Adaptive Capacity of Granulocytic Bone Marrow Stem Cells in Preleukemic AKR Mice

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 127, No. 2, pp. 151-154, February, 1999
Original article submitted April 16, 1998

In leukemia-prone AKR mice, adaptation to 10-h immobilization stress increases the content of sialoadhesin-positive macrophages to the level of intact (CBA×AKR)F_1 hybrids. Hybrid mice responds to stress by a slight reduction of this parameter. The contents of granulocytic hemopoietic islets and committed granulomonocyte precursors in the bone marrow after stress undergo opposite changes. Unlike hybrids, granulocytopoiesis in AKR mice is not activated by stress.

Key Words: *sialoadhesin; hemopoietic islets; granulocyte-macrophagic precursors; bone marrow; stress*

Receptor-mediated interaction of hemopoiesis-inducing microenvironment with hemopoietic cells and extracellular matrix is very important for understanding structural and functional bases of hemopoiesis. In particular, many studies are now focused on sialoadhesin (SA), a macrophage receptor mediating adhesive interaction with lymphocytes and myeloid cells [8]. In the bone marrow, SA is localized within contacts between central macrophage and granulocytes in hemopoietic islets [10], which implies its functional role in granulocytopoiesis [11]. It has been demonstrated that stress hormones glucocorticoids enhance SA expression on cells of the mononuclear phagocyte system (SMP) *in vitro* [11].

In light of this it seems important to study local mechanisms (SA expression, cell associations) of granulocytopoiesis in leukemia-prone AKR mice during adaptation to immobilization stress.

MATERIALS AND METHODS

Experiments were carried out on 48 AKR/JY and (CBA×AKR)F_1 mice of both sexes aged 4 months (collection of Laboratory of Experimental Biomedical Modeling, Tomsk Research Center). The animals were immobilized for 10 h in the supine position with extremities fixed with soft strings. Previously we observed the development of the resistance phase of the general adaptation syndrome on days 4-8 after the start of experiments, which was characterized by marked hyperplasia of the bone marrow hemopoietic tissue [1].

Expression of SA on bone marrow macrophages was assessed in the rosette formation test [7]: to this end, ligands for SA (sheep erythrocytes) were applied on the adherent macrophage monolayer. The preparations were fixed in glutaraldehyde, stained with azure II-eosin, and rosettes were counted.

Hemopoietic islets were isolated as described previously [6] with some modifications [3] using 0.05% collagenase (Sigma) and 0.1% neutral red. Granulocytic hemopoietic islets were identified by morphology of azure II-eosin-stained cells associated with central elements.

Committed precursors of granulomonocytopoiesis (CFU-GM) were cloned by culturing nonfractionated bone marrow cells (3×10^5 nuclears/ml) for 7 days in a methylcellulose tissue culture [3]. Mouse recombinant granulocyte-macrophage colony-stimulating factor (Sigma) in a dose of 4×10^{-9} g/ml was used as a stimulator. Myelogram was counted on bone marrow smears stained with azure II-eosin.

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0007-4888/99/0002-0137$22.00

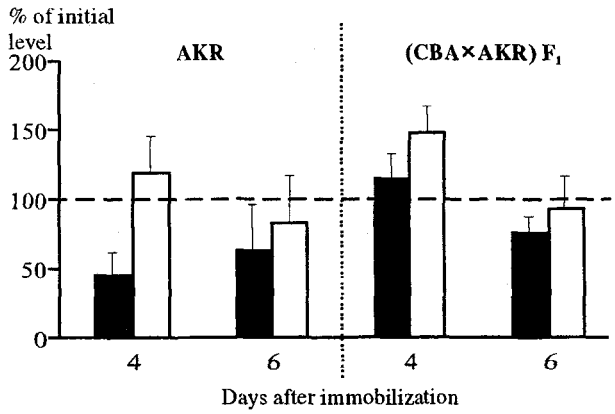

Fig. 1. Number of neutral red-positive (dark bars) and negative (light bars) hemopoietic islets in mouse bone marrow after 10-h immobilization stress.

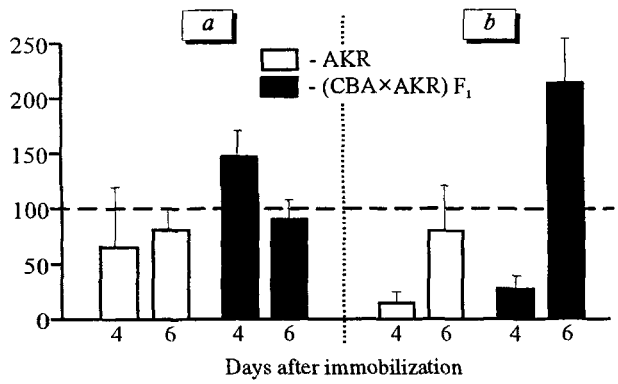

Fig. 2. Content of granulocytic hemopoietic islets (*a*) and granulocyte-macrophage colony-forming units (*b*) in the bone marrow after 10-h immobilization stress. Ordinate: content of cell associations, % of initial level.

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

RESULTS

In 4-month-old AKR mice, SA expression on bone marrow macrophages was lower than in (CBA×AKR)F_1 hybrids (Table 1). However, myelograms revealed no differences between these groups in the parameters of granulocytopoiesis. Receptor insufficiency of SMP cells in AKR mice is probably compensated by its increased (1.5-5-fold) content in the bone marrow. In response to 10-h immobilization SA expression on bone marrow macrophages from AKR mice increased to the level of intact (CBA×AKR)F_1 hybrids, whereas in hybrids immobilization stress slightly reduced this parameter (Table 1).

It can be hypothesized that resident macrophages take part in the maturation of bone marrow granulocytes in myeloblastic clusters (islets) [10]. We found that the number of hemopoietic islets with functionally mature (neutral red-positive) stromal elements in AKR mice sharply decreased in response to immobilization stress (to 45-63% of the initial level), while in hybrids this parameter increased on day 4 of the experiment. Similar and even more pronounced changes were observed for granulocytic hemopoietic islets (Fig. 1).

It is now generally accepted that hemopoietic islets play an important role in the proliferation and differentiation of hemopoietic precursors from committed to mature forms [6] and macrophagic SA close interacts with sialic ligands of myeloid precursors [11]. We have previously demonstrated that 10-h immobilization stimulated granulocytopoiesis via activating cell proliferation and increasing the content of CFU-GM and granulocytic hemopoietic islets in the bone marrow 4-8 days after stress [4]. In our experiments, the post-stress decrease in the number of granulocytic hemopoietic islets in AKR mice was accompanied by a decrease in the concentration of CFU-GM in the bone marrow most pronounced on day 4 of immobilization (Fig. 2). In contrast, in hybrids the concentration of CFU-GM after a transient decrease on day 4 sharply rose and surpassed the level of intact hybrids and immobilized AKR mice.

It should be noted that the stress-induced release of granulocytic hemopoietic islets and CFU-GM in (CBA×AKR)F_1 hybrid mice is accompanied by hyperplasia of monocytic and granulocytic bone marrow stems associated with a significant accumulation of monocytes (more than 3-fold), immature and mature neutrophil granulocytes (1.5-2-fold). Thus, the response of the bone marrow granulocytic stem to immobilization stress in (CBA×AKR)F_1 hybrids is well-balanced and practically does not differ from that of other mouse strains [2]: enhanced proliferation and differentiation of committed precursors in hemopoietic islets.

TABLE 1. Expression of SA (%) on Bone Marrow Macrophages after 10-h Immobilization (*M*±*m*)

Groups	Day 4		Day 6	
	control	experiment	control	experiment
AKR/JY	59.40±10.98+	87.83±3.02*+	67.20±4.49+	77.66±4.75
(CBA×AKR/JY) F_1	81.66±2.10	74.00±2.74*	86.30±3.54	80.66±4.46

Note. $p<0.05$: *compared with the control, +compared with hybrids.

In AKR mice on day 6 of immobilization we observed accumulation of mature neutrophil granulocytes (by 51%, $p<0.05$) in the bone marrow against the background of a reduced concentration of CFU-GM and granulocytic hemopoietic islets and enhanced expression of SA on macrophages, which indirectly indicates the possibility of granulocyte differentiation without enhanced proliferative activity of committed precursors.

Our findings suggest that the reaction of the bone marrow granulocytic stem to stress in AKR mice is limited even at the age of 4 month, which corresponds to the onset of the preleukemia period [5]. This reaction is determined by impaired structural and functional adaptation of the hemopoietic tissue to extreme factors resulting from reversion (probably due to compensatory and adaptive processes) of quantitative and qualitative parameters of bone marrow macrophages. Previously reported functional peculiarities of SMP cells in 4-month-old AKR mice in comparison with 1-month-old animals [5] are probably related to early expression and production of murine leukemia viruses by macrophages [9].

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