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Protective Effect of Immunization with Virus-Infected Glioma Cells against Intracerebrally Implanted Glioma in Mice*

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Abstract—*The effect of immunization with virus-infected tumor cells and uninfected tumor cells was studied in mice with intracerebrally implanted glioma. Immunization with membranes prepared from influenza virus-infected tumor cells (MVT) showed a higher protective effect against subsequent intracerebral challenge with the same tumor cells than that with membranes prepared from uninfected tumor cells (MT). Immunization of tumor-bearing mice with MVT resulted in marked prolongation of survival time and, in some mice, in complete regression of tumor. A delayed hypersensitivity test showed that the induced immunity by MVT was specific to this tumor.*

INTRODUCTION

IMMUNIZATION with tumor cells induces a specific immunity against subsequent challenge of the same tumor cells [1]. Several investigators have shown that the immunogenicity of the tumor cells was considerably increased by the infection of the tumor cells with virus and that the immunization with virus-infected tumor cells induced a higher degree of immunity than did immunization with uninfected tumor cells [2–7].

As the brain is devoid of lymphatic vessels, the immune resistance is generally weak against intracerebrally implanted tumor [8], but the immunization with tumor cells affords some degree of resistance to intracerebral tumor allografts [9,10]. The present experiments were undertaken to determine whether the immunization with virus-infected tumor cells induces an enhanced degree of immunity against intracerebral challenge of glioma cells and whether such an immunization would also be effective in suppression of the growth of preexisting glioma in the brain.

MATERIALS AND METHODS

Animals

C57Bl/6 mice of both sexes, aged from 8 to 12 weeks, were used. All mice were bred in our laboratory from stock which had been purchased from the Shizuoka Experimental Animal Center.

Tumors

Methylcholanthrene-induced mouse glioma (203-glioma), which has been passed serially in C57B1/6 mice by subcutaneous transplantation, was kindly provided by Dr. Y. Ishida, Dept. of Pathology, Gumma University, Japan. A monolayer culture cell line was established and maintained in our laboratory, as described in a previous paper [11].

Intracerebral implantation of 203-glioma cells

A single-cell suspension was prepared by trypsinization of the cultured cells with 0.25% of trypsin. The mice were anesthetized with pentobarbital and 0.01 ml of the suspension was administered intracerabrally using Hamilton's microinjector with Yaoi's needle in the middle of the right eye and right ear.

Viruses

The WSN strain of influenza virus, which had been adapted to the mice by serial passage in the brain of suckling mice, was kindly provided by Dr. C. W. Boone, NIH, U.S.A. The seed virus was propagated by allantoic inoculation into 10-day-old embryonated eggs.

Preparation of cell membranes and soluble extracts from 203-glioma cells

Membranes from virus-infected 203-glioma

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cells (MVT) and uninfected 203-glioma cells (MT) were prepared according to the methods of Boone and Blackmann [5]. MVT was irradiated using a u.v. lamp to inactivate the residual viruses. These preparations were used for immunization. Soluble extracts from 203-glioma cells were prepared by extraction with 3M KCl according to the methods described by McCoy *et al.* [12], and were used for delayed hypersensitivity test. These extracts contained 4.5 mg/ml of protein as measured by Lowry *et al.*'s methods [13]. The reactivity of MT-immunized mice to these extracts was confirmed by delayed hypersensitivity tests.

Delayed hypersensitivity tests

Soluble extracts (0.04 ml), at a dilution of 1:50 or 1:100, was injected into a footpad of MVT-immunized, tumor-bearing or control mice. Thickness of the footpad was measured before and 48 hr after injection.

RESULTS

Effect of immunization with membrane preparations on the tumor growth

The minimum lethal dose of 203-glioma cells was determined by the intracerebral implantation of various numbers of the tumor cells. The mice inoculated with 5×10^5 or more of tumor cells all died (Table 1). The effect of immunization with MVT or MT was tested by subsequent intracerebral challenge of the same tumor cells. Systemic immunization with both preparations protect mice from subsequent intracerebral challenge of the same tumor cells (Table 1). Furthermore, the results obtained by immunization with diluted membrane preparations clearly showed that the immunization with MVT was more protective against subsequent intracerebral challenge of the tumor cells than was the immunization with MT (Table 1).

Effect of immunization with membrane preparation on the survival of tumor-bearing mice

Mice were implanted with 5×10^5 or 1×10^6 of 203-glioma cells intracerebrally and then immunized with MVT or MT 5 or 10 days later. Regression of the tumor and marked prolongation of the survival time were observed only in mice which had been immunized with MVT 5 days after intracerebral implantation with 5×10^5 of the tumor cells (Table 2; Fig. 1).

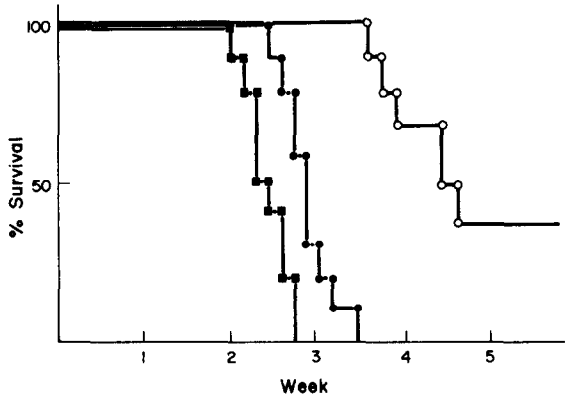

Fig. 1. The effect of immunization on the survival of 203-glioma-bearing mice. 5×10^5 of 203-glioma cells were implanted intracerebrally and 5 days later mice were immunized with membranes prepared from virus-infected 203-glioma cells (○—○) or membranes prepared from uninfected 203-glioma cells (●—●). One group of control was injected with saline (■—■).

Table 1. Protective effect of immunization against subsequent intracerebral challenge of 203-glioma cells

Immunized with:*	Dilution of MT and MVT	No. of cells challenged 2×10^6	1×10^6	5×10^5	2×10^5	1×10^5
Control		0/15§, ‖	0/15	0/15	3/5¶	2/5
MT†	×1	7/10	9/10	10/10		
	×2	3/10				
	×5	0/10				
MVT‡	×1	10/10	10/10	10/10		
	×2	10/10				
	×5	10/10				

*Mice were immunized 14 days before intracerebral challenge of tumor cells.
†Membranes prepared from uninfected tumor cells.
‡Membranes prepared from virus-infected tumor cells.
§No. of mice surviving/No. of mice tested.
‖All mice died within 3 weeks.
¶Mice surviving throughout the 8 week experiment.

Table 2. Effect of immunization on the survival of 203-glioma-bearing mice

Immunization	Challenge dose of glioma cells	No. of survivors at the 8 week Days after implantation 5	10
M.V.T.*	5×10^5	3/10‡	0/10
M.V.T.	1×10^6	0/10	0/10
M.T.†	5×10^5	0/10	0/10
M.T.	1×10^6	0/10	0/10

*Membranes prepared from virus-infected 203-glioma cells.
†Membranes prepared from uninfected 203-glioma cells.
‡No. of mice surviving / No. of mice tested.

Table 3. Delayed hypersensitivity test in immunized and tumor-bearing mice

Mice	Response ratio* Dilution of soluble extract† ×50	×100
Mice immunized with 1:10 dilution of MVT ($n = 10$)	204 + 12.2	189 + 9.30
Mice immunized with 1:50 dilution of MVT ($n = 10$)	201 + 16.5	174 + 11.5
Mice with early tumor‡ ($n = 10$)	163 + 7.30	N.T.
Mice with advanced tumor§ ($n = 10$)	138 + 9.29	N.T.
Untreated mice ($n = 10$)	106 + 2.37	106 + 2.37

*(Thickness of footpad 48 hr after injection/thickness of footpad before injection) ×100.
†Prepared from 203-glioma cells with 3M KCl as described in materials and methods.
‡Two weeks after subcutaneous implantation of 203-glioma cells.
§Eight weeks after subcutaneous implantation of 203-glioma cells.
n:No. of mice in each group.
N.T.:Not tested.

Delayed hypersensitivity test

Mice similarly immunized with MVT were tested by delayed hypersensitivity test with 3M KCl extracts from 203-glioma cells. As shown in Table 3, a positive reaction occurred in all the immunized mice. No reaction was observed in control mice tested with the same dilution of the extracts. These results show that immunization with MVT induced a delayed hypersensitivity to the tumor extracts and that this hypersensitivity was specific to 203-glioma cells. Tumor-bearing mice also showed a positive reaction with the extracts; this reaction was weaker than that seen in immunized mice and appeared to be suppressed with the growth of the tumor.

DISCUSSION

In the present experiments, the systemic immunization with membranes prepared from 203-glioma cells induced an effective immunity against subsequent intracerebral challenge of the same tumor cells. Such an effect was significantly enhanced when the membranes were prepared from 203-glioma cells infected with influenza virus.

Tumors in the central nervous system behave differently from those in other sites of the body. Complete and permanent regression in malignant glioma has not been documented, in contrast to tumors in other anatomical locations, and malignant glioma rarely metastasized from the brain to other sites of the body, whereas other tumors often invade the brain. One explanation is that the brain is an immunologically privileged site [14]. Subsequent experiments showed, however, that intracerebral isotransplants or allotransplants in specifically sensitized animals were rejected and that the animals implanted with tumor cells and then irradiated were resistant against subsequent intracerebral challenge of the same tumor cells. These results suggest that the brain is not an altogether completely privileged site [9,10]. Our results also showed that the systemic immunization was effective for the regression

of intracerebrally implanted glioma cells; however, this effect may be ascribed to the artificial destruction of the blood–brain barrier rather than to an incompleteness of the barrier. In view of the finding that lymphocytic infiltration is often seen in and around the brain tumor and that the prognosis of the patients with brain tumor depends on the degree of the lymphocytic infiltration [15], the blood–brain barrier may not be entirely operative in patients with brain tumor. Thus, such an immunological treatment may be appropriate for the prophylactic therapy against the recurrence of the brain tumor in patients after surgical treatment.

Numerous attempts have been made to treat cancer patients by specific immunization with tumor cells; however, results appear to be disappointing. The weak antigenicity of the tumor cells and impaired immune function may be responsible. Thus, the question is raised as to whether the specific immunization can suppress the growth of pre-existing tumor by the enhancement of the antigenicity by viral infection. In the experiments reported herein, immunization of tumor-bearing mice with MVT resulted in marked prolongation of the survival time and, in some mice, complete regression of the tumor, whereas the immunization with MT prolonged to some extent the survival time.

The mice implanted intracerebrally with 203-glioma cells died within 3 weeks, whereas subcutaneous implantation with the same number of the tumor cells resulted in over 8 weeks survival. The death at an earlier stage in the former may be ascribed to increased intracranial pressure, as is often the case in patients with brain tumor. It was found in our laboratory that the specific killer T-cell function to 203-glioma cells was maximally increased at 2 weeks after implantation of 203-glioma cells and decreased with the growth of the tumor [11]. These support the possibility that patients with brain tumor could be effectively treated by specific enhancement of immunological activity at the earlier stage of the disease.

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