HUMORAL MECHANISM OF THE MEMBRANE-TOXIC EFFECT OF MASTOCYTOMA P815 AND LEUKEMIA EL4 CELLS

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Previous investigations in the writers' laboratory showed for the first time that tumor cells possess the property of membrane toxicity [7]. By the term "membrane toxicity" we understand an increase in membrane permeability of target cells for proteins with a molecular weight of up to 12 kD during incubation with effector cells. In previous publications, cells of leukemias EL4, YAC, YAC-1, L1210, and BW5147, of sarcomas MX-II and SA-1, and of Ehrlich's carcinoma were used as effector cells; the target cells were lymphocytes and also certain tumor cells (K562, YAC-1) [2,5-7,12]. It was shown on conjugates of normal killer cells and tumor cells, obtained in a thin layer of agar, that the membrane toxicity of tumor cells is realized in the zone of contact of their membranes with membranes of normal killer (NK) cells [8, 9]. The aim of this investigation was to study the membrane-toxic properties of mastocytoma P815 and leukemia EL4 cells and also of humoral factors secreted by them.

EXPERIMENTAL METHOD

Experiments were carried out on DBA/2 and C57BL/6 mice aged 2-4 months, obtained from the "Rappolovo" and Stolbovaya" Nurseries, Academy of Medical Sciences of the USSR. P815 and EL4 cells were maintained in vitro and subjected to passage in vivo in syngeneic animals. The sources of humoral factors of tumor cells were 3-day-old cultural supernatants and ascites fluids. Ascites tumors were obtained 7 days after intraperitoneal injection of the tumor into mice in a dose of 5×10^6 cells per mouse. Ascites fluid was separated from cells by centrifugation. Control peptone ascites fluid was obtained 3 days after intraperitoneal injection of 5 ml of 3% peptone ("Serva") into mice. The cells were cultured in medium A: medium RPMI-1640 ("Flow Laboratories"), with the addition of L-glutamine (2 mM), HEPES-buffer ("Flow," 25 mM), 5% embryonic calf serum ("Flow"), and gentamicin (50 μg/ml). The membrane-toxic test was carried out by the method in [7]. Target cells in a concentration of 10×10^6/ml were labeled with ^{3}H-uridine (4 μCi/ml) for 60 min at 37°C, washed 3 times, and treated with actinomycin D ("Serva," 1 μg/ml) for 4 h (in some experiments treatment with actinomycin D was not given). At the end of incubation with actinomycin D, the target cells were washed 3 times. Tumor cells (P815 and EL4), splenocytes of DBA/2 and C57BL/6 mice, and kidney cells of DBA/2 mice were used as effectors. The kidney cells were isolated by the method in [1]. The effectors were treated beforehand with actinomycin D in a dose of 1 μg/10×10^6 cells at 37°C for 60 min to prevent reutilization of hydrolysis products of labeled RNA from the target cells and washed 3 times. The effectors and targets were resuspended in medium A containing 10 μg/ml of RNase ("Sigma"). A mixture of 0.1 ml of effectors and 0.1 ml of target cells (2×10^5 cells/ml) was introduced into each well of a round-bottomed 96-well micropanel (2×10^5 cells/ml). The ratio of effector cells to target cells was 100:1, 50:1, and 25:1. In the control, 0.1 ml of medium A containing 10 μg/ml of RNase was added instead of the effectors. The panels were incubated for 16 h at 37°C in air containing 5% CO_2. At the end of incubation the samples were collected on glass-fiber filters ("Flow") by means of a harvester, and washed successively with physiological saline, 5% ice-cold trichloracetic acid, and 96% ethanol. The index of membrane-toxicity (IMT) was calculated by the formula: IMT = (A — B):A, where A and B denote incorporation of ^{3}H-uridine (in cpm) in control and experimental samples.

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TABLE 1. Membrane-Toxic Activity of Mastocytoma P815 and Leukemia EL4 Cells

Effectors (E)	Targets (T), 2×10^4 cells per well	E-T	IMT, % (M ± m)
P815	Splenocytes of DBA/2 mice	100:1	35±16
+	+	50:1	17±5
+	+	25:1	7±2
+	[1]ConA-blasts	50:1	22±10
+	[2]ConA-blasts	50:1	36±15
Spenocytes of DBA/2 mice	Splenocytes of DBA/2 mice	100:1	2±4
Kidney cells of DBA/2 mice	+	100:1	0±0
EL4	Spenocytes of C57BL/6 mice	100:1	26±11
Splenocytes of C57BL/6 mice	+	100:1	0±0

Legend. [1]ConA-blasts and [2]ConA-blasts denote 3-day-old ConA-blast cells from DBA/2 mice treated with actinomycin D and intact respectively.

TABLE 2. Membrane-Toxic Activity of Humoral Factors of P815 and EL4 Cells

Preparation (dilution)	Targets (T), 2×10^4 cells per weell	IMT, % (M ± m)
Cultural supernatant of P815 cells	Splenocytes of DBA/2 mice	
1:1		34±8
1:10	+	30±13
1:100	+	24±11
Ascites fluid of		
1:1	+	49±20
1:10	+	36±10
1:100	+	17±9
Peptone ascites fluid of DBA/2 mice, 1:1	+	0±0
Cultural supernatant of EL4 cells:	Splenocytes of C57BL/6 mice	
1:1		37±8
1:10	+	29±9
1:100	+	27±15

EXPERIMENTAL RESULTS

It will be clear from Table 1 that mastocytoma P815 cells had a dose-dependent membrane-toxic action on 3-day-old ConA-blasts and splenocytes of DBA/2 mice. EL4 cells also possessed membrane-toxic activity against splenocytes of C57BL/6 mice. Normal spleen cells of DBA/2 and C57BL/6 mice and kidney cells of DBA/2 mice were not membrane-toxic. These data, in conjunction with results published previously, lead to the conclusion that the property of membrane toxicity is possessed only by tumor cells. That membrane toxicity is a real phenomenon is shown by the fact that P815 cells have a membrane-toxic action not only on lymphocytes treated with actinomycin D, but also on intact syngeneic blast cells. In the next stage of the work we attempted to evaluate the contribution made to the phenomenon of membrane toxicity by humoral factors contained in the cultural supernatants of P815 and EL4 cells and also in tumor ascites fluid, used as growth medium for P815 cells in vivo. It follows from Table 2 that humoral factors of tumor cells were membrane-toxic against spleen cells. The control peptone ascites fluid had no membrane-toxic action. In a previous publication [12] we cited data in the literature on the mechanisms of the immunosuppressive activity of tumor cells. During the subsequent years research has been published in which the chemical characteristics of certain humoral factors of tumor cells have been given [10, 11, 13]. These substances belong to different classes of biopolymers: proteins [11], lipids [10], and glycoconjugates [13]. However, the mechanisms of their action on the cell membrane of the immune system have virtually not been studied. The processes of suppression of immunocyte function under the influence of these substances likewise have received little study. Parallel with the investigation of membrane

toxicity of humoral factors of P815 and EL5 cells, we studied their effect on the immunoreactivity of lymphocytes. Suppression of lymphocyte proliferation in response to lectins and alloantigens and also activation of suppressor cells under the influence of immunosuppressive factors of P815 cells were demonstrated [3]. We regard membrane toxicity as increased permeability of the membrane of target cells, but this is not the only possible explanation. Increased uptake by lymphocytes of molecules attached to their membrane (endocytosis) cannot be ruled out. As applied to our system the results are evidence against such an interpretation. On penetrating into a lymphocyte or lymphoblast, RNase damages a high proportion of the newly formed intracellular RNA molecules. The enzyme obtains access to important zones of the cytoplasm (through RNA transport into zones of protein synthesis) and is not deposited in phagolysosomes. This mechanism alone can suppress the functions of lymphocytes, if it is recalled that tumors produce a wide variety of enzymes, and that a fairly high level of ribonucleases, proteases, and other enzymes is found in blood. A solution to the problem enunciated above may be of fundamental importance for elucidation of the causes of the ineffectiveness of antitumor immunity. An answer must also be found to the question whether the same immunosuppressor factor may possess multiple biological activity.

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