STIMULATION OF GROWTH AND ARTIFICIAL HETEROGENIZATION OF TUMORS BY VACUOLIZING VIRUS SV40 AND TYPE 16 ADENOVIRUS

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UDC 616-006-092.9-02:615.7]
-0.22.1:[576.858.5[576.858.6

New antigens of transplantation type have recently been induced by means of certain tumor-producing and infectious viruses in the cells of transplantable sarcomas produced by a clinical carcinogen. This phenomenon has been called artificial heterogenization of tumors [3, 4].

In the present investigation artificial heterogenization of cells by the action of virus SV40 and of type 16 adenovirus was studied. Stimulation of growth of tumors by vacuolizing virus SV40 and by Type 16 adenovirus was reported by the authors previously [1].

EXPERIMENTAL METHOD

The experimental animals and the tumors used have been described earlier [1]. In the present investigation, besides tumors induced by a carcinogen (BA-1 in Syrian hamsters and K-237 in C57 B1/6j mice), sarcoma 874 supplied by I. S. Irlin was also studied. This tumor developed from embryonic fibroblasts of a Syrian hamster transformed by polyoma virus in vitro. This tumor has now been subcultured both in tissue culture and in hamsters. Different numbers of tumor cells were infected with virus SV40 and with Type 16 adenovirus in vitro, the infected and control suspensions of tumor cells being incubated at 37° for 30 min. Cells of tumors whose growth had been stimulated by vacuolizing virus SV40 and by type 16 adenovirus were also used in the experiments. The presence of a new cell antigen in the heterogenized tumors was determined by transplantation into animals immunized with the corresponding virus. The animals were immunized by a single intraperitoneal injection of 2×10^6 $TCPD_{50}$ of virus SV40 and 2×10^2 TCD_{50} of type 16 adenovirus 9-12 days before injection of reacting doses of the cells. The animals were examined by palpation for the presence of tumors every three days.

EXPERIMENTAL RESULTS

It follows from the experimental results given in the table that new cell antigens appear in tumor cells treated in vitro with virus SV40. In the other experiment (see table), when the same treatment with virus SV40 was given to tumor cells of sarcoma 874 induced by polyoma virus, and containing transplantation polyoma antigen, new cell antigens specific for virus SV40 also were found. The differences between the rates of successful transplantation are statistically significant.

As the authors have demonstrated, stimulation of growth of sarcoma BA-1 cells in hamsters took place after injection of 2×10^6 $TCPD_{50}$ of virus SV40 into the heart 48 h after transplantation of tumor cells into the animals [1]. The results in the table show that a new cell antigen appears in the "stimulated" tumors. Resistance to transplantation of sarcoma BA-1 heterogenized with virus SV40 was reproduced with the cells of this tumor after two passages through unimmunized animals.

Quite different results were obtained when experiments of a similar type were carried out with sarcoma K-237 of C57 B1/6j mice; growth of the sarcoma was activated by type 16 adenovirus (see table). A noteworthy feature was the large weight of the tumors in animals immune to the virus which had been injected with 10^4 "stimulated" tumor cells. According to the mean data, they were more than 6 times heavier than tumors in the animals of the analogous control group. A combined assessment of all the tumors developing after injection of 10^5 and 10^6 cells showed a statistically significant difference, indicating resistance

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Heterogenization of Tumors by Virus SV40 and Type 16 Adenovirus

Type of tumor and animal	Tumor cells: infection: virus	Tumor cells: infection: method	Tumor cells: number	Growth of tumors in animals: infected with virus	Growth of tumors in animals: intact
Sarcoma BA-1 of Syrian hamsters	SV40	Treatment in vitro	10^3	0/8	6/8
			10^4	1/8	8/8
			10^5	3/8	8/8
	Not infected		10^3	4/8	3/8
			10^4	6/8	5/8
			10^5	8/8	8/8
Sarcoma BA-1 of Syrian hamsters	SV40	Infection of tumor carriers	10^3	0/5	2/5
			10^4	0/5	4/5
			10^5	2/5	5/5
	Not infected		10^3	3/5	2/5
			10^4	5/5	5/5
			10^5	5/5	4/4
Sarcoma 874 of Syrian hamsters	SV40	Treatment in vitro	10^3	0/9	9/10
			10^4	4/10	9/9
			10^5	8/8	10/10
	Not infected		10^3	8/10	9/9
			10^4	9/9	8/9
			10^5	10/10	8/8
				Mean weight of tumors	
Sarcoma K-237 of C57 bl/6j mice	Ad16	Infection of tumor carriers	10^4	8/10 5,8±1,84	8/10 0,9±0,3
			10^5—10^6	9/15 1,9±0,7	20/20 2,1±0,5

Note. Numerator—number of tumors developing, denominator—number of animals inoculated.

*Mean weight of tumors in grams (analysis by Lord's method).

of the animals of these groups immune to the virus to transplantation of the tumor. The weight of the tumors was essentially indistinguishable in the experimental and control animals. The results obtained show that in this particular experiment, during stimulation of tumor growth by adenovirus artificial heterogenization of the tumor cells apparently took place. The results described above confirm the previously observed heterogenizing action of polyoma virus [3, 5, 7, 8], the virus of herpes simplex [2, 5], and Sendai para-influenza virus [6] when used to infect in vitro the cells of tumors induced by a chemical carcinogen or by another virus [8]. The occurrence of artificial heterogenization of tumor cells after injection of virus SV40 into the blood stream 48 h after transplantation of tumor cells in further evidence of the possibility of obtaining artificial heterogenization in experiments in vivo [6]. The response of an animal immune to the

virus to heterogenized tumor cells does not necessarily lead to suppression of their growth. The results of passage of K-237 cells heterogenized by adenovirus through mice immune to the virus indicates that with certain quantitative relationships it is possible to obtain considerable acceleration of growth of the "heterogenized" tumor. Induction of new cell antigens by virus SV40 is known to accompany both the transformation of the cells in vitro and the appearance of tumors after neonatal infection of Syrian hamsters with SV40 virus. Evidently a similar process takes place when the virus acts on transplatable cells of sarcomas induced by chemical carcinogens or oncogenic viruses. It is important to note that no direct correlation exists between antigens detected by immunofluorescence and by the complement fixation reaction, on the one hand, and transplantation antigens on the other.

The possibility is not ruled out that, by analogy with lysogenic conversion of phages, both the induction of new cell antigens in tumors produced by oncogenic viruses and the artificial heterogenization of tumors of different origin may be connected with introduction of additional genetic information into the genome of normal or tumor cells.*

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*The authors are grateful to Dr. Med. Sci. G. Ya. Svet-Moldavskii for directing this research and they are also indebted to Professor N. P. Mazurenko for his interest and his valuable critical remarks.