Effect of a Brain Cell Suspension from Mice of Different Age on the Growth of Tumors Transplanted under Kidney Capsule

N. I. Lisyanyi, A. P. Romodanov, G. M. Oleinik, and O. V. Markova

UDK 612.82.014.2.017.1]06

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 2, pp. 195–197, February, 1993
Original article submitted June 26, 1992

Key Words: *brain; antitumor activity*

Like immunocytes, brain cells are known to express lympho- andmonokine receptors and to synthesize immunoregulatory molecules.Individual subpopulations of brain cells are able to realize antigenpresentation, as well as to regulate the function of neighbouring cells [1,5-9,11-13]. However, the collaboration of brain cells in the cooperative processes of immunogenesis has not been studied in depth, and their immune functions, including the possible antiproliferative effect of individual brain cell populations have not been defined.

The aim of this work was to study the antitumor activity of brain cells at different stages of the ontogeny (embryonic, newborn, and adult organism).

MATERIALS AND METHODS

The investigations included two main parts: a) an assay of natural killer activity *in vitro* using K-562 target cells; b) assessment of tumor transplant growth under mouse kidney capsule *in vivo* with or without the addition of brain cells. A cell suspension was obtained from the brain of 18-20-day-old embryos and from newborn and adult CBA mice after Moskona [10]. The brain of the decapitated animals was aseptically placed in medium 199, cut with scissors into small pieces, incubated in 0.25% trypsin solution, and further disaggregated by passage through a syringe with needles of progressively smaller diameter. The resulting cell suspension was washed twice in medium 199 supplemented with 3% bovine serum. Cell viability was determined by staining with 0.1% Trypan Blue. Mouse lung adenocarcinoma and human glial tumors were transplanted under kidney capsule after Bogden [4]. The tumor tissue was cut into 1 mg pieces, which were implanted under mouse kidney capsule under hexenal anesthetic. The operations were carried out under sterile conditions under an MBS-1 stereomicroscope. An amount of 0,02 ml brain cell suspension (4×10^6 cells) was injected under the capsule adjacent to the tumor graft. The results were estimated seven days later by evaluation of the percent inhibition of graft growth in the experimental group as compared with the control. The natural killer activity of the brain cells was assayed in a 4-hour cytotoxicity test using ^{52}Cr-labeled K-562 target cells [2]. The effector-target ratio was 10:1. The reliability of the differences between the relative values was estimated by Student's t test.

RESULTS

The growth of tumor allo- and heterotransplants was reliably inhibited in the presence of mouse brain cell inoculate. In both cases the highest level of inhibition was produced by brain cells from embryos and neonates, the level of tumor growth inhibition being equal

Laboratory of Neuroimmunology, Research Institute of Neurosurgery, Kiev

0007–4888/93/0002–0199$12.50

TABLE 1. Effect of a Brain Cell Suspension from Mice of Different Age on the Growth of Tumor Allo- and Heterografts ($M \pm m$)

Donors of brain cells	Lewis lung carcinoma				Human brain glioma			
	n	tumor weight, mg	I	p	n	tumor weight, mg	I	p
Control	3	3.86±0.19			24	1.6±0.1		
18–20-day embryos	3	1.80±0.11	53.4	<0,01	18	0.30±0.04	82.0	<0.001
Neonates	3	1.93±0.18	50.0	<0.01	1	0.74±0.09	56.6	<0.01
Adults	3	3.43±0.13	11.2	>0.5	12	1.38±0.15	19.4	<0.5

Note: n – number of recipients per group. I – percent inhibition, compared with control group.

to or more than 50%. The adult brain cells exhibited a weak inhibitory effect. The growth of heterografts was inhibited by 20%, while that of allografts was inhibited only by 10%. Thus, mouse brain cells possess antitumor activity and inhibit both allo- and heterograft growth under the kidney capsule, young brain cells manifesting higher antitumor activity (Table 1).

The antiproliferative activity of newborn mouse kidney, spleen, and liver cells was studied in order to clarify whether the tumor-inhibiting capacity is a specific feature of brain cells. The spleen and liver are considered to be immunocompetent organs, and their connective tissue consists largely of phagocytosing elements called cells of the reticuloendothelial system (RES). The results of the tests are shown in Table 2. Only spleen cells of neonates were shown to retard allo- and heterograft growth, but to a lesser degree than brain cells. Thus, the antitumor activity inherent in neonate brain cells is not characteristic of liver and kidney cells and is not related to the admixture of elements of circulating blood cells present in the brain, liver, and kidney tissue at the moment of decapitation. The brain antitumor activity exceeded that of the cells of the spleen - the main organ of immunity, contaning all types of cellular components of the immune system.

Taking into account that the liver contains large quantities of connective-tissue elements belonging to the RES, and that the liver cells lacked antitumor activity in our experiments, one may assume that the activity exhibited by the brain cell populations should be attributed to the cells of neurogenic origin (neuroglia, neurons), not to the cells of the connective tissue and/or endothelium. The weak antitumor activity of adult brain cells might be due to two factors: the disappearance of this activity in adults or a decrease in the relative amount of specific cells possessing such activity. In this connection, experiments *in vivo* with varying doses of brain cell inoculate were performed (Table 3). It was shown that a tenfold increase in adult brain cells failed to inhibit allo- and xenograft growth. However, a tenfold reduction of injected brain cells of neonates led to a decrease of antitumor activity.

TABLE 2. Effect of Liver, Kidney, and Spleen Cells from Newborn Mice on the Growth of Tumor Allo- and Heterografts ($M \pm m$)

Source of cells	Lewis lung carcinoma				Human brain glioma			
	n	tumor weight, mg	I	p	n	tumor weight, mg	I	p
Control	3	7.83±0.48			4	1.32±0.06		
Liver	3	7.47±0.27	4.6	>0.5	3	1.10±0.06	16.6	>0.5
Kidney	3	6.73±0.19	14.0	>0.5	3	1.13±0.09	14.4	>0.5
Spleen	3	4.73±0.22	39.6	<0.01	3	0.87±0.05	34.1	<0.5

Note. n – number of recipients per group. I – percent inhibition, compared with control group.

TABLE 3. Effect of Dose of Brain Cells from Newborn and Adult Mice on the Growth of Tumor Allo- and Heterografts ($M \pm m$)

Source and number of cells	Lewis lung carcinoma				Human brain glioma			
	n	tumor weight, mg	I	p	n	tumor weight, mg	I	p
Control	3	7.83±0.48			4	1.32±0.06		
Newborn mice								
4×10^6	3	4.43±0.19	43.4	<0.01	3	0.73±0.03	44.7	<0.01
4×10^5	3	6.23±0.15	20.4	<0.5	3	0.80±0.05	39.4	<0.01
Adult mice								
4×10^6	3	6.75±0.16	13.8	>0.5	3	1.11±0.06	15.9	>0.5
4×10^7	3	6.30±0.23	19.5	>0.5	3	0.95±0.13	28.6	>0.5

TABLE 4. Natural Killer Activity of Mouse Brain Cells

Donors of brain cells	Number of trials	Cytotoxic effect, %	p
Mouse embryos	9	10.2±1.4	<0.05
Newborn mice	12	9.7±1.8	<0.05
Adult mice	10	1.2±0.6	

Thus, first of all, the newborn brain cell-mediated inhibition of tumor grafts under the kidney capsule is dose-dependent. Secondly, this capacity (i.e., to retard tumor growth) is significantly diminished in the cells of the adult brain, and/or this capacity is lost in the course of preparation of a brain cell suspension from adults. The brain cells of embryos and neonates are not so intricately connected with each other via processes as are adult cells and can be easily suspended in a cell culture [3]. For more information about the nature of the brain cell antitumor activity, a cytotoxicity assay *in vitro* was performed, using ^{51}Cr-labeled K-562 target cells (Table 4).

The embryonic and newborn brain cells proved to possess killer activity. The brain cells of the adult animals showed no killer effect in this assay.

Thus, it has been shown that brain cells possess antiproliferative activity both in an *in vivo* test of tumor growth under the kidney capsule and in an *in vitro* direct cytotoxicity test evaluating natural killer activity. The antitumor activity is mostly associated with the brain, because it is lacking in the case of kidney and liver cells and is only weakly manifested by splenocytes. It is therefore reasonable to assume that the antitumor activity revealed in our experiments is a specific feature of cells of neural origin rather than of RES cells.

The low antitumor activity of the adult brain cells may be connected with the loss of this capacity by brain cells and might explain the increase in the number of brain tumors with advancing age. Although the nature of the cells and the mechanism of their antitumor activity have not yet been elucidated, still the data obtained indicate that the brain cells play a part in "local" immune reactions, including antitumor resistance within the brain, where the systemic immune surveillance is limited due to the blood-brain barrier.

REFERENCES

1. V. A. Berezin, *Neirokhimiya,* **9**, № 1, 114-124 (1990).
2. N. I. Lisyanyi, O. V. Markova, and E. N. Zhmareva, *Eksp. Onkol.*, № 3, 57-59 (1988).
3. V. P. Bozhkova, L. A. Brezhestovskii, V. M. Buravlev, *et al., Manual of Neural Tissue Culture. Methods. Techniques. Problems,* [in Russian], Moscow (1988).
4. A. B. Bogden, W. R. Cobb, D. L. Lepage, *et al., Exp. Cell Biol.*, **47**, № 4, 281-293 (1979).
5. D. W. Dickson, *Amer. J. Pathol.*, **135**, № 1, 135-147 (1989).
6. W. L. Farrar, P. Kilian, M. R. Ruff, *et al., J. Immunol.*, **139**, № 2, 459-463 (1987).
7. K. Frei, C. Siepl, P. Groscurth, *et al. Europ. J. Immunol.*, **17**, 1271-1278 (1987).
8. U. V. Malipiero, K. Frei, and A. Fontana, *J. Immunol.*, **144**, № 10, 3816-3821 (1990).
9. R. J. Morris, *Dev. Neurosci.*, **7**, 133-160 (1985).
10. A. A. Moskona, *Intern. Rev. Pathol.*, **1**, 371-428 (1962).
11. S. Schuller-Petrovic, W. Gebhart, H. Lossman, *et al., Nature,* **306**, № 5939, 179-181 (1983).
12. D. L. Tweardy, E. M. Glazer, P. L. Mott, *et al. J. Neuroimmunol.*, **32**, № 3, 269-273 (1981).
13. M. N. Woodrofe, G. M. Hayes, and M. L. Curner, *Immunology,* **48**, № 3-4, 369-375 (1989).