tumor and native cells and between the number of type I and II epithelial cells in affected and unaffected tissue. These findings point at homeostatic stability of epithelial-mesenchymal structures in breast carcinoma, prompting the search for new approaches to its regulation.

REFERENCES

1. E. A. Dikshtein, Yu. P. Burlak, and N. I. Shevchenko, *Arkh. Pat.*, No. 6, 61-67 (1985).
2. E. A. Dikshtein, I. V. Vasilenko, N. M. Rovenskaya, *et al.*, *Ibid.*, No. 11, 51-57.
3. A. E. Dorosevich, *Ibid.*, No. 4, 11-16 (1988).
4. L. I. Dubenskaya and S. M. Bazhenov, *Ibid.*, No. 4, 40-43 (1992).
5. T. B. Zhuravleva, *Proceedings of the Leningrad Scientific Society of Pathologists* [in Russian], No. 23, Leningrad (1981), pp. 155-158.
6. V. I. Kazanin, *Classification of Cell Reactions in Pathology* [in Russian], Moscow (1983).
7. J. Crocker and P. Nar, *J. Pathol.*, **151**, 111-118 (1987).
8. J. Crocker, D. Boldy, and M. Egan, *Ibid.*, **158**, 185-188 (1989).
9. A. Wendy and S. Antony, *Hum. Pathol.*, **20**, 741-746 (1989).

Distribution of Lectin Receptors on the Plasma Membranes of Brain Glioma Cells and Autologous Peripheral Blood Mononuclear Cells as a Function of the Degree of Anaplasia

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 122, No. 10, pp. 441-445, October, 1996
Original article submitted March 1, 1995

The content of the D-mannose-specific LCL-receptor that binds D-mannose-containing lymphokines (interleukin-1 and interleukin-2) is proportional to the degree of glioma anaplasia. This may reflect the mechanism whereby brain glioma utilizes lymphokines to proliferate and to escape immunologic surveillance.

Key Words: *gliomas; carbohydrate receptors to lectins; immunologic surveillance*

There is considerable evidence indicating that the structure of plasma membrane glycoproteins and glycolipids changes during cell growth, differentiation, and malignization [5,12]. A tendency toward the loss of N-acetylneuraminic acid and N-acetylglucosamine and an increase in the amount of D-galactose-specific receptors on the surface of tumor cells irrespective of tumor origin has been documented [5,12]. Receptors containing N-acetyllactosamine, mannose, and L-fucose were identified on metastatic tumors [5,12].

Glycoconjugates of cell membranes can be obtained with the use of lectins, nonimmune proteins capable of binding to simple and complex antigenic determinants [5]. It should be noted that the regulatory effects of lymphocytes are realized via lymphokines and carbohydrate-containing surface receptors [1]. It was found that the effect of lymphokines stimulating lymphocyte proliferation (IL-1 and IL-2) is determined by terminal D-mannose [9,14]. D-mannose is a constituent of the CD4 receptor of T helpers [11], while lymphocytes expressing the dis-

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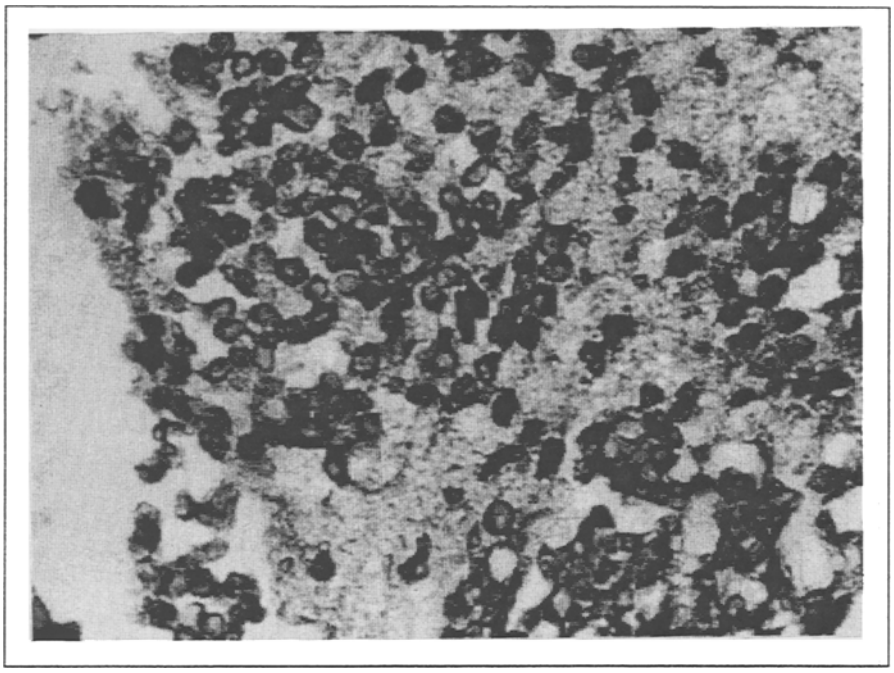

Fig. 1. Anaplastic glioma. Histotopography of SBA receptors. The receptors are located on juxtanuclear membranes of tumor cells. Paraffin section. Direct immunoperoxidase method, counterstaining with methyl green. ×400.

accharide N-acetylneuraminic acid—N-acetylglucosamine reacting with wheat-germ agglutinin (WGA) act as suppressor cells [3]. Cells expressing N-acetylgalactosamine reacting with *Vicia villosa* agglutinin exhibit antisuppressor activity [8].

It should be noted that N-acetylgalactosamine that binds to soybean agglutinin (SBA) is present on the membranes of highly malignant tumor cells [5] and on lymphocytes with antisuppressor activity [8], while the expression on WGA receptors (markers of suppressor cells) is reduced in a number of tumors [5,12].

Study of the relationship between lectin receptors, which reflect the state of the major immunoregulatory receptors on peripheral blood mononuclear cells and cells and blood vessels of gliomas, may be helpful for evaluation of the role of the immune system in the pathogenesis and growth of brain tumors.

Our objective was to study the relationship between the distribution of lectin receptors on peripheral blood mononuclear cells (PBMC), cells and blood vessels of gliomas and the degree of brain tumor anaplasia.

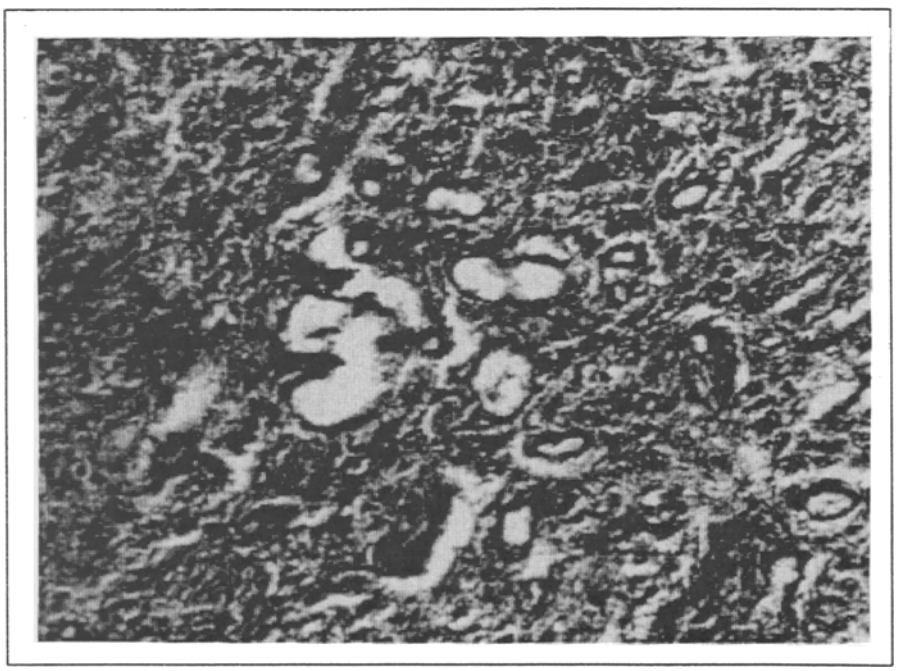

Fig. 2. Anaplastic astrocytoma resembling glioblastoma (III degree anaplasia). Histotopography of SBA receptors on blood vessels of small and medium caliber. Paraffin section. Direct immunoperoxidase method, counterstaining with methyl green. ×100.

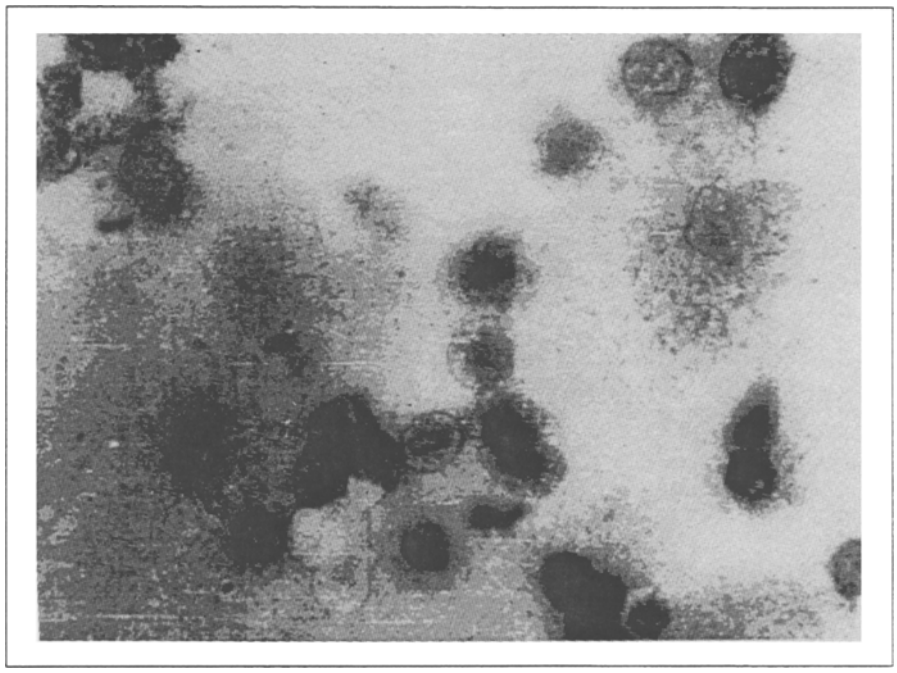

Fig. 3. Ependymoastrocytoma (II degree anaplasia). Tumor cells were isolated on sucrose gradient (1.2 M). LCL receptors are located on the plasma membrane of some tumor cells. "Dried drop" preparation, fixation in formol-acetone, direct immunoperoxidase method, counterstaning with methyl green. ×400.

MATERIALS AND METHODS

Lectin receptors were studied on paraffin sections [5] (Figs. 1 and 2) and "dried drop" preparations [2] (Fig. 3) of fractionated suspension of biopsy material and on PBMC isolated on a Ficoll-Verografin gradient (d=1.077). Blood was collected from 50 healthy donors and 40 patients with gliomas of various degrees of anaplasia: 13 patients with I-II degree, 20 patients with III degree, and 7 patients with IV degree. Autopsy brain tissue from people without a history of oncologic disease served as the control. Suspension of tumor cells or brain tissue (control) was fractionated on a sterile sucrose gradient (0.9, 1.2, 1.32, and 2.0 M) [13]. Tumor cells formed a ring at the 1.2 M level (Fig. 3), while endothelial cells and capillary fragments sedimented on the vial bottom. Both fractions were washed, and processed to obtain "dried drop" preparations for each type of lectin receptors [2]. The direct immunoperoxidase method was employed [2] with the following lectins: SBA (soybean), LCA (laburnum), PNA (peanut), SNA (elder), WGA (wheat germs), and LCL (lentil). The lectins were obtained from Lektintest company (L'vov). The significance of differences was evaluated using Student's *t* test. The preparations were studied under an Opton microscope equipped with an Ibos-2000 image analyzer.

RESULTS

The WHA receptors (suppressor cell markers) predominated on donors' PBMC and on control brain cells (Table 1). This finding confirms the existence of suppressor cells and factors preventing unregulated expansion of cell clones [6].

The following tendencies in the variations of carbohydrate receptors on PBMC and glioma cells and blood vessels were observed: 1) a significant increase in the absolute and relative contents of WGA-receptors on PBMC and tumor cells of patients with I-II degree benign gliomas compared with donors' PBMC and control brain preparations (Table 1); 2) a significantly enhanced expression of D-mannose-containing LCL receptor on glioma blood vessels compared with glioma cells was directly proportional to the degree of tumor anaplasia (Table 1); 3) a relative decrease in the expression of WGA receptors on cells and vessels of III-IV degree gliomas and on PBMC compared with LCL and SBA receptors which predominate on helper and antisuppressor lymphocytes, respectively [8,11], and were identified in various malignant tumors [5,12].

It should be emphasized that LCA receptors containing L-fucose were identified on 40-45% PBMC and on glioma cells and blood vessels in all patients irrespective on the degree of tumor anaplasia (Table 1). It was shown that L-fucose determines the activity of the factor inhibiting macrophage migration and inhibits the cytotoxic activity of macrophages toward tumor cells [10]. Presumably, enhanced expression of the L-fucose-containing receptors on PBMC and glioma cells and blood vessels is one of the mechanisms responsible for the inhibition of immunocytotoxicity in neuro-oncological diseases.

The present study shows that the number of receptors containing D-mannose (LCL), L-fucose (LCA), and N-acetyl galactosamine (SBA) on glioma

TABLE 1. Distribution of Lectin Receptors on Peripheral B Blood Lymphocytes and Cells and Blood Vessels of Gliomas Differing in the Degree of Anaplasia (*M±m*)

Degree of anaplasia	Distribution of receptors for the following lectins, %						Receptor ratio	
	SBA NAcDGal	LCA L-Fuc	PNA D-Gal	SNA NAcNeu	WGA N-AcDGlc	LCL D-Man	WGA/LCL	WGA/SBA
Control								
Donors' lymphocytes	25.7±10.1	21.7±5.2	-	35.2±9.8	35.2±4.6	18.7±3.5	1.9±0.05	1.3±0.11
Brain cells	-	5.0±2.1	-	20.0±5.6	25.1±5.6	11.0±1.5	2.2±0.18	-
Brain blood vessels	-	7.5±2.1	-	33.4±7.8	-	-	-	-
I-II degree								
Lymphocytes	40.6±9.1	45.4±9.3	25.6±9.4	53.8±8.1	65.4±6.0	26.1±7.5	2.5±0.09	1.6±0.07*
Tumor cells	36.6±7.5	43.3±10.1	50.0±1.1	60.0±10.1	50.0±1.1	6.0±2.1*	8.2±0.54	1.3±0.12*
Tumor blood vessels	26.6±5.7	26.6±7.5	16.6±5.7	50.0±11.0	16.6±5.7	16.6±5.7	1.0±0.18	0.62±0.09
III degree								
Lymphocytes	43.3±4.5	41.7±5.7	33.6±4.9	52.9±6.9	61.0±5.2	40.1±5.8*	1.5±0.04	1.4±0.11
Tumor cells	43.3±5.7	60.0±9.1	33.0±5.0	50.0±4.2	33.3±4.2	20.0±9.1	1.65±0.05	0.76±0.08*
Tumor blood vessels	30.0±1.1	66.0±6.8	26.6±6.6	66.6±11.1	43.8±9.7	50.1±5.0	0.86±0.11	1.4±0.18
IV degree								
Lymphocytes	34.8±0.8*	42.1±9.8	34.4±9.3	44.2±11.5	33.4±9.3*	23.0±10.8	1.4±0.12	0.95±0.04*
Tumor cells	26.6±9.7	33.3±10.1	36.6±7.9	66.6±10.1	50.0±2.1	50.1±6.7	0.98±0.01	1.8±0.19*
Tumor blood vessels	66.6±7.9	50.1±2.1	50.9±5.7	83.9±10.1	50.8±5.6	70.8±5.8	0.99±0.66	0.76±0.12

Note. *$p<0.01$ between patients with gliomas differing in the degree of anaplasia.

cells and blood vessels increases proportionally to the degree of tumor anaplasia. An enhanced expression of LCL and SBA receptors by malignant tumors was observed by others [5,12]. It is important that the above-mentioned carbohydrates are the constituents of immunoregulatory receptors [1,4,8,11]. It was reported that D-mannose determines the activity of IL-1 and IL-2 [9,14]. It can be suggested that enhanced expression of D-mannose-containing receptors on glioma cells and blood vessels compared with that on PBMC facilitates the utilization of lymphokines for tumor proliferation and represents a mechanism whereby tumor cells escape immunologic surveillance. Our findings account for ineffectiveness of IL-1 and IL-2 in the treatment of brain tumors [7] and prompt the search for new therapies.

REFERENCES

1. T. A. Danilova, *Immunologiya*, No. 1, 5-11 (1987).
2. V. D. Pinchuk *et al.* (Eds.), *Immunocytochemistry and Monoclonal Antibodies in Oncohematology* [in Russian], Kiev (1990).
3. A. G. Kalinkovich, *Immunologiya*, No. 1, 31-34 (1989).
4. N. I. Lisyanyi, I. A. Gnedkova, and I. A. Brodskaya, *Byull. Eksp. Biol. Med.*, **117**, No. 2, 155-157 (1994).
5. A. D. Lutsik, E. S. Detyuk, and M. D. Lutsik, *Lectins in Histochemistry* [in Russian], L'vov (1989).
6. Y. Paul, in: *Immunology* [Russian translation], Vol. 1, Moscow (1987), pp. 14-45.
7. D. Barba, S. C. Saris, C. Holder, *et al.*, *J. Neurosurg.*, **70**, No. 2, 175-182 (1989).
8. R. Brines and T. Lehner, *Immunology*, **63**, No. 2, 247-254 (1988).
9. D. T. Brody and S. K. Durum, *J. Immunol.*, **143**, No. 4, 1183-1187 (1989).
10. W. H. Churchill and C. Wong, *Cell. Immunol.*, **55**, No. 2, 490-498 (1980).
11. R. A. Dwek, D. A. Ashford, C. I. Edge, *et al.*, *Philos. Trans. R. Soc. Lond.* [*Biol.*], **342**, No. 1299, 43-50 (1993).
12. A. Makita, *Complex Carbohydrates in Medicine*, Sapporo (1989).
13. R. C. Melcangi, E. Celotti, M. Ballabia, *et al.*, *Brain Res.*, **31**, No. 10, 229-236 (1990).
14. A. P. Sherblom, N. Sathyamoorthy, J. M. Decker, *J. Immunol.*, **143**, No. 3, 939-944 (1989).