EXPERIMENTAL BIOLOGY

Functional Capacities of Committed Hemopoietic Progenitor Cells of the Bone Marrow in AKR/JY Mice during the Preleukemia Period

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The content of hemopoietic progenitor cells in 7-month-old AKR/JY mice was higher than in hybrid (CBA/CaLac×AKR/JY)F1 mice of the same age. After stress, the number of erythroid precursors in leukemia-prone mouse strain and hybrids increased, while the content of other precursors in AKR/JY mice (by contrast to that in (CBA/CaLac×AKR/JY)F1 mice) decreased. Thus, functional capacity of the pool of bone marrow committed hemopoietic precursors in AKR/JY mice is reduced during the preleukemia period.

Key Words: *immobilization; colony-forming activity; bone marrow; preleukemia*

Disturbances of colony-forming activity in the bone marrow during the preleukemia period play a great pathogenetic role and are of particular diagnostic and prognostic importance [6]. Committed hemopoietic precursors considerably suppress proliferation of normal and tumor cells [3,4]. The contents of erythroid (CFU-E), granulocyte-macrophage (CFU-GM), and fibroblastic (CFU-F) colony-forming units in the bone marrow of AKR/JY mice decrease in the late preleukemia period [7,11] and during leukemia [7,10] due to inhibitory effects of leukemic cells on the colony formation [7,13]. However, some authors reported no changes in colony formation during leukemia [12].

Therefore, studies of functional reserve of committed hemopoietic precursors in AKR/JY mice during the preleukemia period under stress conditions are of prime interest.

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MATERIALS AND METHODS

Experiments were performed on 40 female AKR/JY mice aging 2 and 7 months, 12 CBA/CaLac mice aging 2 months, and 24 hybrid (CBA/CaLac×AKR/JY) F1 mice aging 2 and 7 months. The animals were obtained from the collection of the Laboratory of Experimental Biomedical Modeling (Institute of Pharmacology, Tomsk Research Center). AKR/JY and (CBA/CaLac×AKR/JY)F1 mice aging 7 months were immobilized in the supine position for 10 h. Indexes of bone marrow hemopoiesis were determined on day 6 after immobilization.

Cloning of CFU-E, CFU-GM, granulocytic CFU (CFU-G), and CFU-F was performed by culturing unfractionated bone marrow cells (3×10^5 nuclears/ml) in methyl cellulose for 3, 5, and 6 days [2]. Human recombinant erythropoietin (0.5 U/ml, Sigma), human granulocyte colony-stimulating factor (6 ng/ml, Vektor), supernatant of CBA/CaLac mouse splenocytes stimulated with 15 μg/ml phytohemagglutinin M (Sigma), and anemic rabbit serum were used as growth

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stimulators [2]. The total cellularity of the femoral bone marrow was determined. Hemograms and myelograms were analyzed by routine methods.

The results were analyzed by Student's t test.

RESULTS

Pathology of the thymus and spleen without considerable replacement (no more than 8%) of the bone marrow hemopoietic tissue by lymphoblasts were observed in 17% of 7-month-old AKR/JY mice. The total cellularity of the bone marrow in leukemic animals decreased to 43% compared with that in healthy AKR/JY mice. In erythropoietin-depleted culture medium containing anemic rabbit serum, the growth of CFU-E in 7-month-old animals was 36% of the level observed in 2-month-old AKR/JY mice. After stimulation with human recombinant erythropoietin, the concentration of CFU-E in the medium considerably increased and 2- and 13-fold surpassed these parameters in 7-month-old (CBA/CaLac×AKR/JY)F1 mice (Fig. 1) and 2-month-old CBA/CaLac mice, respectively. However, the content of bone marrow erythrokaryocytes in 7-month-old AKR/JY mice was 2-3 times lower than in 2-month-old AKR/JY mice and 7-month-old (CBA/CaLac×AKR/JY)F1 hybrids. These results confirm impairment of erythroid differentiation in leukemia-prone AKR/JY mice at the level of hemopoietic islets [1].

On day 4-8 after stress, proliferation and differentiation of bone marrow hemopoietic precursors were enhanced and accompanied by hyperplasia of the hemopoietic tissue due to a high content of committed and mature erythro- and granulomonocytic progenitor cells [8]. Under stress conditions, the contents of CFU-E

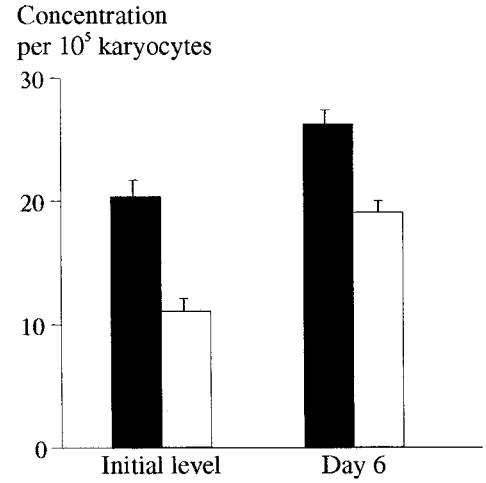

Fig. 1. Concentrations of CFU in the erythroid bone marrow of 7-month-old AKR/JY (dark bars) and (CBA/CaLac×AKR/JY)F1 mice (light bars) after 10-h immobilization.

in 7-month-old leukemia-prone AKR/JY mice and (CBA/CaLac×AKR/JY)F1 hybrids increased to 129 and 172% of the initial levels, respectively (Fig. 1), while the number of erythrokaryocytes in the bone marrow remained unchanged. In light of this, of particular interest is the decreased ability for *in vitro* proliferation of differentiated erythroid cells in elderly mice in response to increasing doses of erythropoietin without disturbances of CFU-E reactions [15]. Our findings probably reflect accelerated aging in AKR/JY mice due to early manifestation of leukemia (7 months in AKR/JY mice *vs.* 24 months in CBA/CaLac×AKR/JY)F1 hybrids). Reduced compensatory abilities of the erythron in leukemia-prone mice are typical of mature cells and committed erythropoietic precursors.

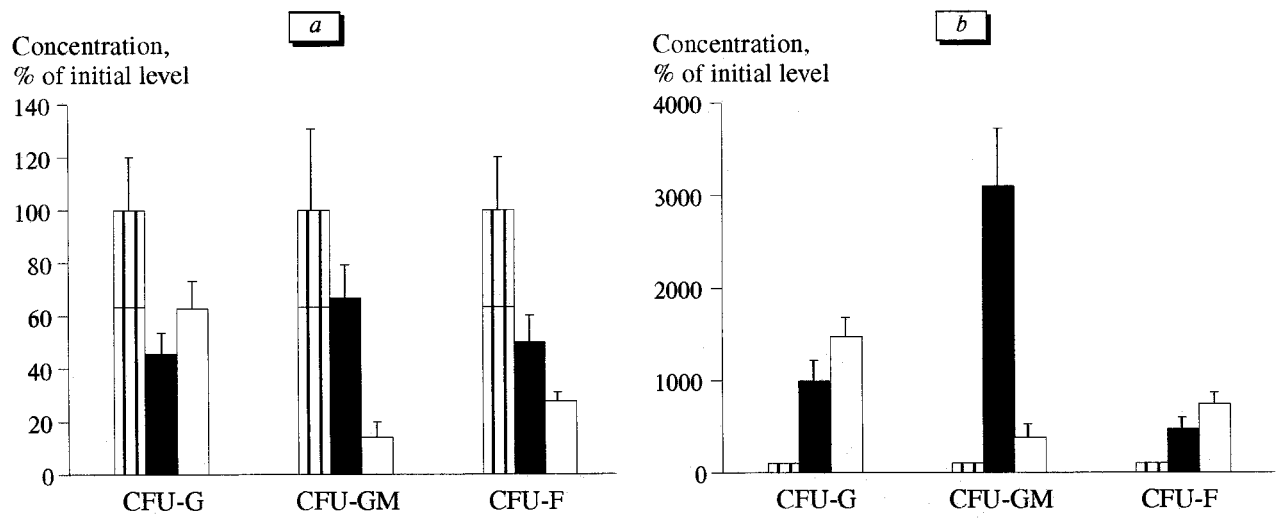

Fig. 2. Concentrations of granulocytic (CFU-G), granulocyte-macrophage (CFU-GM), and fibroblastic (CFU-F) precursors in the bone marrow of 7-month-old AKR/JY (a) and (CBA/CaLac×AKR/JY)F1 mice (b) 6 days after 10-h immobilization. Shaded bars: initial levels (before immobilization); dark bars: 5-day-old cultures stimulated with granulocytic colony-stimulating factor; light bars: 6-day-old cultures stimulated with splenocyte supernatant.

Functional reserve of CFU-G, CFU-GM, and CFU-M in the bone marrow of AKR/JY mice is reduced. Stress inhibited colony formation in the culture stimulated with splenocyte supernatant or human recombinant granulocyte colony-stimulating factor (Fig. 2). Six days after stress, the yields of CFU-GM, CFU-G, and CFU-F in AKR/JY mice decreased by 1.5-7, 1.5-2, and 1.5-3.5 times, respectively, while in (CBA/CaLac×AKR/JY)F1 hybrids, colony formation was considerably accelerated without intensification of neutrophilopoiesis and monocytopoiesis in the bone marrow (Fig. 2). Stress markedly increased the content of mature neutrophilic granulocytes in both groups and induced neutrophilia in the peripheral blood of AKR/JY mice.

It should be noted that the content of bone marrow CFU-GM in intact 7-month-old AKR/JY mice was higher than in 7-month-old (CBA/CaLac×AKR/JY)F1 hybrids and did not differ from that in 2-month-old CBA/CaLac mice. At the same time, the concentrations of CFU-G and CFU-F in AKR/JY mice during the preleukemia period increased to 361 and 711%, respectively, compared with those in CBA/CaLac mice ($p<0.001$). The increase in the number of CFU-F probably reflects age-related changes associated with reduced bone marrow hemopoiesis. The initial CFU-G/CFU-GM ratio in AKR/JY, (CBA/CaLac×AKR/JY)F1, and CBA/CaLac mice was 44:1, 17:1, and 6:1, respectively. In stressed AKR/JY and (CBA/CaLac×AKR/JY)F1 mice, these ratios were 78:1 and 5:1, respectively. On day 6 after stress, the concentration of CFU-GM in (CBA/CaLac×AKR/JY)F1 mice 11-fold surpassed this level in AKR/JY mice.

Our findings confirm that the growth of bone marrow CFU-GM in AKR/JY mice is reduced during the late preleukemia period [7,13] due to enhanced granulocytic differentiation from committed to mature forms, which increased under stress conditions. The granulocytic stem in mice is known to be stimulated by leukemia virus infection [9]. Functional reserve of granulocytopoiesis was exhausted in AKR/JY mice (by contrast to those in 7-month-old (CBA/CaLac×AKR/JY)F1 hybrids), and the number of committed hemopoietic progenitor cells did not increase during stress.

Thus, the preleukemia period in AKR/JY mice (the 7th month of life) is characterized by reduced proliferative (CFU-GM, CFU-G, and CFU-F) and differential (CFU-E) capacities of committed hemopoietic precursors, which determined the absence of hemopoietic reactions to stress. Changes in the bone marrow hemopoiesis in 7-month-old (CBA/CaLac×AKR/JY)F1 hybrids are the same and represent the early stage of leukemia.

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