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CYTOSTATIC ACTION OF CELLS OF THE IMMUNE SYSTEM ON TUMOR CELLS

I. V. Bogdashin

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Several types of cells of the immune system capable of producing lysis of tumor target cells exist. They include activated cells, such as macrophages, T killer cell, normal killer cells (NKC), and polynuclear lymphocytes [2]. However, the cytotoxicity of effector cells is unable to explain certain experimental data obtained during the study of interaction of tumor cells with cells of the immune system. Cheever et al. [5], for instance, raised the question of the existence of "nonclassical" NKC, on the basis of the absence of their cytotoxic action in the test with ^{51}Cr *in vitro*, despite the presence of cytotherapeutic activity *in vivo*. Other workers [3] also have reported the possibility of immunotherapy with cells noncytotoxic for leukemia EL-4 cells.

It has been shown that tumor cells can remain for a long time in the body in a dormant state [8]. These facts are evidence of the possible existence not only to cytotoxic, but also of cytostatic mechanisms of immunologic surveillance of tumor growth.

The aim of the present investigation was to study the ability of cells of the immune system to exert a cytostatic action on tumor cells.

METHODS

To test the cytostatic action of effector cells we used a modified method [9] based on recording inhibition of RNA or DNA synthesis in target cells. As effector cells we used thymus, bone marrow, and spleen cells from C57BL/6 mice, and also splenocytes of DBA/2, CBA, AKR, and BALB/c mice as well as (CBA × C57BL/6)F_1 hybrids. As target cells we used mastocytoma P-815 (H-2^d), leukemia EL-4 (H-2^b), lymphoma YAC (H-2^a), and sarcoma MCh-11 (H-2^b) cells, maintained in the ascites form in mice of the corresponding strains. All the target cells used were insensitive to the cytotoxic action of the effector cells in the test with ^{51}Cr. To prevent incorporation of ^3H-uridine by the effector cells they were treated beforehand with actinomycin D in a concentration of 1 μg/ml per 10^7 cells in 1 ml at 37°C for 1 h. As was shown previously, this kind of treatment does not affect activity of killer cells [4]. Macrophages were removed by adsorption on plastic Petri dishes.

Nonadherent fractions of spleen, bone marrow, and thymus cells were used in the experiments. To obtain peritoneal macrophages, C57BL/6 mice each received an injection of 1.5 ml of 10% peptone, and on the 3rd day after the injection the peritoneal cavity of the mice was flushed out with Eagle's medium with 10 U of heparin. The cells thus obtained were adsorbed on plastic Petri dishes. The adherent cell fraction was harvested with the aid of a siliconized tube. Effector cells and target cells were incubated for 4 h in 96-well plates in medium RPMI-1640, containing 10% fetal serum and 100 U each of penicillin and streptomycin in

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TABLE 1. Cytostatic Activity of C57BL/6 Effector Cells on P-815 Cells

Effector cells	Ratio of effectors to targets	Duration of incubation, h	Inhibition of synthesis in target cells, %	
			DNA	RNA
Splenocytes	50:1	18	84±8	91±4
		8	76±8	77±7
	20:1	18	70±6	78±6
		8	50±8	54±7
Macrophages	20:1	18	62±7	64±7
		8	50±6	28±8

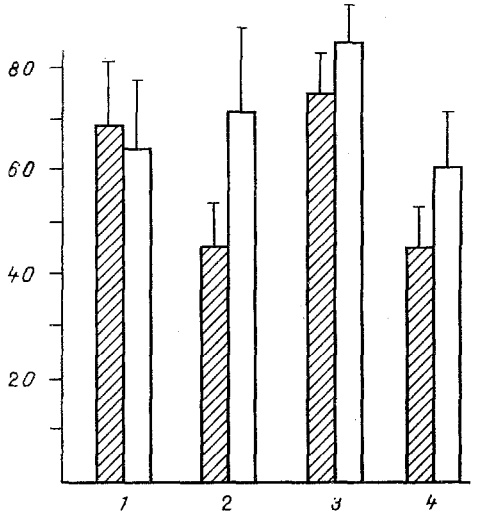

Fig. 1. Inhibition of RNA and DNA synthesis by C57BL/6 effector cells of different organs of immune system with a ratio of effectors to targets of 20:1. Abscissa, 1) thymus, 2) bone marrow, 3) spleen, 4) lymph nodes. Here and in Figs. 2 and 3, ordinate: CSI (in %); unshaded columns, RNA; shaded, DNA.

1 ml. Next, either ^{3}H-uridine or ^{3}H-thymidine was added in a dose of 1 μCi to each well, and the cells were incubated for a further 4 or 18 h. The cells were then transferred to No. 7810505 filters (Flow Laboratories). Incorporation of ^{3}H-thymidine and ^{3}H-uridine into the cells was determined with a β-counter. The cytostatic index (CSI) was calculated by the formula:

$$CSI = \left(1 - \frac{\text{cpm in experimental wells} - \text{cpm in wells with effector cells} \cdot 100\%}{\text{cpm in wells with target cells}}\right).$$

RESULTS

It will be clear from Table 1 that splenocytes and macrophages had a marked cytostatic action on the target cells, tested with reference to inhibition of synthesis of both RNA and DNA. The intensity of the cytostatic action of the effector cells depended on the dose of the cells and the duration of incubation of the target cells with the effector cells. Inhibition of RNA synthesis was more intensive than inhibition of DNA synthesis in target cells in ratios of 50:1 and 20:1.

Results relating to the cytostatic activity of cells from different organs of the immune system of the mouse are given in Fig. 1. The cytostatic activity of thymus cells was about equal in the tests with ^{3}H-thymidine and with ^{3}H-uridine. Bone marrow cells inhibited RNA synthesis more strongly than DNA synthesis. Spleen and lymph node cells inhibited RNA and DNA synthesis about equally.

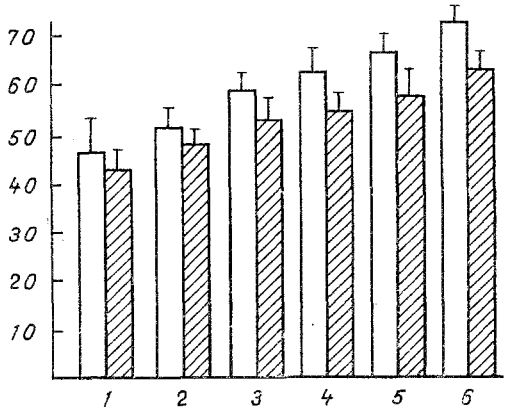

Fig. 2. Cytostatic activity of splenocytes from different strains of mice relative to P-815 cells with effectors to targets in the ratio of 20:1; abscissa: 1) DBA/2; 2) AKR; 3) BALB/c; 4) CBA; 5) C57BL/6; 6) (CBA × C57BL/6)F_1.

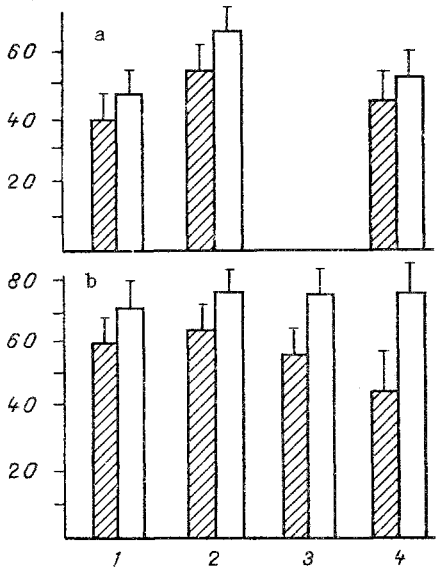

Fig. 3. Inhibition of DNA and RNA synthesis in target cells with effectors to targets in the ratio of 20:1. a) Peritoneal macrophages of C57BL/6 mice; b) splenocytes of C57BL/6 mice. Abscissa: 1) EL-4; 2) P-815; 3) YAC; 4) MCh-11.

The results of another series of experiments showed (Fig. 2) that as regards inhibition of RNA synthesis, the cytostatic activity of AKR and DBA/2 splenocytes was significantly less than the cytostatic activity of splenocytes of the other lines, but as regards inhibition of DNA synthesis, only cells of DBA/2 mice had a weaker cytostatic action than cells of the remaining lines of mice on target cells. No significant difference was found in the cytostatic activity of mice of the BALB/c, CBA, and C57BL/6 lines. Splenocytes of F_1 hybrids had the strongest cytostatic action compared with spleen cells of mice of the other lines as regards inhibition of synthesis of both RNA and DNA. Cytostatic activity, tested with respect to inhibition of RNA synthesis was higher than that discovered with regard to inhibition of DNA synthesis in mice of all lines studied.

The specificity of the cytostatic action of the effector cells was studied in a series of experiments whose results showed (Fig. 3) that the cytostatic action of both macrophages and splenocytes was not restricted in relation to the H-2 complex and was independent of the type of tumor.

Analysis of the results of this investigation shows that the ability of cells of different lymphoid organs to exhibit cytostatic activity is distinct from their ability to exert natural cytotoxic activity. Thymus and bone marrow cells possess cytostatic activity as high as or higher than that of splenocytes, whereas NKC activity is absent in the thymus and low in bone marrow. Splenocytes of CBA and (CBA × C57BL/6)F_1 mice exhibit the strongest cytostatic activity against YAC-1 cells, and those of A/Sn and AKR mice have the lowest activity [1]. Meanwhile the cytostatic activity of splenocytes of BALB/c, CBA, and C57BL/6 mice is about equal, and that of splenocytes of DBA/2 and AKR mice is rather lower. These facts suggest that cells which have a cytostatic action on tumor cells belong to different populations from NKC. Other workers [6, 72] who showed that activity of cytostatic effectors, unlike that of NKC, does not decline during tumor growth, during carcinogenesis, or with age, also reached the same conclusion.

The problem of the place and role of cell-mediated cytostasis in the body and the nature of the effector cells of cytostasis remains unsolved. The population of cytostatic cells may perhaps be heterogeneous. We know that T suppressor cells, cells with the Thy-1,2, Lyt-1^+, $2,3^-$, FcR^+ may exert a marked cytostatic action [7, 11]. It has been shown that the effector cells of cytostasis exercise control over metastasization [10]. These cells with cytostatic action may perhaps participate in the regulation of differentiation and proliferation.

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