Opposite Effects of Dexamethasone on Antitumor and Natural Suppressor Activities of Bone Marrow Cells

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Effects of dexamethasone on the ability of nonadherent bone marrow cells to inhibit proliferation of mastocytoma P815 cells and concanavalin A-stimulated proliferation of syngeneic splenocytes were studied. Antitumor activity of cells increased, but their natural suppressor activity decreased in the presence of dexamethasone. Pretreatment with dexamethasone (for 3 h) did not affect the sensitivity of mastocytoma cells to antitumor factors and the antitumor activity of bone marrow effectors. Pretreatment of bone marrow cells with dexamethasone for 24 h potentiated antitumor activity of their nonadherent fractions.

Key Words: *natural suppressor activity; antitumor activity; bone marrow; dexamethasone*

Tumor growth is accompanied by severe hemopoietic disorders and activation of natural suppressor cells. These cells migrate from the bone marrow to extramedullary hemopoietic foci (spleen and tumor), and produce considerable immunosuppressive effects: inhibit mitogen- and antigen-induced proliferation of T and B lymphocytes and suppress natural killer activity and production of tumor necrosis factor by macrophages [5,7]. At the same time, it was shown that bone marrow cells (BMC) having the same specific features as natural suppressor cells *in vitro* inhibit proliferation of lymphocytes and tumor cells [3,6]. However, these effects are mediated by different mediators and, therefore, their mechanisms are different. Furthermore, subpopulations of antitumor and immunosuppressive effectors can be partially separated [4]. Hence, antitumor and immunosuppressive activities of BMC can be separately and specifically regulated.

It was shown that glucocorticoids inhibit immunosuppressive effects of cultured natural suppressor cells by blocking the production of interferon-γ [2]. Here we studied the influence of dexamethasone on antitumor properties of BMC.

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MATERIALS AND METHODS

Experiments were performed on 20 C57Bl/6J mice (conventional strain) aging 4 months (Laboratory of Experimental Biomedical Modelling, Tomsk Research Center).

Mastocytoma P815 cells were *in vitro* maintained in ascitic fluid. BMC were washed out from the femur with phosphate buffer. The spleen was minced and washed with phosphate buffer to obtain splenocytes. The cells were cultured in RPMI-1640 medium (Sigma) containing 10% fetal calf serum (Novosibirsk), 20 mM HEPES, 0.05 mM 2-mercaptoethanol (Sigma), 50 μg/ml gentamicin, and 2 mM L-glutamine (Flow Lab.) at 5% CO_2.

The suspensions ($3\text{-}5\times10^6$/ml) were incubated in 75-ml plastic flasks (Costar) for 16 h to remove adherent cells. Dexamethasone (10^{-6} M) was added to some flasks 16-18 h or 3 h before the end of culturing. Nonadherent cells were washed, suspended in culture medium, and their natural suppressor (NSA) and antitumor (ATA) activities were analyzed by inhibition of proliferation of targets, splenocytes and tumor cells (mastocytoma P815), respectively. Splenocytes were preincubated for 20-22 h with 4 μg/ml concanavalin A (Con A, Sigma), and the mitogen was then removed.

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TABLE 1. Effects of Dexamethasone on NSA and ATA of Nonadherent BMC ($X \pm m$)

Parameter		Control	With dexamethasone, M	
			10^{-6}	10^{-7}
Proliferation of splenocytes, cpm		29 179±2827	7568±863	—
NSA, %	E:T — 2:1	73.8±1.4	39.7±2.8*	—
	E:T — 1:1	55.6±4.4	30.5±5.7***	—
Proliferation of tumor cells, cpm		40 823±2381	38 007±3933	32 123±1217
ATA, %	E:T — 10:1	50.8±2.1	75.4±3.6**	64.9±3.5***
	E:T — 5:1	23.2±0.5	42.3±5.8***	47.7±5.8***

Note. Here and in Tables 2 and 3: *$p<0.001$, **$p<0.01$, and ***$p<0.05$ compared to the control.

TABLE 2. Effect of Pretreatment of Target Cells on ATA (%) of BMC ($X \pm m$)

Effector:target cell ratio	Control	Preincubation with dexamethasone	In the presence of dexamethasone
10:1	26.0±2.3	22.0±6.3	60.1±3.6**
5:1	8.2±3.0	16.0±5.7	38.0±2.5**

To estimate NSA and ATA, nonadherent BMC or splenocytes in various concentrations were cultured with splenocytes (2×10^5 cells/well) or P815 cells (2×10^4 cells/well) for 36 h in 96-well plates. ^{3}H-thymidine (0.5 μCi/well) was added 16 h before the end of culturing. The inhibition of proliferation of the corresponding target cells was expressed in percents.

The data were analyzed by Student's t test; the differences were significant at $p<0.05$.

RESULTS

In the presence of 10^{-6} M dexamethasone, the ability of nonadherent BMC to inhibit proliferation of Con A-stimulated splenocytes decreased, while their ATA increased (Table 1).

It was shown that dexamethasone indirectly reduces NSA of BMC by inhibiting the production of interferon-γ by T cells, which in turn activates natural suppressor cells [1]. To evaluate whether or not the elevation of ATA was related to the effects of dexamethasone on target tumor cells, these cells and bone marrow effectors were pretreated with dexamethasone, after that the hormone was removed. Proliferative responses of mastocytoma P815 cells in the absence and presence of dexamethasone were 54,705±2408 and 23,707±2672 cpm, respectively. Preincubation of mastocytoma P815 cells with dexamethasone did not change their sensitivity to the antitumor effects of BMC (Table 2).

In the absence of BMC, proliferative responses of mastocytoma P815 cells in the control and in the presence of dexamethasone were 104,423±7672 and 70,938±4467 cpm, respectively. ATA of BMC did not change after their treatment with dexamethasone for 3 h, but significantly increased after incubation for 16 h (Table 3).

Thus, potentiating effect of dexamethasone on ATA of nonadherent BMC is related to its direct influence on bone marrow effectors. Dexamethasone probably stimulates the secretion of antitumor factors or increases the resistance of bone marrow effectors

TABLE 3. ATA (%) of Nonadherent BMC in the Presence of Dexamethasone and After Pretreatment with Dexamethasone ($X \pm m$)

Effector:target cell ratio	Control (without dexamethasone)	With dexamethasone		
		throughout the incubation period	preincubation of BMC	
			3 h	16 h
10:1	59.0±2.0	85.1±2.8**	60.7±9.2	82.5±0.9*
5:1	14.6±3.1	35.6±1.1**	14.0±7.6	27.1±2.5***

to tumor products, which activate antitumor properties of BMC. Adequate cell therapy of malignant neoplasms requires further detailed analyses of the effects of dexamethasone and other compounds selectively regulating the immunosuppressive or antitumor activity of natural suppressor cells.

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