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ACTION OF HUMORAL FACTORS OF MASTOCYTOMA P815 CELLS ON FORMATION OF ALLOSPECIFIC KILLER CELLS IN MIXED LYMPHOCYTE CULTURE AND ON THEIR CYTOTOXIC ACTIVITY

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During tumor growth functional activity of the principal populations of effector cells, namely natural killer cells, macrophages [11], and cytotoxic T lymphocytes (CTL) [10], is depressed. Tumor cells (TC) can exert a similar immunosuppressor action through the secretion of humoral factors (HF), which can influence the activation and differentiation of immunocytes. Reports of the inhibitory action of HF of various TC lines on lymphocyte proliferation induced by mitogens and alloantigens [12], and on functional activity of natural killer cells [8] and macrophages [4], have been published. We could find only one communication dealing with a study of the inhibitory action of P815 cells and their HF on the generation of allo-CTL in mixed lymphocyte culture (MLC) [5]. However, in that study, syngeneity of the responding lymphocytes and TC was not observed. The authors cited also limited themselves to testing the biological activity of tumor cultural supernatants and did not investigate the growth medium of the tumor (ascites fluid) in vivo.

The aim of this investigation was to study the effect of mastocytoma P815 cells and their HF on allo-CTL formation in MLC and on the cytotoxic activity of preformed allo-CTL.

EXPERIMENTAL METHOD

Experiments were carried out on male mice of inbred lines DBA/2 (H-2^d) and C57BL/6 (B6, H-2^b), aged 2-4 months, and obtained from the "Rappolova" and "Stolbovaya" nurseries, Academy of Medical Sciences of the USSR. Mastocytoma P815 and leukemia EL4 cells were maintained in vitro and by passage in vivo through syngeneic mice. The animals were killed by cervical dislocation, the spleens were removed under aseptic conditions, cells were obtained fom them in glass homogenizers, and they were allowed to stand for 3-5 min in centrifuged tubes in medium 199 with 10% inactivated bovine serum. The thrice-washed splenocytes were suspended in medium RPMI-1640 ("Flow Laboratories"), with the addition of 5% embryonic calf serum ("Flow"), 2 mM glutamine, 5×10^{-5} M 2-mercaptoethanol ("Serva"), 25 mM HEPES buffer ("Flow"), and 50 μg/ml of gentamicin. Allospecific killer cells were generated in 24-well panels ("Flow") with responding cells (splenocytes of DBA/2 mice; 5×10^6 cells per well) and stimulators (B6 splenocytes) in the ratio of 5:1. The stimulators and cells added as the third component were treated with mitomycin C ("Sigma," USA) in a dose of 50 μg/ml at 37°C for 30 min, followed by washing 3 times. The cells were cultured at 37°C in air with 5% CO_2 for 5 days. At the end of the reaction the cells were centrifuged, counted, and their cytotoxic activity (CTA) was determined in the microcytotoxic test, by measuring release of ^{51}Cr from labeled target cells (TaC) [3]. TaC in a concentration of 2×10^6 cells/ml were incubated with 100 μCi Na_2CrO_4 (All-Union "Izotop" Combine) for 1 h at 37°C, washed 3 times, and transferred to wells of 96-well micropanels (10^4 TAC/well). Effector cells (EC) were added in a volume of 0.1 ml; the ratio EC:TaC was 30:1. The maximal yield of radioactivity was determined with the aid of 2% sodium dodecylsulfate. The spontaneous yield of radioactive label did not exceed 30% of the maximal. Cytotoxic activity (CTA) was expressed as a percentage of specific lysis, calculated by the known formula [3]. Activity of HF of P815 cells was tested in 3-day cultural supernatants and in 7-day ascites fluids. For this purpose they were added as the third component either to the test system of allo-CTL generation or to the microcytotoxic test sample. The

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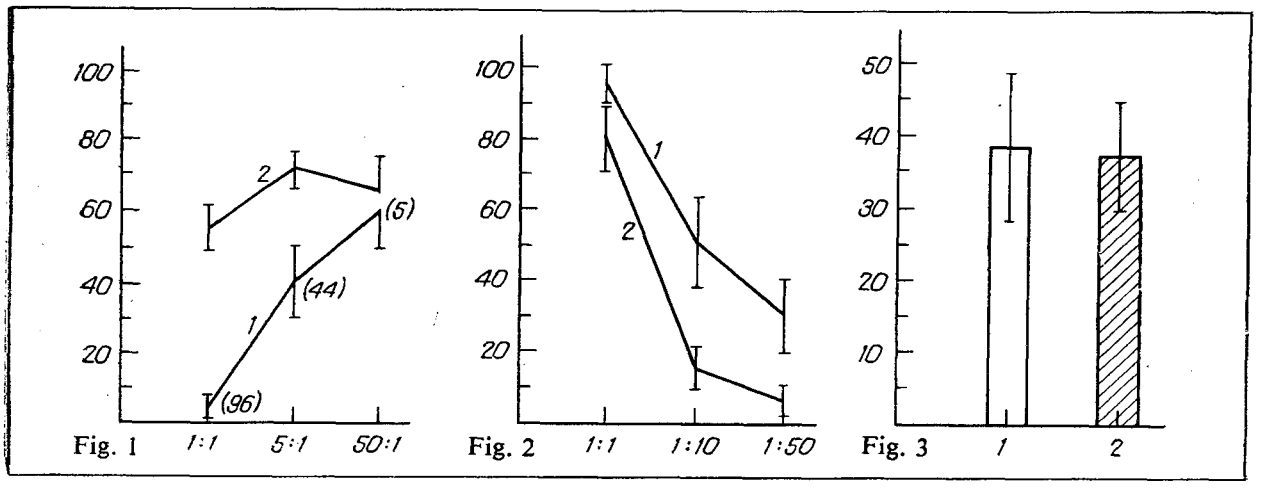

Fig. 1. Inhibition of allo-CTL formation by P815 cells. Abscissa, ratio of responders to cells added as the third component; ordinate, CTA (in %). 1) P815 cells; 2) splenocytes of DBA/2 mice. Numbers in columns are indices of inhibition (in %). Results of three experiments shown (M ± m).

Fig. 2. Suppression of allospecific killer cell generation by humoral factors of P815 cells. Abscissa, dilution of humoral factors; ordinate, indices of inhibition (in %). 1) Cultural supernatant of P815 cells; 2) ascites fluid of P815 cells. Results of three experiments shown (M ± m).

Fig. 3. Effect of cultural supernatant of P815 cells on CTA of mature allo-CTL. Abscissa, variants of substances added in microcytotoxic test; ordinate, CTA (in %). 1) Addition of culture medium; 2) addition of cultural supernatant of P815 cells (dilution 1:10).

3-day peptone ascites fluid from DBA/2 mice was used as the control. To obtain it, mice were given an intraperitoneal injection of 5 ml of 3% peptone ("Serva"). Immunosuppressor activity of P815 cells was tested by adding them as the third component to the test system allo-CTL generation. The index of inhibition (II) was calculated by the formula:

$$II = A - B/A \times 100,$$

where A and B denote release of ^{51}Cr in the control and experimental samples. The possible cytotoxic activity of HF was estimated in the microcytotoxic test. For this purpose HF was added to ^{51}Cr-labeled TaC and incubated for 4 h at 37°C in air with 5% CO_2. At the end of incubation the plate was centrifuged and radioactivity of the supernatants determined on a gamma-counter (LKB). The index of cytotoxicity (IC) was calculated by the formula:

$$IC = A - B/C - B \times 100,$$

where B and C denote the spontaneous and maximal yield of ^{51}Cr and A the yield of ^{51}Cr in wells with the addition of HF.

EXPERIMENTAL RESULTS

In the first stage of the work the effect of mastocytoma P815 cells on allo-CTL formation in MLC was estimated. For this purpose TC (normal splenocytes in the control) were added to MLC as the third component, while observing syngeneity of responders and added cells. It will be clear from Fig. 1 that TC had a dose-dependent suppressor action on allo-CTL formation whereas normal splenocytes had no inhibitory effect. In the next stage of the investigation the inhibitory activity of HF from P815 cells, contained in cultural supernatants and ascites fluids, was tested. Peptone ascites fluid DBA/2 mice was used as the control. It will be clear from Fig. 2 that tumor products caused dose-dependent suppression of allo-CTL formation. The control peptone ascites fluid did not affect this process. The effect was not connected with the cytotoxic action of TC, supernatants, or ascites fluids, for the viability of the cells in the experimental and control samples was similar. Tumor products likewise did not affect the yield of ^{51}Cr from target cells (Table 1). In the next series of experiments the action of HF on cytotoxic activity of allo-CTL

TABLE 1. Testing Cytotoxicity of Humoral Factors of Mastocytoma P815 Cells

Group No.	Target cells	Preparation	Yield of ^{51}Cr from target cells, cpm	IC, %
1	YAC-1	Medium	310±16	—
2	YAC-1	Medium with 2% sodium dodecyl sulfate	3000±33	—
3	YAC-1	Cultural supernatant of P815 cells (dilution 1:10)	324±14	2

already differentiated in MLC was assessed. It was considered important to discover whether the inhibitory activity of humoral substances of P815 cells is limited to their effect on the phase of allo-CTL induction in MLC or whether tumor products can also inactivate the functional activity of mature effector cells. It will be clear from Fig. 3 that HF of mastocytoma cells do not inhibit lysis of target cells by allo-CTL. Thus mediation of tumor immunosuppression by HF takes place at the stage of proliferation and differentiation of CTL. This effect may be due to interference of tumor products with the monolymphokine cascade of lymphocyte activation, and also to induction of suppressors cells, which we described previously [1, 2]. Analysis of data in the literature shows that immunosuppressor substances of different strains of tumor cells can inhibit secretion of mono- and lymphokines themselves [6, 7]. The suppressor-inducing activity of HF of hepatocellular carcinoma cells has been described [9]. The writers showed previously that an important mechanism of lymphocyte damage is the membrane-toxic action of tumor cells and their products [2]. The mechanisms of the immunosuppressor action of tumor cell products, and also their biochemical characteristic, calls for further analysis.

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