INCREASED SENSITIVITY OF IN VITRO TRANSFORMED STHE CELLS TO STHE CYTOLYTIC ACTION OF RESIDENT AND ACTIVATED MACROPHAGES

E. A. Vol'pe

UDC 616-006.04-092.9

KEY WORDS: transformed cells, macrophages, natural resistance, immunomodulators, actinomycin D.

Macrophages (Mph) and other effector cells of the natural resistance system (NK cells, neutrophils) may have a cytolytic action (CLA) on tumor cells in vitro and in vivo. It has been shown that successful realization of CLA on tumor cells depends on activation of Mph by at least two signals (priming and trigger) [12]. The result of interaction of Mph and tumor cells evidently depends, on the one hand, on the level of cytolytic activity of the effector cells and, on the other hand, on the relative sensitivity of tumor cells to such activity. It was shown previously that experimental tumors of mice vary in their sensitivity to the CLA of Mph [9, 13, 16], and that progression of a tumor may be connected with the appearance of versions relatively more resistant to the CLA of Mph [16]. It has been shown [7] in a system of murine sarcoma WEHI 164, sensitive to the CLA of Mph that preliminary treatment of the tumor cells with actinomycin D (which inhibits DNA-dependent RNA-polymerase and, secondarily, protein synthesis) increases the sensitivity of target cells (TC) to the CLA of monocytes and Mph. In Philip's opinion [15], this may be connected with an increase in number of receptors for tumor necrosis factor.

The aims of the present investigation were: 1) to study the sensitivity of Siberian hamster cells with a low level of malignancy (strain STHE) to the CLA of resident and activated Mph, using a series of activators; 2) to attempt to increase the sensitivity of STHE TC to the CLA of Mph by preliminary treatment of the TC with actinomycin D (act D). The obtaining of data of this kind was an essential first step in the study of the possible role of resident and activated Mph in the selection of malignant variants of tumor cells and in tumor progression.

EXPERIMENTAL METHOD

Cells of the STHE strain (Syrian hamster embryonic cells spontaneously transformed in vitro), detailed characteristics of which were given previously [5], and with a low level of malignancy, were used as TC. A monolayer culture of STHE cells was maintained in vitro on Eagle's medium with lactalbumin hydrolysate and 10% bovine serum. Mph of intact noninbred male Syrian hamsters (resident Mph — RMph) or Mph primed by preliminary intraperitoneal injection of 3% medium containing thioglycollate (primed Mph — PMph), were obtained from the peritoneal exudate. The CLA of Mph was determined by the radioisotopic cytolytic test (CLT) [6]. The STHE TC were labeled briefly for 18-20 h with ^{3}H-thymidine (^{3}H) (specific radioactivity 5 Ci/mmole, dose 1 μCi/ml). Act D ("Calbiochem," USA) was added to the medium with the label (1 μg/ml) for the last 2 h of incubation of the TC with ^{3}H. Peritoneal exudate cells (PEC) obtained from the animals consisted of Mph to the extent of 40-74%. Enrichment of the PEC with macrophages was carried out by sedimentation in 96-well "Falcon" and "Linbro" planchets for 2 h. According to their morphological features, 93 ± 3% of adherent cells were Mph. To activate the Mph the following immunomodulators were used: the yeast complex Proper-myl ("Myl Laboratories," Italy) 300 μg/ml, polyfructosan levan (A. Kirchenstein Institute of Microbiology, Academy of Sciences of the Latvian SSR) 1 mg/ml, lipopolysaccharide (LPS) ("Sigma," USA) 20 μg/ml, muramyl dipeptide (MDP) ("Behring Diagnostics, USA) 10 μg/ml, PP-256, a derivative of 1,4-dihydropyridine (Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR) 200 μg/ml, and phorbol-12-myristate-13-acetate (PMA)

Laboratory of Antitumor Immunity, Research Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 1, pp. 59-62, January, 1991. Original article submitted June 5, 1990.

 0007-4888/91/0001-0080$12.50

TABLE 1. Composition of Peritoneal Exudate Cells (PEC) of Hamsters (%, $M \pm m$) after Injection of 3% Medium with Thioglycollate

PEC tested	Time after injection of thioglycollate, days		Neutrophils	Macrophages	Lymphocytes
Resident (control)	—	(n=6)	8,4±1,7	55,9±1,9	35,7±2,1
I Attracted	1	(n=6)	69,0±1,3	21,7±1,1	9,3±0,7
	2	(n=8)	49,2±1,2	39,0±0,9	11,7±1,2
	3	(n=8)	23,5±1,3	50,5±0,8	26,0±1,1
	4	(n=8)	13,5±0,9	52,9±1,1	33,6±0,7
	5	(n=11)	7,0±0,7	67,1±1,3	25,9±1,5
	6	(n=6)	8,7±0,7	53,7±1,1	37,7±0,8
II Resident (control)	—	(n=11)	1,9±0,8	56,4±3,3	41,6±3,5
Attracted	5	(n=13)	2,1±0,7	65,6±1,8	32,4±1,7

TABLE 2. Sensitivity of Cells of STHE Strain and CLA of Macrophages, Activated by Various Immunomodulating Preparations in Vitro (pooled data)

Activator[a]	Cytolytic activity (%)					
	resident	p	Mph	attracted by thioglycollate	p	Mph
Control without activator	5,0±1,5	—	(n=6)[b]	5,3±0,5	—	(n=25)
A. Proper-myl	2,4±1,0	n.s.[c]	(n=4)	18,2±5,4	<0,05	(n=4)
Levan	n.d.			16,6±2,4	<0,05	(n=10)
LPS	n.d.			21,8±1,8	<0,001	(n=15)
MDP	8,8±4,2	n.s.	(n=4)	25,4±1,6	0,001	(n=25)
B. PP. 256	n.d.			23,9±9,0	0,01	(n=5)
PMA	6,2±2,9	n.s.	(n=2)	25,4±1,6	0,01	(n=10)

a) A – bacterial, B – chemical; b) n denotes number of experiments, c) n.s – not statistically significant ($p > 0.05$), d) n.d. – no data.

("Sigma," USA) 2 μg/ml. Preliminary experiments showed that activators were dissolved when needed in the basic RPMI-1640 medium and added to washed Mph for the whole period required for the CLT to ensure their most complete activation [8]. After washing to remove unincorporated label the TC were added to Mph in the ratio of 1:20. Radioactivity of the samples was determined after 42 h and the specific cytolytic activity was calculated by the standard method [15]. Statistical analysis was performed by Student's test.

EXPERIMENTAL RESULTS

As a first step, to determine the optimal time of obtaining PEC, we studied the dynamics of attraction of Mph into the peritoneal cavity of individual hamsters by thioglycollate daily for 6 days after priming. As the data in Table 1 show, PEC of the hamsters consisted of neutrophils, Mph, and lymphocytes, and the maximal number of PMph (2/3 of the composition of PEC) was found on the 5th day after priming. The study of the PEC pool from several (3-7) animals gave similar results. The content of RMph in PEC did not differ significantly from that of PMph, and it averaged 56%. In the subsequent experiments, during tests of the CLA of Mph and the CLT, a pool of PEC obtained from 3-5 hamsters on the 5th day after thioglycollate priming was usually used.

The sensitivity of the STHE TC to the CLA of Mph was tested in 25 experiments. Depending on the experimental conditions, various bacterial and chemical preparations were used to activate Mph: either one or a set of several activators, which are known (depending on the schedule of administration) are known to possess immunomodulating properties [1, 2, 10]. These preparations, as the present writers and other workers showed previously, facilitate the secretion of active forms of oxygen by macrophages and neutrophils on contact with them [3, 11, 14]. The study of activator preparations on toxicity for STHE TC showed that the doses of activators which we used had no toxic action on TC. Smaller doses of activators activated Mph under our conditions for the CLT either weakly or not at all.

The pooled results of the study of sensitivity of STHE cells to the CLA of activated Mph are given in Table 2. They showed that in all cases only activated Mph (primed with thioglycollate in vivo and activated in vitro) had statistically significant

TABLE 3. Effect of Actinomycin D on Sensitivity of STHE Cells to Cytolytic Action of Resident and Activated Macrophages (pooled data)

Treatment of TC with actinomycin D (− or +)	Cytolytic activity (%) of macrophages.						
	without additional treatment of macrophages		p	after additional treatment with MDP	p	after additional treatment with PMA	p
−	Resident	6,6±3,1 (n=2)	–	9,9±2,2 (n=2)	n.s.*	4,5 (n=1)	
+	Resident	18,5±6,1 (n=2)	n.s.	20,0±5,4 (n=2)	n.s.	9,7 (n=1)	
−	Attracted by thioglycollate	9,6±3,2 (n=5)	–	22,9±4,0 (n=5)	<0,05	20,9±9,9 (n=3)	n.s.
+	Attracted by thioglycollate	36,1±11,5 (n=5)	<0,01	57,7±3,9 (n=5)	<0,001	56,0±13,3 (n=3)	<0,001

*n.s.) Not statistically significant ($p > 0.05$).

CLA on STHE cells. Depending on the level of activation of PMph, the test preparations did not differ significantly from one another. The most marked and stable activating action in this series of immmunomodulators was possessed by LPS and MDP. Resident Mph and PMph (not subjected to additional activation in vitro) did not develop significant cytolytic activity on STHE cells.

On incubation of TC with act D for 2 h a significant increase (two-threefold) in cytolytic activity of PMph (both activated by the 2nd signal and unactivated) on STHE cells was observed, and was reproduced in five experiments. The pooled data of this series of experiments are given in Table 3. Even though a twofold increase in the values of cytolytic activity of RMph on STHE cells treated with act D also was observed, this did not affect lysis of TC, for the level of cytolytic activity in this case did not exceed that of the unactivated PMph. In addition, after 42 h the CLT showed a very small increase in spontaneous release of the ^3H label from TC (from 17% on average in the control to 24.6% in STHE cells treated with act D).

The investigation thus showed that cells of the STHE strain, with low malignancy are highly sensitive to the CLA of activated but nonresident Mph; short-term treatment of the TC by actinomycin D makes them sensitive to the CLA of RMph and significantly increases the CLA of activated Mph. It is possible that the action of act D, in inhibiting protein synthesis, plays an essential role in the regulation of sensitivity of TC to the CLA of macrophages.

The author is grateful to Senior Laboratory Scientist L. M. Kashkina, Candidate of Biological Sciences, for reviewing the morphological picture of the PEC preparations.

LITERATURE CITED

1. I. G. Veksler, Éksp. Onkol., **3**, No. 4, 10 (1981).
2. E. A. Vol'pe, G. K. Zakenfel'd, and N. Z. Minchenok, Current Problems in Tumor Immunotherapy [in Russian], Part 1, Riga (1988), pp. 78-79.
3. E. A. Vol'pe, L. M. Kashkina, and M. O. Prilutskaya, Proceedings of the 7th Conference of Oncologists of Lithuania, Latvia, and Estonia [in Russian], Part 1, Vilnius (1990), pp. 57-58.
4. G. I. Deichman, Progress in Science and Technology. Oncology, Vol. 13 (Immunology of Tumors) [in Russian], Moscow (1984), pp. 46-97.
5. G. I. Deichman, T. E. Kluchareva, L. M. Kashkina, et al., Int. J. Cancer, **30**, 349 (1982).
6. A. Benomar, D. Gerlier, and J.-F. Dore, Int. J. Cancer, **38**, 419 (1986).
7. F. Colotta, G. Peri, A. Villa, and A. Matovani, J. Immunol., **132**, 936 (1984).
8. K. Frei, C. Siepl, P. Groscurth, et al., Eur. J. Immunol., **17**, 1271 (1987).
9. D. Gemsa, W. Kramer, I. Napierski, et al., J. Immunol., **126**, 2143 (1981).
10. A. G. Johnson and J. Regal, Springer Semin. Immunopathol., **8**, 347 (1985).
11. R. B. Johnston, Jr., C. A. Godzik, and Z. A. Cohn, J. Exp. Med., **148**, 115 (1978).
12. M. S. Meltzer, J. Immunol., **127**, 179 (1981).
13. K. M. Miner and G. L. Nicholson, Cancer Res., **43**, 2063 (1963).
14. M. J. Pabst and R. B. Johnston, Jr., J. Exp. Med., **151**, 101 (1980).
15. R. Philip, J. Immunol., **140**, 1345 (1988).
16. K. Yamashina, A. Fulton, and G. Heppner, J. Nat. Cancer Inst., **75**, 765 (1985).