EFFECT OF DEGREE OF MALIGNANCY ON SENSITIVITY OF CELLS TO CYTOSTATIC ACTION OF RESIDENT SYRIAN HAMSTER PERITONEAL MACROPHAGES

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The in vivo selection of cell variants possessing a higher level of carcinogenicity and metastasization is an essential part of tumor progression [2]. According to some workers, malignant variants of cells selected in vivo may differ from the original parental cells in their greater resistance to the cytolytic and cytostatic action (CSA) on activated macrophages [6, 8, 10, 11, 13, 14], evidence in support of the possible role of activated Mph in selection. Less is known about the sensitivity of cells with different degrees of malignancy to inactivated Mph, which evidently also participate in selection in vivo. Mechanisms responsible for the resistance of tumor cells (TC) to Mph have received little study. One such mechanism may be the release by TC of the immunodepressant PGE_2, which inhibits Mph activity [2].

It was decided to compare the sensitivity to CSA of inactivated Mph on highly malignant cells of strain HET-SR (transformed in vitro by Rous sarcoma virus – RSV) and of cells of strain HETR (spontaneously transformed in vitro), with low malignancy, and five of its highly malignant variants selected in vivo, and also to determine whether the PGE_2 produced by highly malignant cells of these strains [4] affects the value of CSA.

EXPERIMENTAL METHOD

The following monolayer cultures of Syrian hamster tissues were studied: strain HETR (embryonic cells spontaneously transformed in vitro) with low degrees of malignancy and metastasization, and its variants with high malignancy and high degree of metastasization selected in vivo: HETR-LM^1, HETR-LM^4, HETR-LM^6, HETR-LM^8, and HETR-75/18 [7], and also cells of strain HET-SR (embryonic cells transformed in vitro by RSV, strain Schmidt-Ruppin), whose high degree of malignancy (ML) and experimental metastasization (EM) are unconnected with selection, but arise during virus transformation [3]. An unfractionated population of Syrian hamsters [5] or peritoneal Mph, washed to remove nonadherent cells, were used as infectors. The cytostatic test with peritoneal cells (PC) was carried out by the technique described previously [5], a characteristic feature of which is addition of effector cells to the previously sedimented TC. Mph purified by adsorption on plastic were not added to the previously sedimented TC because removal of Mph from the plastic injured them. In this case the TC were added to Mph, previously sedimented on plastic and washed to remove nonadherent cells, which reduced the level of cytostasis (Table 1). The observed reduction of cytostasis was evidently unconnected with separation of nonadherent cells from Mph, but was due to the order of sedimentation of the effectors and target cells, for a similar reduction of cytostasis took place on the addition of TC to the previously sedimented whole PC population (Table 1).

Accordingly, the main part of the work was done with unfractionated PC by the standard method [5].

CSA was determined as a percentage by the formula: $[(cpmK - cpmE)/cpmK] \times 100$, where K stands for TC grown without effector cells was not more than 3% of the incorporation of label into TC in the control. To determine the statistical significance of differences between the strains in their sensitivity to CSA of PC, Student's t test was used. To study the causes of

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TABLE 1. Dependence of CSA of PC on Conditions of Reaction

Dose of effector cells	Per cent CSA of effector cells		
	PC*	PC**	Mph***
$5 \cdot 10^3$	60,6±2,61	41,8±1,8	39,8±2,9
$5 \cdot 10^2$	29,3±3,3	15,4±2,7	9,9±4,6

Legend. *) PC added 2 h after TC, **) PC added 2 h before TC, ***) PC added 2 h before TC and washed free from nonadherent cells (Mph > 92%).

TABLE 2. Comparison of Cells Differing in Degree of Malignancy for Their Sensitivity to CSA of PC (addition to PC to previously sedimented TC; $M \pm m$)

Target cells studied	Number of experiments	Mean value of percent CSA of PC on target cells, with dose of PC of		
		$5 \cdot 10^4$	$5 \cdot 10^3$	$5 \cdot 10^2$
HETR	18	80±2,0	53±2,6	26±2,2
HETR-LM[1]	6	73±5,4	31±9**	n.d.***
HETR-LM[4]	10	57±5,0*	22±0,6*	n.d.
HETR-LM[6]	6	61±4,7*	22±5,5*	n.d.
HETR-LM[8]	13	74±1,9	56±2,1	27±3,3
HETR-75/18	13	49±2,8*	30±2,7*	7±2,6*
HET-SR	6	0 (3±6,9)*	0 (4±8,9)*	n.d.

Legend. *$p < 0.001$, **$p < 0.05$ compared with CSA of PC on HETR cells, ***) no data given.

TABLE 3. Comparison of Cells Differing in Degree of Malignancy for Sensitivity to CSA of PC and Mph (addition of TC to previously sedimented effector cells)

Dose of effector cells	Per cent CSA on TC							
	HETR		HETR-LM[8]		HETR-LM[6]		HETR-75/18	
	TC	Mph	TC	Mph	TC	Mph	TC	Mph
$5 \cdot 10^5$	n.d.	н. д	98,1	96,1	96,2	81,0	73,5	72,9
$5 \cdot 10^4$	73,5	66,7	63,8	67,2	26,4	4,3	35,3	37,5
$5 \cdot 10^3$	40,7	36,0	38,9	45,2	16,3	11,0	13,5	17,1
$5 \cdot 10^2$	15,5	8,4	14,8	10,0	3,0	—11,7**	5,7	—11,4

Legend. *) No data, **) "—" indicates intensification of proliferation of TC.

the low CSA and the role of PGE_2, secreted by TC or Mph, in this situation, the TC and/or Mph were pretreated with indomethacin ($10^{-6} - 5 \times 10^{-5}$ M, from "Sigma," USA) for 4 and 21 h in the monolayer, followed by thorough washing, and in some experiments indomethacin also was added to the test system.

EXPERIMENTAL RESULTS

The comparative study of the sensitivity of different cell lines to CSA of PC showed (Table 2) that parental HETR cells were highly sensitive to CSA of PC, whereas all variants of that strain selected in vivo, except HETR-LM[8], were significantly less sensitive to CSA of PC. The greatest differences in sensitivity between the strains compared were observed with PC in a dose of 5×10^3 cells per well, with a dose of 5×10^4 cells per well the differences were less marked but remained statistically significant for all variants except HETR-LM[1], while with a dose of 5×10^5 cells per well (data not shown) all the TC were equally highly sensitive to CSA of PC: by 97-99%, from which it follows that high concentrations of PC cannot be used to detect differences between strains.

Results similar in principle were obtained by the use of Mph, freed from nonadherent cells, as effectors (Table 3). Under these conditions of the cytostatic test (see: Experimental Method) differences in sensitivity to CSA of Mph between the cell strains compared still remained, but the level of CSA was lower than under standard test conditions (compare with Table 2) and was similar to the level of CSA exerted on TC by previously sedimented PC (Table 3). The higher CSA of the effectors on their addition to TC can perhaps be explained by the action of short-living substances, released by effector cells at the time of primary contact with plastic [1], or with the surface of TC, on TC.

It follows from the results that selection in vivo leads not only to the intensification of ML and EM [7] but also, as a rule, to a decrease in sensitivity of the cells to CSA of inactivated PC and Mph.

During the study of RSV-transformed HET-SR cells, whose ML and EM were higher not only than those of the corresponding levels for HETR, but also for variants of HETR selected in vivo, we found that they had maximal resistance to CSA of PC, which was significantly higher than the level of resistance of the malignant variants of HETR (Table 2). The actual value of cytostasis of these cells was close to zero (with these doses of PC). We thus found a higher level of resistance to CSA of inactivated PC and peritoneal Mph in four of five strains of TC which had gone through selection in vivo, and also in RSV-transformed cells of the HET-SR strain, correlating with higher survival rate in vivo compared with cells of the HETR strain. Correlation between the level of malignancy of TC and their resistance to CSA of activated Mph has been described by many authors [8, 10, 11, 13]. In our investigation, it was first demonstrated with the use of inactivated resident Mph. Meanwhile, highly malignant HETR-LM^8 cells, like HETR cells, were highly sensitive to CSA of PC. It might be postulated that HETR-LM^8 cells lost their malignant properties by the time of our investigations, but repeated experiments to determine ML and the ability to undergo spontaneous metastasization showed that HETR-LM^8 cells remained highly malignant. The high sensitivity of HETR-LM^8 cells to CSA of Mph may perhaps be connected with certain of their properties, essential for the development of cytostasis in vitro but not playing a role in survival in vivo (spreading out, increased affinity for serum factors, etc.). On the other hand, the ability of HETR-LM^8 cells to survive in vivo may be connected with their acquisition of alternative properties, compensating for their high sensitivity to Mph.

All the strains which we studied differed from HETR cells in their high production of the immunodepressant PGE_2, in response to their direct contact with normal killer (NK) cells, Mph, and neutrophils, and in their ability to suppress the cytotoxic activity of NK cells on account of their rapid release of PGE_2 [4]. Release of PGE_2 by macrophages on contact with malignant, but not benign, TC also has been reported [15]. To discover whether this low CSA of PC on relatively malignant cells is connected with lowering of the sensitivity of these cells to PC or with suppression of PC activity by prostaglandin E_2 (of macrophagal or tumor origin), we blocked any possible PGE_2 production in HET-SR and HETR-75/18 cells and/or in PC by their preliminary treatment with indomethacin, and we also added indomethacin to the test system. None of the schedules and doses of indomethacin treatment used led to any increase in CSA of PC relative to resistant cells. In parallel experiments, the cytotoxic activity of NK cells was preserved while release of PGE_2 from TC was suppressed by indomethacin [9]. Our results are in full agreement with data in the literature according to which PGE_2 inhibits the work of activated, but not of intact Mph [12]. Since the activity of the PC which we tested was not suppressed by PGE_2 of TC, the low CSA of PC on cells selected in vivo and on RSV-transformed cells is evidently due to a decrease in their sensitivity to cytostatic factors of PC on account of some other, as yet unknown, mechanism.

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ACTIVE COBALAMIN TRANSPORT IN L-1210 MOUSE LEUKEMIA CELLS

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A decisive role in the mechanism of the supply of cobalamin coenzyme precursors to cells, which are essential for their normal proliferation and maturation, is played by the formation of a transcobalamin-II cobalamin complex (TC-II+Cbl) with the membrane surface receptor. Specific receptors of this type have been found on cells of the villi of the mature human placenta, the spleen, normal hepatocytes, and L-1210 mouse leukemia cells [6, 12]. Changes in functional activity of TC-II receptors of the cell membrane surface may be responsible for differences in the intensity of cobalamin transport [1].

The effect of temperature and ionic composition of the medium on affinity of hormone receptors for the ligand is known [10]. The number of membrane surface receptors may vary depending on the concentration of the ligand in the medium and in the course of cell differentiation [7, 11]. However, the factors changing the number of TC-II receptors on the surface membrane of tumor cells are not yet known. We therefore investigated the effect of temperature, cobalamin concentration in the medium, and their intracellular concentration on the level of binding of radioactive [TC-II+Cbl] with surface receptors of L-1210 mouse leukemia cells. The functional activity of the receptors and endocytosis of cobalamins were investigated during the mitotic cell cycle in vitro.

EXPERIMENTAL METHOD

Experiments were carried out on L-1210 mouse leukemia cells isolated from ascites fluid on the 7th day after intraperitoneal injection of 2×10^6 mouse spleen cells of line DBA/2. Because of the absence of species-specific differences in protein transport, in order to saturate the surface receptors we used saturated ^{57}Co-cyanocobalamin (^{57}CoCNCbl) TC-II, obtained by special treatment of rabbit serum [4], as the standard. For quantitative determination of the receptors we used the ability of the proteolytic enzyme papain to remove protein molecules from the surface membrane cells, by cleaving their polypeptide chain [2]. The successive stages of determination were as follows: saturation of the cell surface receptors with the radioactive complex, separation of ligand-receptor complexes with radioactive label by papain from the cell surface membrane, determination of radioactivity on a γ-counter on solubilized receptors and inside the cells. Receptors of leukemic cells $(1.0\text{-}1.5) \times 10^7$ were estimated during short-term culture (between 5 and 100 min) in Eagle's medium with 10% standard serum, containing [TC-II+^{57}CoCNCbl]. The leukemic cells were analyzed in the experiments directly after isolation from ascites fluid and after their preliminary incubation (5 h, 37°C) in medium deficient in cobalamins, but containing TC-II. The pool of endogenous cobalamins in the L-1210 cells was determined by a microbiological method with *E. coli* 113/3 [1]. Receptors of the cell membrane surface were estimated at different periods of the mitotic cycle during longer-term culture (25 h) in the same medium with the addition of 10% embryonic calf serum. Binding of radioactive TC-II with receptors, accumulation of ^{57}CoCNCbl in the cytoplasm, and the number of cells synthesizing DNA were investigated at the same times. In this series of experiments, with the aim

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