GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Activity of Sympathoadrenal System and Myelokaryocyte Death during Aging in AKR/JY Mice

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Accelerated bone marrow cell death and activation of the sympathoadrenal system were observed during aging of highly leukemic 2-7-month-old AKR/JY mice compared to that in (CBA/CaLac×AKR/JY)F1 strain. Close correlation was revealed between activity of the sympathoadrenal system and necrotic and apoptotic forms of cell death. This can promote tumor process, because maximum changes in hemopoietic cells occur during advanced stage of the disease.

Key Words: *adrenal glands; bone marrow; apoptosis*

The concept of two different forms of cell death (necrosis and apoptosis) proposed by J. Kerr *et al.* [15] is now widely accepted. It is known that glucocorticoids induce apoptotic death of various cell types [3, 8,9] via the cAMP system [8]. However, the role of hormones of the sympathoadrenal system (SAS), a basic stress-realizing element of the organism, in the regulation of apoptosis remains unclear [11,14]. Disturbances in apoptotic processes play a role in the pathogenesis of many diseases, including neoplastic processes [3]. The development of stress reaction during tumor growth is beyond doubt. However, the data on the role of SAS in tumor processes are contradictory [1].

Our aim was to study the state of SAS and apoptotic death of hemopoietic cells during aging of AKR/JY mice, which in 91% cases die of thymus leukemia by day 300 of their life [2].

MATERIALS AND METHODS

Experiments were carried out on 32 female AKR/JY mice aging 2-7 months, 12 (CBA/CaLac×AKR/JY)F1 hybrids aging 4 and 7 months, and 6 CBA/CaLac mice aging 2 months (Laboratory of Experimental Biomedical Modeling, Institute of Pharmacology, Tomsk Research Center).

Viability of myelokaryocytes was verified by 0.5% trypan blue exclusion test [6]. The concentration of catecholamines (CA) in the adrenal medulla was determined by chromaffin reaction according to Hillarp and Heckfield [7]. The data are expressed in optical density units. Apoptotic cells and bodies were determined according to morphological criteria [16] on cytological preparations of the bone marrow stained with azure II and eosin.

The results were analyzed statistically using Student's *t* test and Spearman's rank correlation coefficient.

RESULTS

The concentration of CA in the adrenal medulla of AKR/JY mice underwent waveform oscillations during aging, being decreased in comparison with that of 2-month-old CBA/CaLac mice (Fig. 1, *a*). A significant decrease in CA level (by 12-21%) was observed at the age of 4, 6, and 7 months in comparison with that in young AKR/JY mice. The concentration of

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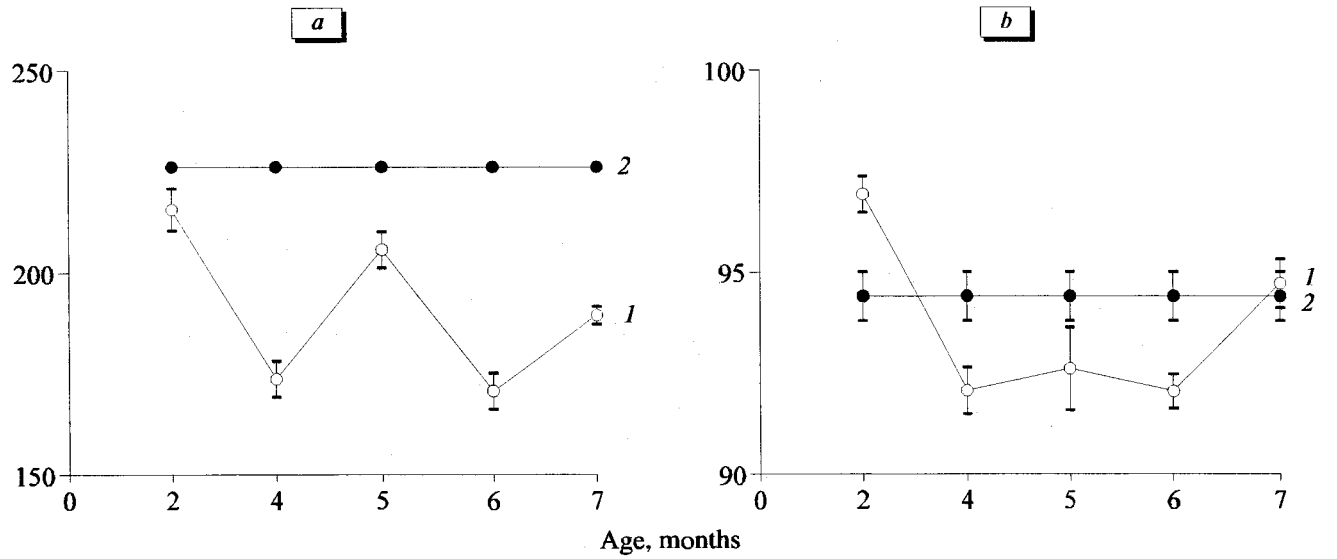

Fig. 1. Concentration of catecholamines in the adrenal glands (*a*) and viability of myelokaryocytes in the bone marrow (*b*) during aging of AKR/JY (*1*) and (CBA/CaLacxAKR/JY)F1 (*2*) mice. Ordinate: concentration of catecholamines, optical density units (*a*), and the fraction of viable cells, % (*b*).

SAS hormones in 4- and 7-month-old AKR/JY mice did not exceed 86-88% of that in age-matched (CBA/CaLacxAKR/JY)F1 hybrids ($p<0.05$, Table 1).

Depletion of CA stores in the adrenal medulla is the marker of stress-reaction, in particular during immobilization [5,7] and cytostatic treatment [7].

The decrease in thymus weight at the age of 4-6 months and accumulation of myelokaryocytes observed in AKR/JY mice during aging are also characteristic symptoms of stress [7]. Presumably, hemoblastosis developing in AKR/JY mice during aging is attended by the general adaptation syndrome, which is probably related to circulation of a Gross-type leukosogenic virus [10,12].

On the other hand, viability of myelokaryocytes in AKR/JY mice also decreased during aging (Fig. 1, *b*). It is evidenced by the close correlation between adrenal CA concentration and viability of bone marrow cells ($r=0.9$; $p<0.05$). It is noteworthy that viability of bone marrow cells in AKR/JY mice significantly decreased to month 7 (but not to month 4) of life in comparison with that in (CBA/CaLacxAKR/JY)F1 mice ($p<0.05$).

Thus, 4-7-month-old AKR/JY mice are characterized by more or less pronounced accumulation of trypan blue-positive myelokaryocytes. An increase in stain absorption is a sign of cell necrosis [8]. The correlation analysis suggest that SAS hormones promote the death of bone marrow cells in highly leukemic AKR/JY mice.

Published data focuses on readiness of the hemopoietic cells to apoptosis [3]. In AKR/JY mice, the content of bone marrow nuclears undergoing apoptosis 2-fold surpassed that in (CBA/CaLacxAKR/JY)F1 mice (Table 1). In 7-month-old AKR/JY mice with morphologically verified lymphoma, the number of apoptotic cells in the bone marrow reached 4.6% (0.39×10^6 cells/femur) *vs.* 1.40±0.15% in the control. At the same time, the total celularity of the bone marrow decreased more than 2-fold in comparison with that in healthy AKR/JY mice. The content of CA in

TABLE 1. Concentration of CA in Adrenal Glands and Content of Apoptotic Cells and Bodies in Bone Marrow during Aging of AKR/JY and (CBA/CaLacxAKR/JY)F1 Mice (*M*±*m*)

Index	Age, months			
	4		7	
	AKR/JY	(CBA/CaLacx AKR/JY)F1	AKR/JY	(CBA/CaLacx AKR/JY)F1
Concentration of CA, optical density units	163.82±1.99	186.63±6.41	197.72±4.39	222.67±1.89
Number of apoptotic cells and bodies, 10^6/femur	0.32±0.02	0.17±0.02	0.29±0.03	0.15±0.01

the adrenal medulla was also decreased in leukemic mice (185.95±7.07 *vs.* 203.60±4.33 optical density units in healthy mice, $p<0.05$).

The correlation analysis revealed strengthening of the inverse correlation between the number of apoptotic bone marrow cells and CA concentration in the adrenal glands during aging: the rank correlation coefficient was -1.0 ($p<0.001$) in 7-month-old mice and -0.8 ($p>0.02$) in 4-month-old mice. No correlations were revealed in (CBA/CaLac×AKR/JY)F1 mice.

In conclusion, the high death rate of bone marrow cells in AKR/JY mice during aging is closely related to activation of SAS. Similar relationship was observed previously during damage to hemopoietic bone marrow cells induced by cytostatics [4].

Damage to hemopoietic precursors in AKR mice was observed by M. Hellebostad *et al.* [13]. Presumably, AKR/JY mice a defect of hemopoietic bone marrow cells, which is aggravated by CA. These changes are most pronounced during advanced stage of the disease and probably promote the tumor process.

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