Suppressive and Antitumor Activities of Bone Marrow Cells and Splenocytes of AKR Mice during Aging

Yu. P. Bel'skii, N. V. Bel'skaya, M. G. Danilets, E. S. Stal'bovskaya, and S. A. Kusmartsev

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 127, No. 4, pp. 452-454, April, 1999
Original article submitted April 16, 1998

In 4-month-old leukemia-prone AKR mice, the ability of bone marrow cells to inhibit proliferation of concanavalin A-stimulated splenocytes and mastocytoma P815 cells *in vitro* was sharply increased in the preleukemic period. In 7-month-old mice, differences in natural suppressive activity of bone marrow cells were significant, but less pronounced than in 4-month-old mice. The immunosuppressive activity was not found in the spleen. In 4-month-old AKR mice, in the inhibition of proliferation of mitogen-stimulated splenocytes was increased due to enhanced NO production by bone marrow cells. These findings suggest that the increased antiproliferative activity observed in the bone marrow of AKR mice long before the appearance of clinical manifestations of leukemia is associated with disturbances in differentiation of myeloid progenitor cells and accumulation of natural suppressor cells in the bone marrow.

Key Words: *natural suppressor cells; nitric oxide; leukemia; granulocyte-macrophage precursors*

The development of leukemia in AKR mice [1] is accompanied by deep alterations at the level of stem hemopoietic cells. The activity of natural suppressor cells (NSC) [5,9] that belong to immature hemopoietic cells increases under activated hemopoiesis, *e.g.*, during tumor growth [7]. In this case, NSC appear in the spleen and play an important role in the pathogenesis of immunosuppression. Effects of NSC are mediated by soluble factors. NO is known to be a mediator of the suppressive effects of intact bone marrow NSC [2]. On the other hand, NSC isolated from the bone marrow of cancer patients produce considerable amounts of tumor growth factor-β [8].

Besides immunosuppressive properties, NSC can inhibit proliferation of tumor cells [6] and hemopoiesis [4]. These data suggest that dysfunction of bone marrow suppressor cells plays an important role in the pathogenesis of leukemia.

Here we studied suppressive and antitumor activities (SA and AA, respectively) of bone marrow cells and splenocytes in the latent period of leukemogenesis in AKR mice and the mechanism of antiproliferative effects of these cells.

MATERIALS AND METHODS

We used 50 male and female AKR/JY mice at the age of 2, 4, and 7 months and 4-month-old F_1 (CBA/CaLac× AKR) hybrid mice. AKR/JY mice (conventional strain) were obtained from the collection of the Laboratory of Experimental Biomedical Modeling (Tomsk Scientific Center). Bone marrow cells were washed out from the femur with phosphate buffer. Spleens were minced and washed with phosphate buffer to obtain splenocytes. These suspensions (5×10^6/ml) were incubated in 75-ml plastic flasks (Costar) in RPMI-1640 medium with 10% fetal calf serum at 37°C and 5% CO_2 for

Laboratory of Experimental Biomedical Modeling, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences, Tomsk

0007-4888/99/0004-0410$22.00

16-20 h to remove adherent cells. Nonadherent cells were washed, suspended in the culture medium, and used for further studies.

Suppressor activity was determined by inhibition of proliferation of splenocytes from F_1 hybrids preincubated with 4 μg/ml concanavalin A (Sigma) for 20-22 h. AA was estimated by inhibition of mastocytoma P815 cell proliferation.

Nonadherent bone marrow cells and splenocytes in various concentrations were incubated with preactivated splenocytes (2×10^5/well) or with P815 cells (2×10^4/well) in 96-well plates for 36 h to estimate SA and AA. ^{3}H-Thymidine (1 μCi/well) was added 16 h before the end of culturing. The results were expressed in percents of inhibition of proliferation of the corresponding target cells.

NO production in supernatants was assessed by the content of nitrites using Griess reagent [3]. This reagent (0.1 ml) was mixed with equivalent volume of the supernatant. The absorption was measured at 550 nm. The concentration of nitrites was determined by the calibration curve constructed using sodium nitrite.

RESULTS

Gross virus-induced leukemia transferred from parents to offspring is known to be typical of AKR mice. The death of experimental animals began at the age of 7 months. Total death of mice was observed at the age of 8-10 months. Therefore, we used 3 experimental groups consisting of 2-, 4-, and 7-month-old animals. Four-month-old mice carrying no Gross virus (hybrids) were used as the control.

Suppressor activity of bone marrow cells in 4- and 7-month-old AKR mice increased compared with that in 2-month-old AKR mice and hybrids (Table 1). SA attained maximum in 4-month-old AKR mice at the effector:target cell ratios of 1:1 and 0.5:1. This parameter was two times higher than in hybrid mice. The differences between these parameters in hybrid mice and 7-month-old AKR mice were also significant. Proliferation of mitogen-stimulated splenocytes in the absence of effectors was 73,500±3,605 cpm and 29,179±2,827 cpm. Bone morrow cells and splenocytes cultured separately produce no NO.

The highest AA was observed in 4-month-old AKR mice at all effector:target cell ratios (Table 2). It was significantly higher than in hybrid mice. AA in 4-month-old AKR mice was higher than in 2-month-old AKR mice at the effector:target cell ratio of 10:1. At the effector:target cell ratio of 10:1, AA in 7-month-old AKR mice was higher than in 2-month-old AKR and hybrid mice. Proliferation of mastocytoma P815 cells in the absence of effectors was 43,797±5,612 cpm and

TABLE 1. SA of Bone Marrow Cells and NO Production ($X\pm m$)

Animals	Age, months	Effector:target cell ratio					
		2:1		1:1		0.5:1	
		% of suppression	NO, μM	% of suppression	NO, μM	% of suppression	NO, μM
(CBA/CaLac×AKR) F_1	4	72.0±1.8	38.5±2.6	38.1±8.3	27.8±4.3	23.6±8.1	18.6±2.6
AKR	2	—	—	53.0±0.6	—	28.3±6.8	—
	4	89.9±3.2***	53.7±4.1*	75.6±5.8***+	43.4±3.4*	57.2±5.7*+	32.7±9.9
	7-8	—	—	69.6±4.9***+	—	56.9±1.7***+	—

Note. Here and in Table 2: *$p<0.05$, **$p<0.02$, and ***$p<0.01$ compared with hybrid mice; +$p<0.05$ and ++$p<0.01$ compared with 2-month-old AKR mice.

TABLE 2. AA of Bone Marrow Cells and NO Production ($X\pm m$)

Animals	Age, months	Effector:target cell ratio					
		20:1		10:1		5:1	
		% of suppression	NO, μM	% of suppression	NO, μM	% of suppression	NO, μM
(CBA/CaLacrAKR) F_1	4	43.4±0.1	<2	3.4±7.7	<2	2.9±5.8	<2
AKR	2	—	—	14.4±3.4	—	10.8±3.2	—
	4	62.2±5.5*	<2	46.4±6.4****++	<2	23.8±5.8*	<2
	7-8	—	—	42.0±9.3*+	—	14.4±6.7	—

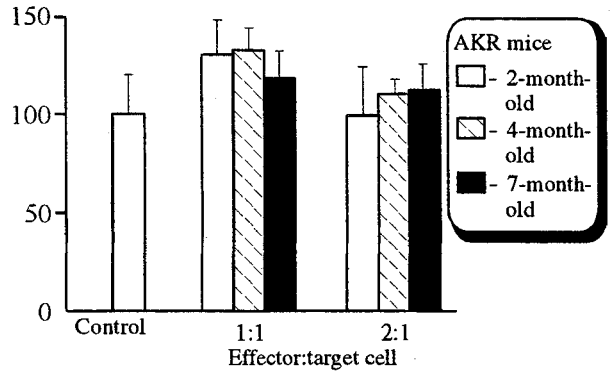

Fig. 1. Suppressive activity of splenocytes from AKR mice of various ages. Ordinate: cell proliferation, % of proliferation of target splenocytes in the absence of effectors (56,560±5,872 cpm and 41,820±1,136 cpm).

35,275±1,270 cpm. Bone marrow and tumor cells did not produce NO.

In the periods studied, no antiproliferative activity with respect to mitogen-stimulated splenocytes and tumor cells was revealed in the spleen (Fig. 1).

During incubation of bone marrow cells with mitogen-stimulated splenocytes, NO production in 4-month-old AKR mice was significantly higher than in hybrid mice (Table 1). Bone marrow cells (in the absence of splenocytes) and splenocytes produced no NO. During culturing of myelocytes with mastocytoma P815 cells, NO production was not detected in all groups studied. This indicates the presence of other factors responsible for the antitumor effects (Table 2).

Thus, SA and AA in the bone marrow (but not in the spleen) of 4-month-old AKR mice increased considerably long before the appearance of clinical manifestations of leukemia. This is probably due to disturbances in differentiation of myeloid progenitor cells observed at this period (accumulation of granulocyte-macrophage precursors in the bone marrow [1]). These differences in the mechanisms of inhibition of proliferation of tumor cells and splenocytes form the basis for the search for more selective factors in relation to bone marrow suppressor cells.

REFERENCES

1. E. D. Gol'dberg, Yu. P. Bel'skii, M. G. Danilets, *et al.*, *Byull. Eksp. Biol. Med.*, **125**, No. 3, 266-268 (1998).
2. I. Angulo, R. Rodriguez, B. Garcia, *et al.*, *J. Immunol.*, **155**, 15-26 (1995).
3. L. C. Green, D. A. Wagner, J. Glogowski, *et al.*, *Anal. Biochem.*, **126**, 131-143 (1982).
4. S. C. Moore, S. A. Theus, J. B. Barnett, and L. S. F. Soderberg, *Exp. Hematol.*, **20**, 11780-1183 (1992).
5. J. L. Subiza, J. E. Vinuela, R. Rodriguez, *et al.*, *Int. J. Cancer*, **44**, 307-314 (1989).
6. K. Sugiura, M. Inaba, H. Ogata, *et al.*, *Cancer Res.*, **50**, 2582-2586 (1990).
7. M. R. I. Young, G. Mccloskey, M. A. Wright, and A. S. Pak, *Cancer Immunol. Immunother.*, **38**, 9-15 (1994).
8. M. R. I. Young, M. A. Wright, M. Coogan *et al.*, *Ibid.*, **35**, 14-18 (1992).
9. M. R. I. Young, M. A. Wright, and M. E. Young, *Ibid.*, **33**, 146-152 (1991).