Structural and Functional Organization of Bone Marrow of AKR/J Mice during Aging

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Expression of sialoadhesin and erythroblast receptors on macrophages and structural and functional organization of the bone marrow during aging were studied on AKR/J mice. It is shown that progressive accumulation of granulocyte hemopoietic islets can be a compensatory reaction to a decreased capacity of their central stromal elements to bind young granulocytopoietic cells. Expression of erythroblast receptors on macrophages from 4-month-old AKR/J mice is considerably higher than in young and old mice and than in 4-month-old (CBA×AKR)F_1 mice. The high concentration of erythroid precursors in the bone marrow of AKR mice is not accompanied by enhanced erythropoiesis, probably due to a decreased yield of erythroid hemopoietic islets. Thus, a marked imbalance in structural and functional organization of the bone marrow during aging is noted in highly leukemic AKR/J mice, which provides a basis for the development of reliable diagnostic and prognostic criteria of leukemic progression.

Key Words: *sialoadhesin; erythroblast receptor; macrophages; hemopoietic islets; hemopoietic precursors*

Macrophages among other stromal cells play an important role in the regulation of hemopoiesis. Bone marrow macrophaes express various membrane surface receptors, in particular, sialoadhesin (SA) and erythroblast receptors (EBR) [8,11], and participate in the formation of structural and functional units (hemopoietic islets) [6]. SA is a highly specific structure, a member of the Ig SF family. It mediates binding of macrophages to hemopoietic cells bearing surface sialic acid or its derivatives [8], primarily to immature granulocytic and some lymphoid cells [5]. Specific sialic ligands for this receptors are also expressed on sheep erythrocytes. EBR primarily mediates binding of macrophages to erythroid cells, but not to mature erythrocytes. It is assumed that these membrane receptors play an important role in the terminal phase of differentiation and maturation of myeloid and erythroid cells [9]. AKR/J mice are characterized by high occurrence of leukemia, which usually develops at the age of 8 months [1]. Our goal was to study expression of SA and EBR on macrophages and structural and functional organization of the bone marrow in AKR/J mice during aging.

MATERIALS AND METHODS

Experiments were carried out on 39 AKR/J mice of both sexes aging 2, 4, and 7 months and 27 (CBA× AKR)F_1 hybrids aging 2 and 4 months. The animals were obtained from strain collection of the Laboratory of Experimental Biomedical Modeling (Tomsk Research Center). Expression of receptors was evaluated by the method of rosette formation [7]. Myelokaryocytes were washed out from the femur with phosphate buffer containing 0.05% collagenase, in-

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cubated for 3 h in the same medium at 37°C and 5% CO_2, suspended, and washed 3 times. The cell suspension in RPMI-1640 medium supplemented with 10% fetal calf serum was placed on slides and incubated for 3 h at 37°C and 5% CO_2, nonadherent cells were removed, and the final monolayer was covered with ligands specific for SA (sheep erythrocytes) or EBR (erythroid cells isolated from the liver of mouse embryos on gestation days 13-15). The preparations were fixed in glutaraldehyde, stained with Azure II and eosin, and rosettes were counted.

Hemopoietic islets were isolated as described previously [6] with some modifications [2] using 0.05% collagenase (Sigma). Granulocytic, erythroid, and erythrogranulocytic hemopoietic islets were identified by morphological peculiarities of cells associated with the central element and stained with Azure II-eosin.

Erythropoietic (CFU-E) and granulomonocytopoietic (CFU-GM) colony-forming units were cloned by culturing nonfractionated bone marrow cells (3×10^5 nuclears/ml) on methylcellulose for 3 and 7 days, respectively [2]. Cell propagation was stimulated with human recombinant erythropoietin (2 U/ml, Sigma) and mouse granulocyte-monocyte colony-stimulating factor (4 ng/ml, Sigma). Myelogram was counted on bone marrow smears stained with Azure II and eosin.

The data were processed statistically using the Student *t* test.

RESULTS

Expression of SA on bone marrow macrophages from young AKR/J mice constituted 81.11±3.96%, which is comparable to this parameter in age-matched (CBA×AKR)F_1 hybrids (Table 1). The capacity of macrophages from AKR/J mice to bind specifically sheep erythrocytes decreased with age: in 4- and 7-month-old mice it constituted 63.3±6.9 and 49.67±9.11%, respectively.

On the other hand, the percentage of granulocytic hemopoietic islets gradually increased (Table 2). Hemopoietic islets are structural and functional units of the bone marrow, where proliferation and differentiation of hemopoietic cells from committed to mature forms occur [6]. Interestingly, the concentration of CFU-GM in the bone marrow of AKR mice increases with age (Table 2), the content of immature (7.9 and 8.31%, respectively) and mature (39.33 and 39.25%, respectively) neutrophil granulocytes being unchanged. It can be hypothesized that progressive accumulation of granulocytic hemopoietic islets is a compensatory reaction to a reduced capacity of their central stromal elements to bind young granulocytopoietic cells. It should be noted that this compensatory reaction maintains granulocytopoiesis and a high level of mature neutrophil granulocytes in peripheral blood, which far surpasses this parameter in (CBA×AKR)F_1 mice.

In contrast to granulocyte precursors, the content of erythroid hemopoietic islets in AKR mice slightly decreased with age (Table 2), their absolute number being significantly lower than in control (CBA×AKR)F_1 mice. In particular, in 4-month-old animals the number of erythroid hemopoietic islets was 2.44±0.45 *vs.* $3.93\pm0.47\times10^3$ per femur in the control ($p<0.05$). Expression of EBR in these animals was increased in comparison with young and old animals (Table 1), as well as in comparison with (CBA×AKR)F_1 mice of the same age (by 16%, $p<0.05$). The concentration of CFU-E in 4-month-old AKR/J mice was also increased (Table 2). However, the higher capacity of macrophages to bind erythroid precursors was not accompanied by enhanced erythropoiesis.

Functional disturbances in the mononuclear phagocyte system in AKR/J have been described by many researchers. These disturbances manifest themselves in 4-month-old animals [4], precede thymocyte alteration and probably contribute to the development of lymphoid neoplasia [10]. In light of this, the sharp increase in EBR expression on macrophages can be considered as an early marker of the preleukemic stage in AKR/J mice.

TABLE 1. Expression of SA and EBR on Bone Marrow Macrophages of (CBA×AKR)F_1 Hybrids and AKR/J Mice of Different Ages (%, $X\pm m$)

Mice	Age, months	Receptors	
		SA	EBR
(CBA×AKR) F_1 (control)	2	84.77±3.31 (n=9)	80.75±8.08 (n=12)
AKR/J	2	81.11±3.96 (n=5)	62.4±7.39 (n=3)
	4	63.3±6.98 (n=10)**	82.73±3.34 (n=11)*
	7	49.67±9.11 (n=4)**	59.67±4.33 (n=6)*

Note. *$p<0.05$, **$p<0.02$ compared with the control; *$p<0.05$ compared with 2-month-old AKR/J mice.

TABLE 2. Contents of Hemopoietic Islets and Hemopoietic Precursors (per 10^5 Nuclears Cells) in the Bone Marrow of AKR/J Mice during Aging (%, $X \pm m$)

Age, months	Hemopoietic islets		CFU-GM	CFU-E
	granulocytic	erythriod		
4 (n=9)	31.56±3.37	16.44±1.57	3.00±0.84	23.83±4.17
7 (n=4)	44.33±2.05**	12.50±2.18	4.14±0.35	13.28±2.11*

Note: *$p<0.05$, **$p<0.02$ compared with 4-month-old mice.

Thus, we revealed pronounced disturbances in the receptor expression and an imbalance in structural and functional organization of the bone marrow in highly leukemic AKR mice during aging. The compensatory increase in the number of granulocytic hemopoietic islets in response to impaired capacity of macrophages to bind young granulocytic cells presumably restricts the resources of erythropoisis. The high concentration of CFU-E and the low content of erythroid hemopoietic islets in the bone marrow of AKR/J mice point to disturbances in the mechanisms of erythroid differentiation. Restricted differentiation potential of hemopoietic cells in AKR/J mice has been reported by others [3].

Our experiments unveil some delicate mechanisms contributing to the development of leukemia. These data provide the basis for creation of pathogenetically justified methods for correction of hemopoietic disturbances and can be used as diagnostic and prognostic markers of leukemic progression.

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