RESISTANCE OF TUMOR CELLS ON MALIGNANT PHENOTYPE, SELECTED IN VIVO, TO THE CYTOLYTIC ACTION ACTIVATED PERITONEAL MACROPHAGES IN VITRO

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One of the factors involved in tumor progression is selection of malignant variants of cells in vivo, in which an essential role may be played by effectors of the natural resistance (NR) system [4]. As a rule, in the process of selection such variants, unlike parental transformed cells, may acquire marked malignant properties (tumorigenic and metastatic activity), and also a high level of resistance to effectors of NR (macrophages and monocytes, NK cells, and neutrophils) [5]. Recently, the role of various effectors of NR in carcinogenesis and in tumor progression has been studied intensively in our laboratory. Investigations of cell variants obtained in the laboratory by in vivo selection from Syrian hamster cells, transformed in vitro (spontaneously or by Rous sarcoma virus — RSV) have shown that, besides acquiring a malignant phenotype (tumorigenic activity — TGA), or spontaneous and experimental metastatic activity (SMA, EMA) the selected variants also acquire resistance to hydrogen peroxide (H_2O_2) [6, 7], they secrete an increased quantity of prostaglandins of type E_2 (PGE_2) on contact with NK cells [8, 9], and in some cases these two features may be combined into a cluster [5, 8, 9]. Possibly it is these discrete characteristics of tumor cells (resistance to H_2O_2 and PGE_2 secretion) that determine their resistance to effectors of NR. Correlation has been shown between these features and TGA and EMA [5]. Variants of the strain STHE (spontaneously transformed hamster embryonic cells), selected in vivo, and RSV transformants with different degrees of malignancy are resistant (to different degrees) to the cytolytic action (CLA) of neutrophils [2] and of NK cells [10] and to the cytostatic action of macrophages (Mph) and neutrophils [1, 11]. Meanwhile the sensitivity of malignant tumor cells to CLA of activated Mph (producing a large quantity of cytotoxic factors, including H_2O_2, proteases, cytolytic factor, etc.) has not been adequately studied. Data in the literature is contradictory in character. Some workers, for instance, have reported resistance of more malignant variants of cells to the CLA of activated Mph [16, 17], whereas others in general did not find any difference between CLA of Mph on tumor cells with varied degrees of malignancy, and assumed that the sensitivity of tumor cells to the CLA of Mph is a common property of all tumor cells [15], whereas a third group found an opposite relationship: cells with a more malignant phenotype were more prone to lysis by macrophages [12, 13].

The aim of this investigation was to study the sensitivity of parental cells of the STHE strain, with low malignancy, and variants with malignant phenotype selected from it in vivo, and also of tumorigenic RSV transformants to the CLA of activated Mph.

The preliminary results of these investigations were described previously [3].

EXPERIMENTAL METHOD

The following were used as target cells (TC) in the cytolytic test (CLT) with Mph: cells of the parental STHE strain (Syrian hamster embryonic cells spontaneously transformed in vitro, with low malignancy), malignant variants of that strain obtained from lung metastases (STHE-LM-4, STHE-LM-8, STHE-75/18), highly tumorigenic cells of strain HET-S-1 obtained by transformation in vitro of Syrian hamster embryonic cells by Rous sarcoma virus (Schmidt—Rupin strain), and cells of strain

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TABLE 1. Resistance of Variants of Strain STHE and Rous Sarcoma Virus Transformants (strain Schmidt—Ruppin) to CLA of Macrophages Activated by Various Immunomodulators (pooled data)

Cells tested	Control without activator	CLA (per cent) of macrophages activated by immunomodulators			
		levan	LPS	MDP	PMA
STHE (control)	4,1±0,1	16,6±2,4	18,7±0,3	22,4±0,6	23,6±4,8
(n=10)	—	(p<0,001)	(p<0,001)	(p<0,001)	(p<0,01)
STHE-LM-4 (n=3)	5,8±3,6	1,6±2,9	1,5±1,2	3,2±0,1	4,3*
STHE-LM-8 (n=3)	4,2±4,3	6,2±0,9	6,7±2,6	6,6±4,9	5,8*
STHE-75/18 (n=3)	—2,4±4,2	1,6±0,9	—4,5±5,5	—1,1±1,2	—2,6±1,7
TU-SR (n=6)	1,3±0,6	1,1±1,0	2,6±0,6	4,4±4,6	4,5±4,5
HET-SR-1 (n=4)	—0,3±0,6	4,5±0,1	0,8±0,6	3,3±1,5	4,5±0,8

Legend. n) number of experiments, asterisk indicates tested in one experiment.

TU-SR, obtained from a primary tumor induced in vivo in a newborn Syrian hamster with the aid of RSV-SR. By contrast with cells of the STHE strain with low malignancy, the STHE variants selected in vivo (STHE-LM-4, STHE-LM-8 STHE-75/18), and also the RSV transformants, selected in vivo (TU-SR) or transformed in vitro (HET-SR-1) nave a malignant phenotype (high levels of TGA and MA) [8, 9], are resistant to the action of H_2O_2, and secrete PGE_2 on contact with NK cells [8, 9]. Details of the method of obtaining the test strains of TC and their malignant characteristics were described previously [6-9]. The TC were maintained in culture on Eagle's medium with lactalbumin hydrolysate, 10% cow serum, and antibiotics, and underwent passage twice a week. Syrian hamster peritoneal Mph, obtained 5 days after stimulation (priming) with 3% medium with thioglycollate, and activated in vitro by various immunomodulators, were used as effectors in the CLT. Peritoneal exudate cells (PEC) obtained from the animals consisted to the of 65.6 ± 1.8% of Mph. The radioisotope CLT was carried out in 96-well flat-bottomed planchets [14]. The TC were labeled for 18-20 h with ^{3}H-thymidine (specific radioactivity 5 Ci/mmoles, dose 1 μCi/ml). For complete activation of the Mph in the well of the planchet containing Mph and TC in the ratio 20:1, throughout the procedure of the CLT the following immunomodulators were added (in a final concentration): polyfructosan levan (A. Kirchenstein Institute of Microbiology, Academy of Sciences of the Latvian SSR) 1 mg/ml, *E. coli* 026:B6 lipopolysaccharide (LPS; "Sigma," USA) 20 μg/ml, muramyl dipeptide (MDP) ("Boehring Diagnostics, USA) 10 μg/ml, and phorbol-12-myristate-13-acetate (PMA; "Sigma," USA) 2 μg/ml. Radioactivity of the samples was determined after 42 h, specific CLA being calculated by the standard method [2]. The results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULT

After washing to remove nonadherent PEC, 92 ± 3% of the cells used in CLT as effectors were Mph, and the remaining cells were lymphocytes. Cells of the STHE strain sensitive to the CLA of activated Mph were used in all the experiments as the positive control. There were two series of experiments. In the first, sensitivity of parental cells of the STHE strain and its malignant variant selected in vivo to the CLA of Mph was tested, whereas in the second series, the sensitivity of highly tumorigenic RSV transformants was tested. The results of the two series of experiments are summarized in Table 1.

During the investigation of the sensitivity of malignant variants of STHE (STHE-LM-4, STHE-LM-8, and STHE-75/18) to CLA of Mph, significant CLA of activated Mph was not found in any of the three experiments. The level of resistance of strains STHE-LM-4 and STHE-75/18 to the CLA of Mph was equally high: CLA of activated Mph did not exceed the level observed for inactivated Mph and is was significantly lower ($p < 0.001$) than the level of CLA of Mph with the control cells of strain STHE.

Hamster embryonic cells transformed by RSV — strains TU-SR and HET-SR-1 were found to be highly resistant to the CLA of activated Mph (in the last case, these were cells which had not gone through selection in vivo).

In no case had inactivated Mph (including those primed by thioglycollate) any CLA on the control STHE cells or on malignant variants of STHE selected in vivo (STHE-LM-4, STHE-LM-8, and STHE-75/18) or on tumorigenic RSV transformants (TU-SR and HET-SR-1).

The investigation described above is the next step in the study of the role of macrophages in tumor progression. We have shown that highly malignant cells with a high level of TGA and MA, resistant to the action of H_2O_2 and secreting PGE_2 [6, 8, 9], selected in vivo, also acquire high-resistance to the CLA of activated Mph. It is still not clear how important for survival and progression of tumors is the acquisition of resistance to the CLA of activated Mph by cells selected in vivo. Considering that this resistance to CLA of Mph can also be acquired by cells which have not gone through selection in vivo (as was shown in the present investigation for cells of strain HET-SR-1, which have a malignant phenotype — high levels of TGA and MA, resistance to H_2O_2, and secreting PGE_2 [8] — it is interesting to study the role of macrophages in the selection of tumor cells in vitro. At present we are studying the possibility of undertaking such selection in vitro with resident and activated macrophages.

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