CYTOSTATIC ACTION OF SYRIAN HAMSTER PERITONEAL CELLS ON TRANSFORMED CELLS

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Effector cells (EC) of natural resistance and immunity (lymphocytes, macrophages, granulocytes) have the ability to depress multiplication of tumor cells (to exert a cytostatic action – CSA) [2-5, 8-10]. The intensity of CSA depends on the level of activation of EC and on the sensitivity of the target cells (TC), which evidently varies widely. Inactivated EC of natural resistance, the first barrier defending the body against a tumor, are in our opinion particularly interesting. However, CSA of inactivated EC has received much less study than that of activated EC. According to data obtained by some workers, inactivated EC are highly reactive [2, 8, 10], whereas according to others, their activity is altogether impossible to detect [4, 9], or it is detectable only if EC predominate strongly over TC (50:1 or 100:1) [3]. In a previous publication devoted to the study of CSA of inactivated Syrian hamster peritoneal cells (PC), PC depressed incorporation of ^{3}H-thymidine into hamster cells of strains STHE and OPH-SR in a comparatively low EC:TC ratio, namely 5:1 [1].

Since the effect of quantitative ratios between reacting cells on the results of the cytotoxic test has not been adequately studied, it was decided to investigate: 1) the minimal ratio of EC to TC at which PC of intact Syrian hamsters can exhibit CSA; 2) the dynamics of this reaction.

EXPERIMENTAL METHOD

The CSA of PC was determined by recording the decrease in incorporation of ^{3}H-thymidine by STHE cells in the presence of PC. Cells of the STHE strain are embryonic Syrian hamster cells, transformed in vitro. STHE cells were seeded at the rate of (1-4) × 10^4 cells per well in 0.1 ml of growth medium (equal volumes of media L15 and DMEM with 10% bovine serum: 0.3 mg/ml glutamine, 0.2 mg/ml gentamycin, and 10 mM HEPES buffer). After 2 h 0.1 ml of growth medium was added to the control wells and the same volume of PC in doses differing by a factor of 10 was added to the experimental wells (10^5, 10^4, 10^3, 10^2, and sometimes 10^1). PC were collected from intact male Syrian hamsters aged 5-9 months by flushing out the peritoneal cavity with 15 ml of growth medium without heparin. The PC thus obtained were not washed and were added as quickly as possible to STHE cells. The PC used in this series of experiments consisted of 30-56% of lymphocytes, 40-65% of macrophages (Mph), 1-7% of neutrophils, and 0-2.5% of mast cells. The reaction was read every 2-3 h, 12-hourly time intervals being chosen for continuous working in the course of 34 h of contact between PC and STHE. The counting procedure was described in detail previously [1]. The percentage of CSA was determined by the formula:

$$\text{CSA, \%} = (1 - \text{cpm C}) \times 100,$$

where C (control) denotes STHE cells in the absence of PC, and E (experiment) denotes STHE cells mixed with any dose of PC. Incorporation of the label into PC did not exceed 3% of its incorporation into STHE cells in the control.

The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The results of one experiment to study the time course of CSA of PC on STHE cells with different ratios between EC and TC are given in Fig. 1. In this experiment the STHE cells

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TABLE 1. Dependence of CSA of PC of Intact Syrian Hamster on Doses of PC and STHE Target Cells within Interval of Contact of 23-34 h

Dose of STHE, cells/well	Dose of PC	EC/TC	% CSA of PC on STHE cells				
			23 h	25 h	28 h	31 h	34 h
$1{,}1 \cdot 10^4$	$1{,}1 \cdot 10^5$	10:1	79,1	79,0	77,3	73,1	62,4
	$1{,}1 \cdot 10^4$	1:1	62,8	64,5	54,5	47,0	40,8
	$1{,}1 \cdot 10^3$	1:10	34,9	37,1	33,3	37,3	25,0
	$1{,}1 \cdot 10^2$	1:100	2,3*	17,7*	7,6**	5,2*	—1,8*
$4{,}4 \cdot 10^4$	$1{,}1 \cdot 10^5$	2,5:1	98,0	94,1	96,8	94,8	93,0
	$1{,}1 \cdot 10^4$	1:4	94,6	88,3	88,9	75,8	74,8
	$1{,}1 \cdot 10^3$	1:40	73,2	64,9	66,4	27,6	31,4
	$1{,}1 \cdot 10^2$	1:400	34,9	36,9	30,4	2,3*	0,3*
	$1{,}1 \cdot 10^1$	1:4000	4,7*	—	—	—	—

Legend. EC/TC) Ratio of effector PC to target STHE. *$p > 0.05$.

were seeded in a dose of $1.1 \cdot 10^4$ cells per well, and four concentrations differing by a factor of 10 were added to them.

On addition of the maximal number of PC (10^5 cells/well) to the STHE cells, a statistically signfiicant ($p < 0.05$) cytostasis appeared after 3 h of contact (26.3%). It gradually increased to reach 77.3-79.1% after 23-28 h of contact. After 34 h, the CSA decreased. The presence of a maximal reaction on average after 25 h of contact between EC and TC, and a decrease in CSA on average after 32 h was confirmed in four experiments. The reason for the observed decline and subsequent time course of CSA is not yet clear.

As Fig. 1 shows, CSA of PC in a concentration of 10^4 and 10^3 cells per well appeared later, after 6 and 12 h, respectively, and by 25 h had reached 64.5 and 37.1%, respectively, significantly lower than in the reaction with 10^5 PC. PC in a concentration of 10^2 cells per well had no significant CSA on STHE cells, although here also a peak of CSA occurred at 25 h (17.7%).

It is thus clear that CSA of PC was directly proportional to their number and to the ratio of PC to TC.

By increasing the number of TC in the well four-tenfold (leaving the number of PC unchanged), and thereby reducing the ratio of EC to TC, we did not however, obtain the expected reduction of cytostasis but, on the contrary, in most cases the intensity of cytostasis was considerably increased. Typical results of one of the eight experiments in which CSA of different doses of PC was studied relative to two doses of STHE, differing by a factor of 4 ($1.1 \cdot 10^4$ and $4.4 \cdot 10^4$ cells/well), are given in Table 1. CSA of PC in this experiment was studied during the interval from 23 to 34 h.

It will be clear from the data in Table 1 that for every time and every dose of PC, CSA as a rule was significantly higher with a larger dose of STHE cells. PC (10^2 cells/well), not depressing incorporation of the label into STHE cells taken in the minimal dose, i.e., with a ratio of EC/TC of 1:100, depressed incorporation of the label into STHE cells taken in the maximal dose, i.e., with a ratio of EC/TC of 1:400. We found no reports in the literature on the antitumor action of inactivated EC of natural resistance in such small absolute and relative numbers.

The population of Syrian hamster PC which we studied was heterogeneous. The contribution of neutrophils and mast cells to the phenomenon described above (where 10^2 PC can induce CSA), if it exists at all, must be unimportant, becasue these effectors are often absent altogether among PC or are present in very small numbers. Nonadherent cells in the peritoneal cavity of intact hamsters, consisting to the extent of 95-97% of small lymphocytes, according to our preliminary data do not exhibit CSA. In our view, the phenomenon observed is due to activity of resident Mph (RMph) in the peritoneal cavity of the hamsters or to the synergic action of RMph and other PC.

Virtually all workers who have studied CSA of activated or stimulated Mph from the peritoneal cavity of mice and rats have reported strong suppression by these cells of DNA synthesis in the cells of a variety of tumors: lymphomas [6, 7], adenocarcinomas [9], melanomas [4, 7], mastocytomas [7, 10], and several others [7] after contact for 24-28 h. Meanwhile the majority of authors [4, 6, 9] state that RMph are incapable of CSA. In one investigation,

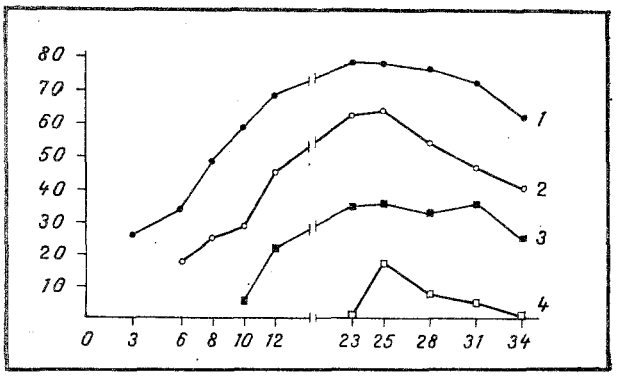

Fig. 1. Dynamics of CSA of various doses of PC relative to cells of the STHE strain. CSA determined in time intervals of 0-12 h and 23-24 h. STHE cells $1.1 \cdot 10^4$/well. Doses of PC: 10^5 (1), 10^4 (2), 10^3 (3), and 10^2 (4). Abscissa, time of contact (in h); ordinate, CSA (in %).

appreciable CSA was observed in RMph: this was CSA against mastocytoma P 815 [10]. Evidently TC differ very strongly in their sensitivity to CSA and only some TC are sensitive to CSA, namely inactivated Mph. The extremely high sensitivity of STHE cells to CSA of PC may be due to a peculiarity of the origin of cells of the STHE strain, which are cells transformed spontaneously in vitro, and have never gone through selection in vivo. Those workers who found that RMph are incapable of CSA were working with target cells of tumor origin, i.e., cells which had gone through in vivo selection. According to our data, tumors and metastases arising in animals after subcutaneous or intravenous injection of STHE cells, unlike the original version of STHE, were much more resistant to CSA of PC.

The fact that PC of Syrian hamsters depress incorporation of label by STHE cells in very low relative numbers (1:400) suggests that CSA of PC of intact Syrian hamsters is humoral in character, i.e., is realized through factors secreted by PC into the surrounding medium. The possibility of transmission of CSA by means of humoral cytostatic factors has been described, in principle, in [11-13]. However, all the cytostatic fators described are products of activated Mph or of activated lymphocytes. We do not know on account of what factors the CSA of inactivated Syrian hamster PC is realized.

Our discovery that CSA of PC is directly proportional to the number of TC in the well suggests, perhaps, that TC in our system are activators of the reaction. On the other hand, the increased density of TC in the well may help to spread the state of cytostasis induced by one EC in one TC to other TC by means of signals transmitted through intercellular junctions.

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