Injection of MC thus leads to the development of immunodepression and a connected increase in the level of adrenocortical hormones (especially of free 11-HCS) in the blood plasma of mice 7 days after a single injection of various doses of the carcinogen. Considering the important role of free 11-HCS in the mechanism of the response to the specific components of the stimulus, the high glucocorticoid level in the recipients can be presumed to be one of the factors leading to the development of immunodepression under the influence of MC.

In the writers' opinion these data can be regarded as evidence that adrenocortical hormones play an important role in the genesis of immunodepression induced by a chemical carcinogen.

LITERATURE CITED

1. A. S. Belokhvostov, N. K. Zelenkova, V. P. Kalinovskaya, et al., Vopr. Onkol., No. 10, 66 (1976).
2. V. G. Vinter, Byull. Éksp. Biol. Med., No. 10, 68 (1968).
3. I. E. Kovalev, Vopr. Onkol., No. 9, 72 (1969).
4. E. A. Korneva, in: Mechanisms of Injury, Resistance, Adaptation, and Compensation (Abstracts of Proceedings of the 2nd All-Union Congress of Pathophysiologists) [in Russian], Vol. 2, Tashkent (1976), pp. 250-251.
5. E. M. Samundzhan, The Adrenal Cortex and Tumor Process [in Russian], Kiev (1973).
6. Yu. A. Umanskii, L. V. Yakimenko, and R. A. Semenova-Kobzar', in: Proceedings of an All-Union Conference on General Immunology and Antiviral Immunity [in Russian], Part 1, Moscow (1970), pp. 44-45.
7. N. A. Yudaev, Vest. Akad. Med. Nauk SSSR, No. 7, 12 (1969).
8. L. V. Yakimenko, in: Carcinogenesis, Methods of Diagnosis and Treatment of Tumors [in Russian], Kiev (1971), pp. 162-164.
9. H. Besedowski, E. Sorkin, M. Veller, et al., Proc. Soc. Exp. Biol. (New York), 150, 466 (1975).
10. P. Demoor and O. Steeno, Acta Endocrinol. (Copenhagen), 33, 297 (1960).
11. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).
12. H. Masaki, K. Takatsu, T. Himaoka, et al., Gann, 63, 633 (1972).
13. J. Stjernswärd, J. Nat. Cancer Inst., 35, 885 (1965).
14. J. Stjernswärd, J. Nat. Cancer Inst., 36, 1189 (1966).

THERMOSENSITIVITY OF SPECIFIC TRANSPLANTATION ANTIGENS OF THE TUMOR CELL MEMBRANE

T. E. Klyuchareva

UDC 616-006-092:612.014.43

A study of tumor specific transplantation antigen (TSTA) on the cell membrane of SV40-induced tumors and spontaneous hepatomas of inbred Syrian hamsters and also of monkey cells infected in vitro with tsA-mutants of SV40 virus demonstrated its high temperature sensitivity. Heating the cells to 56°C for 30-60 min led to the total loss of their immunogenic activity. Moreover, in animals immunized by tumor cells heated to 56°C, stimulation of growth of the test tumor cells was regularly observed.

KEY WORDS: tumors; transplantation antigens; immunogenicity; thermosensitivity.

Inactivation of tumor cells by heating followed by their use for experimental purposes for specific immunization against tumors has been a frequently used method of action on tumors. The loss of the immunogenic properties of tumor cells during the use of this method, as also of certain other physical or chemical methods of treatment of tumor cells (lyophilization, extraction, glutaraldehyde treatment) has been observed

---

Laboratory of Immunology of Tumors, Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 3, pp. 351-353, March, 1978. Original article submitted May 27, 1977.

0007-4888/78/8503-0378$07.50

TABLE 1. Thermosensitivity of TSTA in Irradiated Cells from a Tumor Induced in a Hamster by SV40 Virus and of a Spontaneous Hamster Hepatoma (results of transplantation test)

Immunizing material	Number of cells injected	Temperature of heating,* °C	Results of transplantation test for cells of test tumor					
			E-1			Gt-11B		
			log TD_{50}†	log IR‡	log PT**	log TD_{50}	log IR	log PI
Control	—	—	1,63	—	—	2,45	—	—
Cells of tumor induced by SV40 virus (E-1)	$2,1 \cdot 10^7$	37	>4,83	>3,2	—	2,44	0	—
		56	0,96	—	0,7	1,44	—	—1,0
Cells of spontaneous hamster hepatoma (Gt-11B)	$3,5 \cdot 10^7$	37	1,30	—0,33	—	>4,7	>2,35	—
		56	0,39	—	1,24	0,97	—	—1,48

*All samples of cells heated to this temperature for 30 min.
†Log TD_{50} – logarithm of 50% take dose of test tumor cells calculated by method of Reed and Muench [7].
‡Log IR – logarithm of index of resistance: difference between logarithms of TD_{50} in control and experiment.
**Log PI – logarithm of potentiation index difference between log TD_{50} in experiment and control.

TABLE 2. Thermosensitivity of TSTA Induced by SV40 Virus and Its tsA Mutants in Monkey Cells (results of transplantation test)

Expt.	Immunizing material	Number of cells injected	Temperature of heating, °C	Results of determination with SV40 test tumor†		
				log TD_{50}	log IR	log PI
1	Control	—	—	1,95	—	—
	Uninfected CV-1 cells	$3,0 \cdot 10^7$	37	2,46	0,51	—
	CV-1 cells infected with tsA-239 virus*	$2,8 \cdot 10^7$	37	>4,3	>2,35	—
			56	1,46	—	0,5
2	Control	—	—	1,46	—	—
	CV-1 cells infected with tsA-30 virus	$3,5 \cdot 10^7$	37	>3,90	>2,44	—
			56	0,90	—	0,56
	CV-1 cells infected with WTSV40 virus	$4,2 \cdot 10^7$	37	3,36	1,9	—
			56	4,2	2,7	—

*Before heating cells were infected with viruses for 45 h at 32°C.
†See legend to Table 1.

by several workers [3, 6, 5]. Moreover, some workers have observed that tumor growth is sometimes stimulated in animals immunized with lyophilized or heated cells [4].

In the investigation described below the effect of heat treatment on immunogenicity of the specific transplantation tumor antigen induced in monkey cells by SV40 virus and its temperature-sensitive (TS) mutants was studied.

EXPERIMENTAL METHOD AND RESULTS

Cells of a tumor of strain E-1, transplantable in vitro and induced by SV40 virus in a Syrian hamster of strain ICV, were removed from the glass with versene and heated in a water bath at two temperatures (37 and 56°C) for 30 min. The cells were then irradiated in a dose of 12,000 rad and injected into syngeneic Syrian hamsters in a known immunogenic dose. A culture of spontaneous hepatoma cells of hamsters of the same strain was subjected to the same treatment. The immunogenic activity of the tumor-specific transplantation antigens (TSTA) in cells heated to different temperatures was estimated by the transplantation test in the modification described earlier [1].

The results are given in Table 1. Injection of irradiated tumor cells heated to 37°C caused a high level of specific resistance in the hamsters, evidence of the presence of highly active TSTA on the cell membrane of both types of tumor cells. Heating the cells to 56°C led to total inactivation of TSTA both in the cells of the virus-induced sarcoma and in the spontaneous hepatoma cells, and injection of these cells into hamsters led to stimulation of growth of the test tumor cells. The phenomenon of stimulation of growth of these tumors, by contrast with antitumor immunity, was nonspecific in character, cells heated to 56°C caused increased growth of each of the transplantable test tumors.

To study the temperature sensitivity of TSTA induced in vitro by infection of a culture of green guenon kidney cells (strain CV-1) with SV40 virus, besides the wild-type strain of this virus (WT40) strains of tsA mutants of this virus defective for TSTA synthesis in hamster cells also were used. By the use of the latter it was possible to disregard the presence of thermostable SV40 virus in the cells tested. As the data in Table 2 show, heating cells infected with all variants of SV40 virus to 37°C for 60 min did not lead to loss of their resistance-inducing activity. Cells infected with strain WTSV40 and the tsA mutants, heated to 56°C for 30 min, immunized differently. Injection of cells infected with tsA mutants of SV40 virus and heated to 56°C did not induce specific resistance to subsequent injection of the test tumor cells in the hamsters. Heated cells, infected with the wild-type strain of SV40 virus induced a high level of specific resistance in the hamsters, which was evidently connected not with TSTA but with the presence of SV40 virus in them.

The study of TSTA specific for SV40 virus in cells transformed or infected by this virus, and also in spontaneous hepatoma cells of Syrian hamsters, demonstrated its high thermolability. The results are in contradiction with data demonstrating the thermostability of purified preparations of soluble TSTA, extracted from cell membranes in the presence of protease inhibitors [2]. The thermolability of TSTA on the cell membrane is perhaps connected with its rapid destruction by proteases during heating of the cells and the impossibility of restoring this synthesis in heat-inactivated cells.

LITERATURE CITED

1. Ya. M. Murka, Byull. Éksp. Biol. Med., No. 12, 11 (1965).
2. J. L. Anderson, R. G. Martin, C. Chang, et al., Virology, 76, 254 (1977).
3. J. H. Coggin, V. M. Larson, and M. R. Hilleman, Proc. Soc. Exp. Biol. (New York), 124, 774 (1967).
4. G. I. Deichman, T. E. Klychareva (Kluchareva), and W. L. Vendrov, in: Onco-Development Gene Expression, edited by W. H. Fishman and S. Sell, New York (1976), p. 337.
5. J. E. Morgan and C. P. Eng, Eur. J. Cancer, 8, 293 (1972).
6. M. D. Prager, S. Der, A. Swaan, et al., Cancer Res., 31, 1488 (1971).
7. L. Reed and R. Muench, Am. J. Hyg., 27, 493 (1938).