MEMBRANE-TOXICITY OF TUMOR CELLS FOR LYMPHOCYTES.
ROLE OF HOMOLOGY FOR THE IC SUBREGION OF THE H2 COMPLEX

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The writers showed previously [4] that mouse leukemia and sarcoma cells and also peripheral blood cells of patients with chronic lymphatic leukemia are membrane-toxic for the widely used targets of normal killers (NK) namely K-562 human erythroleukemia cells and EL-4 mouse leukemia cells after a long period of passage. In an attempt to find cytotoxicity of tumor cells for intact lymphocytes, a near-threshold effect was discovered (under 10%). Meanwhile, it has recently been shown [12] that the NK lymphocyte is inactivated by contact with the K-562 target cell, and after contact has ended, its ability to injure the same target cells is restored. This action of the tumor cell on the lymphocyte can be regarded as cytostatic.

It has been shown on a model of NK against target cells [11] that the degree of damage and death of targets depends on protein synthesis in the target cell. Protein synthesis evidently determines the ability of the target to repair the plasma membrane.

The object of this investigation was to determine whether sensitivity of the lymphocytes to the cytotoxic action of the tumor cell increases during a considerable reduction in protein biosynthesis in that cell.

According to published data [10] the level of cytotoxicity of NK for target cells is controlled by genes situated at the D end of the H2 complex. It has been shown [5] that the level of reactivity of NK is controlled by the H-2D-Hh-1 sublocus of the principal histocompatibility complex. This point of view contradicts observations [7] according to which the specificity of NK for a concrete target is controlled, and not the general level of cytotoxic activity of the NK. The NK is known to act on tumor cells in both syngeneic and allogeneic systems. We have no data on how the level of cytotoxicity depends on differences or agreement between NK and the target cell for genes of the H2 complex.

An attempt also was made to discover whether the level of cytotoxicity of the tumor cell for lymphocytes depends on similarities and differences between them relative to genes of the H2 complex.

EXPERIMENTAL METHOD

The cytotoxicity of the tumor cell for the target cell was tested by the 14-hour test [5], in the writers' own modification [3]. To prevent reutilization of degradation products of ^{3}H-RNA in the tumor effector cells, they were treated for 1 h with actinomycin D (1 μg/ml, from Sigma, USA) and washed 3 times with large volumes of medium 199 with the addition of 10% fetal serum (from Flow Laboratories). It was shown beforehand that treatment in this manner does not affect the cytotoxic activity of tumor effector cells.

Splenocytes from various lines of mice, including congeneic and recombinant lines, were used as targets (Table 1). Experiments also were carried out in which B lymphocytes isolated from a suspension of splenocytes, from which macrophages had first been removed, were used as targets. The isolation was carried out with the aid of mass cytolysis with antimouse an-

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TABLE 1

Line No.	Number of experiments	Tumor cells	H-2 complex of effector tumor cell								Mouse splenocytes	H-2 complex of splenocyte targets								Ratio of effectors to targets	Cytotoxicity (%)
			K	IA	IB	IJ	IE	IC	S	D		K	IA	IB	IJ	IE	IC	S	D		
1	7	MCh–II	b	b	b	b	b	b	b	b	B10/Sn	b	b	b	b	b	b	b	b	20:1 50:1 100:1	25:3,2 47+1,7 74+0,75
2	7	MCh-II	b	b	b	b	b	b	b	b	BALB.c	d	d	d	d	d	d	d	d	20:1 50:1 100:1	7+1,5 19+3,1 28+2,5
3	6	MCh-II	b	b	b	b	b	b	b	b	C57BL'6	b	b	b	b	b	b	b	b	20:1 50:1 100:1	21+1,9 43+1,4 70+1,2
4	3	MCh-II	b	b	b	b	b	b	b	b	B10.SM	v	v	v	v	v	v	v	v	20:1 50:1 100:1	5+2,7 11+1,4 23+0,75
5	3	L—1210	d	d	d	d	d	d	d	d	B10.D2	d	d	d	d	d	d	d	d	20:1 50:1 100:1	20+1,4 45+2,1 72+0,95
6	3	MCh-II	b	b	b	b	b	b	b	b	B.10A (5R)	b	b	b	k	k	d	d	d	20:1 50:1 100:1	7+1,04 11+1,2 21+1,25
7	3	MCh-II	b	b	b	b	b	b	b	b	B10.A (3R)	b	b	bb	k		d	d	d	20:1 50:1 100:1	9+0,7 17+1,9 23+2,1
8	3	MCh-II	b	b	b	b	b	b	b	b	B6 (M505)	bd	eb	b	b	b	b	b	b	20:1 50:1 100:1	25+0,8 44+0,7 62+1,3
9	6	SA/1	k	k	k	k	k	d	d	d	CBA	k	k	k	k	k	k	k	k	20:1 50:1 100:1	5+1,4 14+0,9 22+2,7
10	6	L—1210	d	d	d	d	d	d	d	d	A/Sn	k	k	k	k	k	d	d	d	20:1 50:1 100:1	22+2,3 45+1,6 68+0,95
11	3	L—1210	d	d	d	d	d	d	d	d	B10.A (3R)	b	b	b	b	k	d	d	d	20:1 50:1 100:1	23+2,8 40+2,35 70+1,4
12	3	MCh-II	b	b	b	b	b	b	b	b	B10.D2 (R—107)	b	b	b	b	b	b	b	d	20:1 50:1 100:1	21+0,9 44+0,85
13	3	L—1210	d	d	d	d	d	d	d	d	B10.D2 (R107)	b	b	b	b	b	b	b	d	20:1 50:1 100:1	5+0,75 9+0,9 20+1,9
14	3	SA/1	k	k	k	k	k	d	d	d	B10.D2 (R101)	d	d	d	d	d	d	d	b	20:1 50:1 100:1	21+2,1 72+1,7

ti-T-serum (anti-thymocytic immunoglobulin, from Microbiological Associates) and with rabbit complement. The killed cells were removed on a Hypaque-Ficoll gradient (1.09 g/cm^3). To inhibit protein synthesis in the lymphocytes [11] they were treated after uridine labeling (5 μCi/ml, specific activity 24 Ci/mmole) with actinomycin D in a concentration of 1 μg/ml in RPMI-1640 medium with 10% fetal serum (from Flow Laboratories) for 8 h at 37.C, and then washed 3 times with large volumes of culture medium. The action of actinomycin was estimated from inhibition of incorporation of ^{3}H-leucine (5 μg/ml, specific activity 25 Ci/mmole) in the splenocytes.

Cells of two mouse leukemias, EL-4 ($H2^b$) and L-1210 ($H2^d$), and also two mouse sarcomas, MCh-11 ($H2^b$) and SA-1 ($H2^a$), which had previously undergone passage through syngeneic mice in the ascites form, were used as effectors. The experimental results were subjected to analysis by the confidence intervals method.

EXPERIMENTAL RESULTS

The relationship between the duration of incubation of the splenocytes with actinomycin D and the degree of inhibition of protein synthesis in them is shown in Fig. 1. After incubation for 6 h protein synthesis was inhibited by more than 65%. In the next experiments splenocytes were incubated with actinomycin D for 8 h. Death of the splenocytes after exposure to actinomycin D did not exceed 20% by the test with trypan blue and eosin.

As Table 2 shows, the index of cytotoxicity after treatment with actinomycin D when the ratio of effectors to targets was 100:1 exceeded 70%. In a separate group of experiments the cytotoxicity of MHc-11 ($H2^b$) tumor cells for B lymphocytes of B10 mice was investigated. In ratios of 20:1, 50:1, and 100:1 it was 18 ± 1.08, 34 ± 0.95, and 56 ± 1.2% respectively. On

TABLE 2. Investigation of "Cold" Inhibition of Membrane Toxicity of Effector Tumor Cells against Splenocytes of Mice of Congeneic and Recombinant Lines

Line No.	Number of experiments	Effector tumor cells	Mouse splenocyte target cells	Ratio effectors/targets	Cytotoxicity (%)	Mouse splenocytes "cold" inhibitors	Effectors/targets "cold" inhibitors ratio	Inhibition, %
1	3	MCh-II	C57BL/6	50:1	46±1,2	C57BL/6	50:1:50	79±1,2
2	3	MCh-II	C57BL/6	50:1	46±1,2	BALB/c	50:1:50	18±0,9
3	3	SA/1	B10.D2 (R101)	50:1	43±0,9	B10.D2 (R101)	50:1:50	82±2,1
4	3	L-1210	B10.D2	50:1	44±1,4	B10.D2	50:1:50	75±1,4
5	3	L-1210	A/Sn	50:1	45±2,1	A/Sn	50:1:50	84±1,3
6	3	MCh-II	B10.D2 (R107)	50:1	44±0,8	B10.D2 (R107)	50:1:50	75±1,05

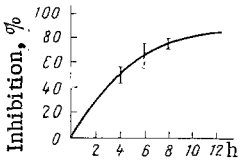

Fig. 1. Inhibition of incorporation of ^{3}H-leucine into splenocytes as a function of duration of exposure to actinomycin D (1 μg/ml). Splenocytes labeled with ^{3}H-leucine for 1 h, then transferred to filters with the aid of a 12-channel harvester and radioactivity estimated on counter. Genetic analysis of membrane toxicity of tumor cells for splenocytes.

the basis of the results of this experiment, a "suicide" effect of the normal killers, T killers, and macrophages on contact with the tumor cell could be ruled out, because the B lymphocyte does not possess the corresponding effector system.

The question of the role of the H2 complex in the cytotoxic action of tumor cells on splenocytes was studied in experiments whose results are summarized in Table 1. If effectors and targets were syngeneic the index of cytotoxicity reached a maximum (Table 1, line 1). Mutation of the targets for the K region (Table 1, line 8) did not lower the cytotoxicity index. Both minimal and considerable differences in the genetic base did not reduce the cytotoxicity index (Table 1, lines 3 and 5). In an allogeneic combination (Table 1, line 2) the cytotoxicity index was lowered by more than half. An equally considerable decrease in the cytotoxicity index took place if effectors and targets were identical for their genetic base and with complete incompatibility for the H2 complex (Table 1, line 4). Coincidence between effectors and targets as regards the K region and 1A, 1B, 1I, and 1E subregions (Table 1: lines 6, 7, and 9), coupled with noncoincidence for the D end of the H2 complex resulted in an index of cytotoxicity which was just as low as that in the completely allogeneic situation. The same result was obtained when coincidence between effectors and targets was limited to the D subregion of the H2 complex (Table 1: line 13). Meanwhile, coincidence between effectors and targets for the D end of the H2 complex (Table 1: lines 10 and 11) resulted in high cytotoxicity values characteristic of a syngeneic system. Noncoincidence confined to the D subregion (Table 1: line 12) gave a cytotoxicity index that was just as high.

The facts described above suggest that coincidence between the effector tumor cell and splenocyte target with respect to the IC subregion and the S region of the H2 complex is essential for cytotoxicity to reach a maximum. In fact, coincidence for IC and S only (Table 1: line 14) resulted in cytotoxicity reaching the maximum characteristic of the syngenic system (Table 1: line 1). The S region, as we know [9], does not code for cell surface proteins, and its influence on cytotoxicity can therefore be disregarded. It can be concluded that coincidence for the IC subregion only determines maximal cytotoxicity of the tumor cell relative to the lymphocyte.

The addition of unlabeled cold inhibitors, i.e., splenocytes treated for 8 h with actinomycin D, to the mixture of effector tumor cells and labeled targets gave the following results (Table 2). In an allogeneic combination there was virtually no "cold" inhibition (Table 2, line 2), whereas inhibition took place if the effector tumor cells and "cold" inhibitors coincided with respect to the H2 complex, the D end, and simply the IC subregion. The same rule was thus found for interaction between the effector tumor cells and the "cold"

inhibitor as in the cytotoxic test. It can also be concluded that for the membrane of the splenocyte targets to be injured, direct contact is necessary between the effector tumor cells and the splenocyte targets.

The experiments thus showed that if protein synthesis is depressed the lymphocyte becomes a target for the membrane-toxic action of the tumor cell; the level of membrane toxicity, moreover, depends essentially on coincidence or noncoincidence between effector and target for the IC subregion of the H2 complex. The hypothesis that we were dealing with an important mechanism of suppressive action of the tumor of the immune system had to be tested. In particular, the possibility of a physiological (more exactly, pathophysiological) fall in the level of protein synthesis in cells of the immune system, as a result of which their sensitivity to the membrane-toxic or cytostatic action of the tumor cell was increased, requires analysis.

The test with ^{3}H-uridine, which we used to estimate the membrane toxicity of the effector tumor cell, is based on recording the effect of penetration of the enzyme ribonuclease (molecular mass 12 kilodaltons) through the membrane of the target lymphocyte. Such an effect must essentially be described as membrane-toxic, for we do not know how often it ends in death of the target cell. An answer to this question, and also to the question of the physiological importance of reparable membrane toxicity, i.e., the situation in which injury to the membrane of the target cell does not end in its death, will have to be answered.

The need for homology for the IC subregion of the H2 complex has recently been demonstrated [1] for interaction in a totally different system — interaction between responders in a mixed culture and T suppressors. Analysis of the mechanism and role of cell interaction with respect to products of the IC region of the H2 complex will be a task for future research.

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