Mechanisms of Preleukemic Hypoplasia of the Bone Marrow Erythroid Stem in AKR/JY Mice

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During the preleukemic period, the expression of erythroblast receptors on bone marrow macrophages, the number of neutral red-stained hemopoietic islets and their erythroid and erythrogranulocytic types, and the content of erythrokaryocytes decreased in AKR/JY mice compared with (CBA×AKR/JY) F_1 mice. The concentration of erythroid colony-forming units increased. The impairment of qualitative (receptor) and quantitative properties of bone marrow resident macrophages inhibits the differentiation of erythroid cells from committed to mature forms and is probably one of the mechanisms of hypoplasia of the erythroid stem in AKR/JY mice.

Key Words: *erythroblast receptors; macrophages; hemopoietic islets; erythroid precursors; erythrokaryocytes*

Proliferation and differentiation of erythroid cells in erythroblast islets [4] is a specific feature of the bone marrow hemopoiesis. Macrophages were shown to have erythroblast receptors (EBR) [9] that are involved in the cell-cell interaction. However, the biological role of these receptors remains unclear [11]. High incidence of anemia associated with impaired of erythropoiesis in the bone marrow [3] and spleen [10] was observed in AKR mice during the development of leukemia. The imbalance between the expression of macrophage receptors and structural and functional organization of the bone marrow of AKR/JY mice during aging has been reported [1].

Here we studied the mechanisms of erythropoiesis inhibition in AKR/JY mice during the preleukemic period.

MATERIALS AND METHODS

Experiments were performed on 56 female AKR/JY mice aging 2-7 months and 18 female hybrid (CBA× AKR/JY) F_1 mice aging 7 months. The animals were obtained from the collection of the Laboratory of Experimental Biomedical Modeling (Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences). A rosette formation test was used to analyze the expression of macrophage receptors. EBR ligands (erythroid cells isolated from mouse embryonal liver on gestational days 13-15) were added to the adherent monolayer [7], the preparations were fixed with glutaraldehyde, stained with azure II-eosin, and rosettes were counted.

Hemopoietic islets (HI) were isolated by the modified [2] method described previously [6] using 0.05% collagenase (Sigma). The suspension was mixed with equivalent volume of 0.1% neutral red in physiological saline. Basing on the results of a morphological analysis of cells associated with the central element and stained with azure II-eosin, erythroid (E-HI) and erythrogranulocytic HI (EG-HI) were identified.

Erythropoietic precursors (CFU-E) were cloned by culturing unfractionated bone marrow cells (3×10^5 nuclears/ml) in methyl cellulose for 3 days [2]. Human recombinant erythropoietin (2 U/ml, Sigma) was used as a growth stimulator. Myelograms were analyzed using bone marrow smears stained with azure II-eosin.

Results were analyzed using Student's *t* test.

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RESULTS

The number of bone marrow erythroid cells in AKR/JY mice decreased during aging (to 58% and 36% of those levels in 2-month-old animals on the 5th and 7th month, respectively, Fig. 1). On the 7th month, the relative and absolute contents of erythrokaryocytes were 40% ($p<0.001$) and 39% ($p<0.05$), respectively, of those levels in (CBA×AKR/JY) F_1 hybrids of the corresponding age. Lymphomas were morphologically detected in 17% of cases in AKR/JY mice at the end of observation.

The analysis of structural and functional organization of the bone marrow in 7-month-old AKR/JY mice showed $16.11\pm1.86\times10^3$ HI/femur with central elements stained with neutral red. This parameter was 2.5 times lower than that in hybrid mice ($45.11\pm2.70\times10^3$/femur). The percentage and absolute content of E-HI and EG-HI in the bone marrow changed similarly (Fig. 2). The number of E-HI in AKR/JY mice was $3.55\pm1.05\times10^3$/femur (25% of the level in hybrid mice). This indicated a sharp decrease in the number of macrophages capable of bounding erythroblasts.

Close contact between immature erythroid cells and the central macrophage via EBR [7] is necessary for maintaining their proliferative and differentiation properties [6,11]. The expression of EBR on bone marrow macrophages of AKR/JY mice was $40.80\pm1.92\%$ *vs.* $48.40\pm0.58\%$ in (CBA×AKR/JY) F_1 hybrid mice ($p<0.05$). EBR ligands were usually located on CFU-E [8], whose concentration in the bone marrow of AKR/JY mice was $20.37\pm1.04/10^5$ nuclears (185% of the level in hybrid mice, $p<0.001$). Thus, during the late preleukemic period, the decreased ability of macrophages in AKR/JY mice to bind various cells with the formation of HI is accompanied by accumulation of CFU-E. This decreases the number of erythrokaryocytes. There are several assumptions on the biological role of EBR expression on macrophages [11]. Our findings demonstrate its possible involvement in the differentiation of erythroid cells from committed to mature forms in HI.

Thus, hypoplasia of the erythroid stem in AKR/JY mice is associated with functional deficiency of the hemopoietic microenvironment (changes of qualitative (receptor) and quantitative properties of bone marrow resident macrophages). The impaired functions of mononuclear phagocytes in AKR/JY mice was also described by other authors [5].

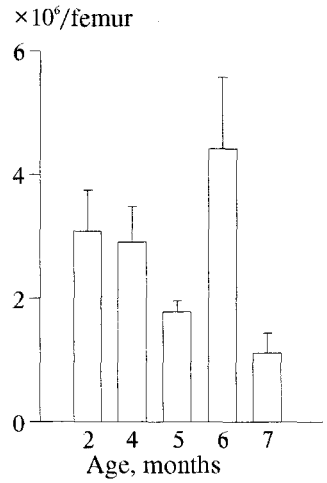

Fig. 1. Dynamics of the content of erythrokaryocytes in the bone marrow of AKR/JY mice during the preleukemic period.

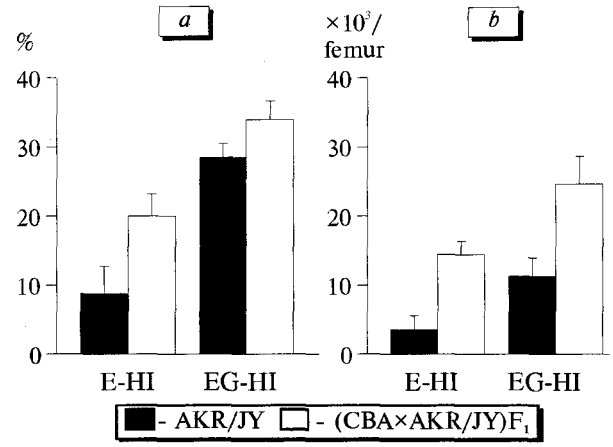

Fig. 2. Relative (*a*) and absolute (*b*) contents of erythroid (E-HI) and erythrogranulocytic (EG-HI) islets in the bone marrow of 7-month-old AKR/JY and (CBA×AKR/JY) F_1 mice.

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