Role of Mononuclear Phagocyte System in Hemopoiesis Regulation in AKR/JY Mice during Preleukemic Period

L. A. Stavrova, A. M. Dygai, V. V. Zhdanov, E. D. Gol'dberg, and S. B. Tkachenko

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* 131, No. 1, pp. 52-54, January, 2001
Original article submitted October 12, 2000

Feeder activity of bone marrow adherent cells and peritoneal macrophages is decreased and colony-stimulating and erythropoietic activity of unfractionated bone marrow increased in highly leukemic AKR/JY mice in comparison with CBA/CaLac mice. At the same time maturation of hemopoietic precursor cells in AKR/JY mice is delayed compared to controls. This indicates compensatory activation of nonadherent elements of the hemopoiesis-inducing microenvironment against the background of suppressed activity of adherent elements. Hence, leukemogenic virus produced a systemic damage to target cells (e.g. mononuclear phagocyte system), which probably represent a mechanism of leukemic transformation in AKR/JY mice.

Key Words: *macrophages; hemopoiesis; feeder activity; preleukemia*

Morphological and functional integrity of the hemopoiesis-inducing microenvironment (HIM) is a prerequisite for adequate regulation of hemopoietic processes [8]. Mononuclear phagocyte system is one of the most important HIM components, directly and indirectly involved in the regulation of proliferation and differentiation of hemopoietic precursors [6]. Structural and functional disturbances in the bone marrow [2] associated with a proliferation-differentiation imbalance of hemopoietic precursors [3] were previously described in AKR/JY mice, characterized by high incidence of spontaneous leukemia of viral etiology [1]. Primary involvement of bone marrow macrophages in leukemogenic viral infection in the studied mouse strain [5] prompted us to investigate the role of components of the mononuclear phagocyte system in disorders of hemopoiesis regulation during the preleukemic period.

MATERIALS AND METHODS

Experiments were carried out on 20 male AKR/JY females aged 4-8 months and 6 CBA/CaLac mice aged 8 months (controls) from the collection of Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences.

Feeder activity of bone marrow cells towards erythro- and granulomonocyte precursor cells was evaluated by culturing nonadherent bone marrow nuclears from CBA/CaLac mice (10^5/ml) on a sublayer of adherent bone marrow cells from AKR/JY mice (2×10^5/ml) in a semisolid medium without stimulants for 3 and 7 days, respectively [4]. Feeder activity of peritoneal macrophages was evaluated similarly.

Colony-stimulating (CSA) and erythropoietic (SPA) activities of conditioned media of bone marrow cells was evaluated in 96-well plates (10^5/ml nonadherent nuclears from CBA/CaLac mice, 20% myelokaryocyte supernatant from AKR/JY mice) [4].

Colony forming (CFU) and cluster forming (ClFU) units of erythro- and granulomonocytopoiesis were cloned by culturing nonfractionated bone marrow cells (3×10^5 nuclears/ml) for 3 and 7 days, respectively, in a methylcellulose tissue culture [4] with recombinant erythropoietin (2 U/ml, Recormon) and human granulocyte colony-stimulating factor (1 ng/ml, Vektor). Coefficient of precursor cell differentiation of the respective bone marrow stem was evaluated as the ClFU/CFU ratio.

Institute of Pharmacology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences

0007-4888/01/1311-0041$25.00

RESULTS

With aging, EPA of conditioned media of bone marrow cells from AKR/JY mice increased by 89-115% in comparison with that of CBA/CaLac mice ($p<0.05$; Fig. 1). Feeder activity of adherent bone marrow cells with regard to both erythro- and granulomonocyte precursors in 4-month-old animals decreased to 32% compared to the control ($p<0.05$; Fig. 2, *a*). Decreased feeder activity of adherent bone marrow cells in the presence of increased CSA and EPA of intact bone marrow probably reflects a primary role of its nonadherent component (lymphocytes) in the production of humoral erythropoiesis regulators. This attests to compensatory activation of nonadherent HIM elements during suppression of adherent cells and is in line with previous data on erythropoiesis disorders during viral leukemic transformation [7]. Decreased feeder activity of adherent bone marrow cells from highly leukemic mice is apparently due to receptor insufficiency of the mononuclear phagocyte system, which results in decreased number of hemopoietic islets [2]. Hemopoiesis precursors are differentiated from committed to mature forms in hemopoietic islets [6]. The intensity of hemopoietic precursor cell maturation in AKR/JY mice proved to be much lower than in the control. Coefficient of erythroid precursor differentiation in 4- and 8-month-old AKR/JY mice was no more than 63% of that in CBA/CaLac mice ($p<0.01$; Table 1). Coefficient of granulomonocytopoiesis precursor differentiation progressively decreased in highly leukemic strain (Table 1).

Since the expression of leukemic virus is apparently not confined to bone marrow microenvironment, we investigated feeder activity of peritoneal macrophages from AKR/JY mice. The number of erythroid

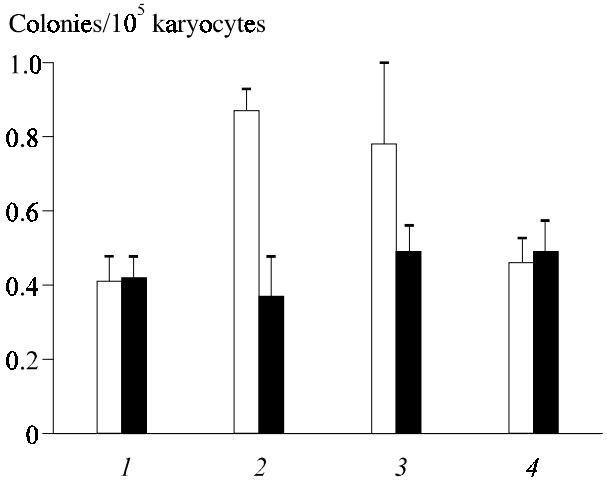

Fig. 1. Erythropoietic (light bars) and colony-stimulating (dark bars) activities of supernatants of myelokaryocytes of AKR/JY mice. Here and in Fig. 2: *1-3*) AKR/JY mice aged 4, 6, and 7 months, respectively; *4*) control (CBA/CaLac mice).

colonies in 3-day bone marrow cultures was the same in the experimental and control groups, but with aging feeder activity of peritoneal macrophages from highly leukemic animals increased from 67 to 210% of that in CBA/CaLac mice (Fig. 2, *b*). A significant decrease in the content of granulocyte-monocyte CFU was observed: in 7-month-old AKR/JY mice the number of granulocyte-monocyte colonies in bone marrow culture did not exceed 33% of the control ($p<0.01$; Fig. 2, *b*). In both cases the time course of parameters was similar to that observed with the adherent bone marrow fraction as the adherent component of cell culture.

Hence, leukemic virus specifically damage target cells (elements of the mononuclear phagocyte system) and this effect is systemic. Impairment of the regulatory functions of bone marrow macrophages suppres-

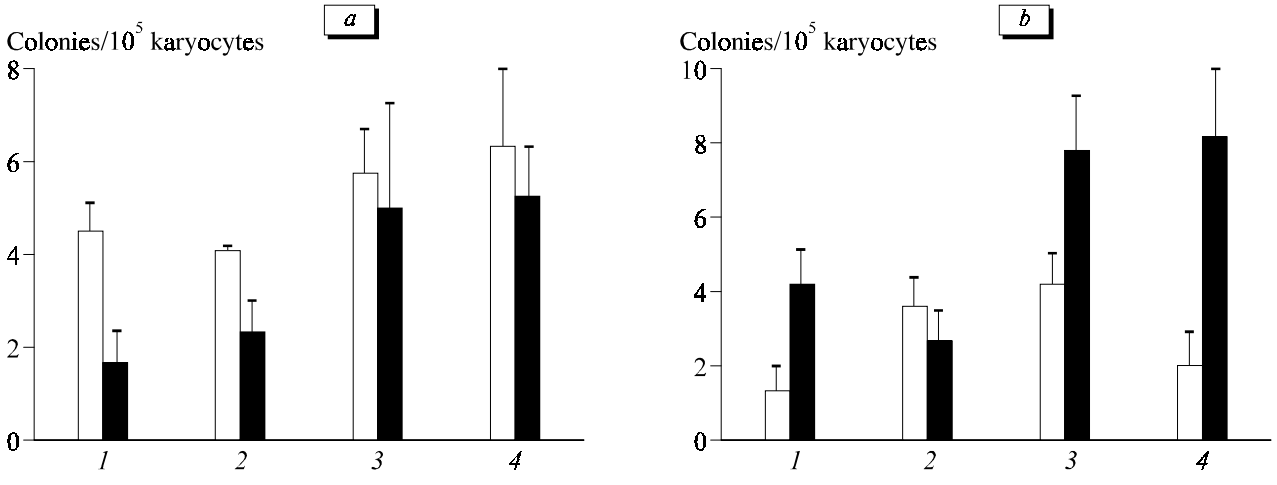

Fig. 2. Feeder activity of adherent bone marrow cells (*a*) and peritoneal macrophages (*b*) of AKR/JY mice. Light bars: erythroid stem; dark bars: granulomonocytic stem.

TABLE 1. Coefficients of Differentiation of Erythro- and Granulomonocyte Precursors in Bone Marrow of AKR/JY Mice of Different Age ($X \pm m$)

Cell type	Control (CBA/CaLac, 8 months)	Age, months		
		4	6-7	8
Erythropoiesis precursors	8.39±0.07	5.06±0.63*	7.19±0.93	5.28±0.52*
Granulomonocyte precursors	2.09±0.12	1.86±0.07	1.99±0.20	1.52±0.11**

sed formation of hemopoietic islets. This results in decelerated maturation of hemopoietic precursors during the preleukemic period, which seems to provide favorable conditions for leukemic transformation of potential tumor cells in AKR/JY mice.

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