ARTIFICIAL HETEROGENIZATION OF MALIGNANT TUMORS.

SECOND PRINCIPLE OF IMMUNOLOGICAL TREATMENT OF TUMORS

(UDC 612.6.054.017.1)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 9,
pp. 85-88, September, 1965
Original article submitted June 4, 1964

For decades investigators have attempted to demonstrate specific antigens in tumors in order to use these antigens for immunological treatment of those tumors [1]. In most cases the elicited antigens were not specific for tumors but proved to be antigens of tissue incompatibility. It was recently shown that both antigen simplification and the appearance of new antigens occur during malignant degeneration [1, 10, 12]. It was established that these antigens, especially in tumors induced by carcinogens, are weak; therefore, the possibility of a therapeutic immunological effect on natural tumor antigens is quite small.

We proposed a different approach—artificial heterogenization of tumors [5]—and we will give an account of its experimental substantiation below. Into an organism with a tumor are introduced substances or biological agents which selectively accumulate or multiply in tumors and create in some manner new antigen determinants in the tumor cells. These determinants are immunologically affected by actively acquired or passively introduced antigens or immune lymphoid cells. More than substances, "infectious" and "tumorogenic" viruses and bacteria, rickettsia and protozoa hold promise as agents causing heterogenization of tumor cells. The numerous experiments carried out in our laboratory on heterogenization of tumors by antigens of staphylococci and salmonella yielded a weak positive effect. In the present work we studied the artificial induction of new tumor antigens by "infectious" and "tumorogenic" viruses.

EXPERIMENTAL

In the experiments, we used herpes simplex virus (strain El 2 passed for a long time in a chick embryo tissue culture and weakly pathogenic for mice. Its titer was 10^4 PFU*/ml. The strain was presented to us by A. A. Shatkin. SE-polyoma virus, obtained from S. Stewart, was passaged in a mouse embryo tissue culture. The culture fluid contained 4960 hemagglutinating units per milliliter. The experiments were carried out with sarcoma 237 induced by dimethylbenzanthracene in our laboratory in mice of the C57BL line; the sarcoma cells underwent 2-5 passages in this line. The sarcoma was gound without trypsinization. The cells were filtered through gauze, mixed with the appropriate undiluted virus and inoculated into the animals in various doses (10^4, 10^5, 10^6 cells). The groups of mice were doubly immunized with live herpes, vaccinia and polyoma viruses. The tumor cells were inoculated a month after immunization.

RESULTS

The sarcoma 237 cells were mixed with herpes simplex virus and inoculated into the mice, then the cells of the grown tumor were again mixed with the herpes virus and again inoculated into the mice. This procedure was repeated three times. Then the herpes-infected tumor cells were inoculated in doses of 10^4, 10^5, and 10^6 cells into fresh mice and mice immunized by herpes simplex and vaccinia viruses. We see from Table 1 that the growth of tumors treated with herpes simplex virus is inhibited in the herpes-immune mice and that this phenomenon is specific.

*Plaque-forming units

TABLE 1. Heterogenization of Tumor Cells by Herpes Simplex Virus

Mouse group	No. of tumor cells infected with herpes simplex virus			Number of noninfected tumor cells		
	10^4	10^5	10^6	10^4	10^5	10^6
Immune to herpes simplex virus	3/14	6/15	15/15	14/14	14/14	14/14
Immune to vaccinia virus	14/14	13/14	14/14	Not investigated		
Not immune	14/14	12/14	14/14	12/12	14/14	13/14

Note: In this and the following table the total number of mice inoculated with tumor cells is indicated in the denominator and the number of mice in whom tumors developed is indicated in the numerator.

TABLE 2. Induction of "Polyoma" Antigen in Sarcoma 237 Caused by a Carcinogen

Mouse group	No. of tumor cells infected with polyoma virus			Number of noninfected tumor cells	
	10^4	10^5	10^6	10^4	10^5
Immune to herpes simplex virus	0/18	4/20	19/19	18/19	20/20
Immune to vaccinia virus	20/20	18/19	20/20	Not investigated	
Not immune	20/20	20/20	19/20	17/19	19/20

In the following experiments the sarcoma 237 cells were mixed in vitro with SE-polyoma virus. After contact for two hours the cells were precipitated by centrifugation, after which the supernatant and cells were resuspended in Earle's solution. The grown tumors were again ground, treated with polyoma virus and in doses of 10^4, 10^5, and 10^6 cells, transplanted to mice immunized with polyoma or vaccinia virus and to the nonimmune control mice in which the absence of antibodies was checked by the hemagglutination-inhibition test with polyoma virus. We see from Table 2 that the growth of sarcoma 237 infected with polyoma virus is markedly inhibited in the polyoma-immune mice. Artificial heterogenization of tumor cells by herpes simplex and polyoma viruses, as clearly evidenced in these experiments, is proved by the passive transfer of inhibition by cells of the lymph nodes.

Fresh C57BL mice were inoculated with sarcoma 237 treated with polyoma virus in a dose of 10^4 cells per mouse. After 48 h, half of the mice were injected with lymphoid cells from mice of the same line actively immunized with polyoma virus in a dose of 10^4 lymphoid cells intravenously and 10^6 intraperitoneally; the other half of the mice were injected with the same number of lymphoid cells from mice immunized with vaccinia virus. Tumor growth in these groups was noted in 3 mice out of 14 and in 14 out of 14, respectively. These experiments attest to the possibility, in principle, of exerting an immunological effect on an artifically heterogenized tumor. Artificial heterogenization of the tumor cells apparently takes place in the described experiments, and the specific immunity has an inhibiting action on the newly induced antigens in the tumor cells.

It is usually considered [7, 8] that the primary cell reaction to virus multiplication is proliferation or destruction. We proposed [2, 3] that the most common type of primary cell reaction to virus is heterogenization of the cells in regard to other structures of the organism. The new data on antigens induced in cells by polyoma, SV_{40} and leukemia viruses indicate from this point of view partial manifestations of this general regularity. The experiments on artificial heterogenization of tumors by herpes simplex virus confirm the position that natural heterogenization of a cell is not only a feature of tumorogenic viruses but is also the basic primary cell reaction to virus [2, 3]. The inflammatory infiltration in viral infections of mammals and birds is a reaction to the virus-altered cells [4].

The next task of greatest importance is to produce artificial heterogenization of a tumor already grown in an organism and to immunologically treat it. For this purpose we must make an extensive study of artificial heterogenization by the most diverse biological agents. It is quite probable that during viral oncolysis the cells remaining stable will prove to be heterogenized in a number of cases.

The possibility of artificial heterogenization of tumors by SV_{40} viruses was recently demonstrated in our laboratory.

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