with a direct effect of regulatory T cells or their interaction with adherent HIM cells. The interaction of Thy $1,2^+$ cells with macrophages and stromal mechanocytes results in a cascade reaction of the system for local regulation of hemopoiesis, which manifested in increased hemopoietic islet formation and stimulation of hemopoietin production by HIM cells. These changes led to stimulation of blood cell proliferation and differentiation [4]. During PSD the interaction of Thy $1,2^+$ cells with adherent bone marrow cells led to activation of granulocyte-macrophage colony formation. However, the intensity of this process during PSD is much lower compared to conflict situation. Suppression of T cell function and modified interaction between hemopoietic microenvironmental cells suppress the formation of hemopoietic islets, inhibit secretion of humoral hemopoiesis stimulators by adherent HIM cells, desynchronize proliferation and differentiation of granulocyte-macrophage precursors, and decrease proliferative activity and maturation of erythroid cells [4].

Previous experiments revealed adrenergic receptors on the membrane of lymphoid cells [9-14]. Published data show that the number of lymphocytes and individual subpopulation of cells in the bone marrow depends on activity of several neurotransmitter systems (adrenergic, dopaminergic, serotoninergic, and muscarinic cholinergic systems) [4,6]. The question arises whether neurotransmitters can regulate the lymphoid mechanisms hemopoiesis during neuroses. Differences in functional activity of Thy $1,2^+$ cells in a conflict situation and PSD are related to specific activity of neurotransmitter regulatory mechanisms. They serve as a derivative of neurochemical and neurotransmitter mechanisms of the pathogenesis of a conflict situation and PSD.

REFERENCES

1. A. I. Vorob'ev, *Manual on Hematology* [in Russian], Ed. A. I. Vorob'ev, 3rd Edition, Moscow (2002).
2. E. D. Gol'berg, A. M. Dygai, and V. V. Zhdanov, *Role of the Hemopoiesis-Inducing Microenvironment in Regulation of Hemopoiesis during Cytostatic-Induced Myelosuppressions* [in Russian], Tomsk (1999).
3. E. D. Gol'berg, A. M. Dygai, V. V. Zhdanov, and I. A. Khlusov, *Byull. Eksp. Biol. Med.*, **127**, No. 5, 484-494 (1999).
4. E. D. Gol'berg, A. M. Dygai, N. V. Provalova, *et al.*, *Role of the Nervous System in Regulation of Hemopoiesis* [in Russian], Tomsk (2004).
5. E. D. Gol'dberg, A. M. Dygai, and V. P. Shakhov, *Tissue Culture Methods in Hematology* [in Russian], Tomsk (1992).
6. L. V. Devoino and R. Yu. Il'yuchenok, *Neurotransmitter Systems in Psychoimmunomodulation: Dopamine, Serotonin, GABA, and Neuropeptides* [in Russian], Novosibirsk (1993).
7. V. V. Zhdanov, S. G. Aksinenko, A. M. Dygai, and E. D. Gol'berg, *Byull. Eksp. Biol. Med.*, **125**, No. 5, 509-513 (1998).
8. T. A. Klygul' and N. A. Krivopalov, *Farmakol. Toksikol.*, No. 2, 241-244 (1966).
9. N. A. Fedorov, *Biological and Clinical Significance of Cyclic Nucleotides* [in Russian], Moscow (1979).
10. D. D. Kharkevich, *Byull. Eksp. Biol. Med.*, **107**, No. 1, 55-57 (1989).
11. R. Bosing-Schneider and M. Haug, *Cell. Immunology*, **27**, 121-125 (1976).
12. A. Depelchin and J. J. Letesson, *Immunol. Lett.*, **3**, 207-213 (1981).
13. R. M. Landman, E. Burgisser, M. Wesp, *et al.*, *J. Recept. Res.*, **4**, 37-50 (1984).
14. K. Miles, S. Atwen, G. Otten, *et al.*, *Int. J. Immunopharm.*, **6**, 171-177 (1984).
15. M. Thakkar and B. N. Mallick, *Sleep*, **16**, No. 8, 691-694 (1993).