The Glucose Transporter and Blood-Brain Barrier of Human Brain Tumors

Christopher Guerin, MD,*† John Laterra, MD, PhD,‡** Ralph H. Hruban, MD,§ Henry Brem, MD,*† Lester R. Drewes, PhD,†† and Gary W. Goldstein, MD‡¶**

The glucose transporter of the human brain has been localized to endothelial cells expressing the blood-brain barrier, but little is known regarding its mechanism of induction or whether its expression is exclusively linked with restricted vascular permeability. We investigated glucose transporter expression by vessels in human astrocytic tumors and pulmonary metastases to the brain using immunohistochemical techniques. Vessels in 9 of 10 low-grade astrocytomas and 8 of 10 anaplastic astrocytomas were positive for glucose transporter. Glioblastoma vessels were transporter-positive in only 2 of 10 specimens. Vessels in all three metastatic tumors were negative for the glucose transporter. The decrease in transporter expression observed in higher-grade tumors occurred independently of increases in vascular permeability. In low-grade astrocytomas and glioblastomas transporter expression and contrast enhancement were inversely related, but vessels in 6 of 9 anaplastic astrocytomas were transporter-positive despite contrast enhancement. These findings suggest that separate mechanisms induce the glucose transporter and the permeability restrictions of the human blood-brain barrier. They also have potential implications for the therapy and prognosis of astroglial neoplasms.

Guerin C, Laterra J, Hruban RH, Brem H, Drewes LR, Goldstein GW. The glucose transporter and blood-brain barrier of human brain tumors. Ann Neurol 1990;28:758–765

The blood-brain barrier is composed of a complex array of physical, metabolic, and transport properties located at the capillary endothelium. Structural endothelial specializations, consisting of tight intercellular junctions, an absence of fenestrations, and few cytoplasmic vesicles, impose a permeability barrier limiting the diffusion of polar compounds [1]. In addition, a collection of endothelial transporter proteins and enzymes serves to regulate delivery of important substances to the brain and comprises the biochemical component of the blood-brain barrier [2].

Under normal conditions, the brain derives its energy almost entirely from glucose oxidation. In the face of permeability restrictions imposed by the blood-brain barrier, a large and constant glucose supply is maintained by specialized facilitative transporters located at the endothelium [2, 3]. Since microvessels account for less than 0.1% of brain weight, and every molecule of glucose used must cross the endothelial cell, the density of such transporters on endothelia must be considerably higher than on surrounding neurons and glia. Using quantitative analyses, Kalaria and colleagues showed the glucose transporter concentration on human brain microvessels to be among the highest of any tissue studied [4].

Recently, using immunohistochemistry and cytochalasin B–binding assays, the glucose transporter of the human brain was localized almost exclusively to vessels expressing a permeability barrier [4, 5]. Whereas cerebral and cerebellar microvessels stain intensely, those of the area postrema and adenohypophysis are not stained. Neuropil is stained minimally or not at all. Ultrastructurally, immunoelectron microscopic examination revealed that the transporter is indeed located at the endothelial cell plasma membrane [5]. Given these findings and the brain's dependence on glucose, it may be postulated that expression of high glucose transporter levels by cerebral microvessels is tightly linked to the presence of the permeability barrier which limits interendothelial diffusion.

The metabolism and vasculature of human brain tumors, especially those of the astrocytic series, have been studied extensively. These tumors may use up to three times as much glucose as normal brain [6]. Their capillaries have a range of morphologies, from essentially normal in low-grade tumors to markedly abnormal in higher-grade tumors [7–9]. Abnormally high vascular permeability is demonstrated by contrast-enhanced imaging studies. One might logically suspect that the need for specific glucose transporters is de-

From the Departments of *Neurosurgery, †Oncology, ‡Neurology, §Pathology, and ¶Pediatrics, The Johns Hopkins University School of Medicine, and **The Kennedy Institute, Baltimore, MD, and the ††Department of Biochemistry, University of Minnesota School of Medicine, Duluth, MN.

Received Mar 26, 1990, and in revised form Jun 11. Accepted for publication Jun 17, 1990.

Address correspondence to Dr Guerin, Department of Neurosurgery, The Johns Hopkins Hospital, Meyer 7-109, 601 North Wolfe St., Baltimore, MD 21205.

creased or eliminated in tumors with permeable vessels since glucose should passively diffuse into brain parenchyma, as do the much larger proteins. Alternatively, increases in vascular permeability might not be associated with decreased transporter expression if the mechanisms that establish these two barrier properties are distinct.

Many blood-brain barrier properties can be induced in vitro by astrocytes [10–13], but glucose transporter induction has not been addressed. In astrocytic tumors, astroglial cells markedly predominate. Therefore, glucose transporter expression by vessels of astrocytic tumors would suggest a role for the astrocyte in transporter induction in vivo.

To clarify these issues, we used the diverse characteristics of human astrocytic tumors to explore the relationships between glucose transporter expression, blood-brain barrier permeability, and astroglial-derived cells.

Materials and Methods

Tumor Specimens

Recently resected adult supratentorial tumors were chosen for study. Preference was given to lobectomy specimens, because they usually included normal brain tissue that could serve as a positive control during immunohistochemical staining. Specimens were fixed in 10% neutral buffered formalin for 6 to 18 hours and then processed to paraffin blocks. The microscopic slides from all specimens were reviewed and the neoplasms categorized into one of three grades: low-grade astrocytoma, anaplastic astrocytoma, or glioblastoma multiforme, according to Burger and Vogel [14]. Briefly, low-grade astrocytomas were remarkable for mild to moderate hypercellularity of uneven distribution and mild nuclear pleomorphism with occasional mitoses. Anaplastic astrocytomas were characterized by marked hypercellularity and nuclear pleomorphism. The diagnosis of glioblastoma multiforme was made only when coagulative necrosis involving the neoplastic astrocytes was identified [15]. The presence of vascular proliferation was not used as a criterion for grading. Ten low-grade astrocytomas, 10 anaplastic astrocytomas, 10 glioblastomas, and 3 metastases (all primary lung carcinomas) were studied.

Immunohistochemical Staining

Serial 5-μm-thick sections were deparaffinized, rehydrated, and stained with antiserum to glucose transporter, *Ulex europaeus* I (UEA I) lectin, or antibody to Factor VIII–related antigen (FVIIIrAg). The glucose transporter antiserum is a rabbit antiserum to the carboxy terminus of the human erythrocyte transporter, developed and characterized by one of us (L. R. D.) [5]. This antibody and similar antibodies produced by other groups have been shown to selectively stain vessels expressing a permeability barrier in normal brain [4, 5].

Rehydrated sections were incubated for 15 minutes at room temperature with 3% hydrogen peroxide (H_2O_2) in 0.05 M Tris-buffered normal saline solution, pH 7.6, and then for 10 minutes with 2% bovine serum albumin (BSA, Sigma Chemical, St. Louis, MO) in 0.5 M Tris buffer, pH 7.6. Sections were then incubated overnight at 4°C with 1:1,000 glucose transporter antiserum. Controls consisted of substituting nonimmune rabbit serum for immune serum. Sections were then incubated at room temperature in 1:100 biotinylated goat anti-rabbit immunoglobulin (Vector, Burlingame, CA) for 30 minutes, followed by 1:100 horseradish peroxidase-streptavidin conjugate (Dako, Santa Barbara, CA) for 30 minutes, and developed with 3-amino-9-ethylcarbazole chromogen (Biomeda, Foster City, CA) for 10 minutes. Sections were counterstained with hematoxylin. Dilutions were made in 0.5 M Tris buffer containing 1% BSA, pH 7.6, unless noted otherwise, and slides were rinsed between incubations with 0.05 M Tris-buffered normal saline solution, pH 7.6.

Stains for endothelial cell markers were performed as just described, with the following modifications. UEA I lectin staining was done with 1:1,000 UEA I lectin (Vector) and 1:2,000 biotinylated goat anti-UEA I (Vector) substituted for primary and secondary antibodies, respectively. Sections for staining with anti-FVIIIrAg were digested with Pronase (Calbiochem, San Diego, CA), 1 mg/ml, in 0.05 M Tris-buffered normal saline solution containing 0.2% ethylene diaminetetraacetic acid (EDTA) for 30 minutes at 37°C just prior to incubation with 1:20 normal horse serum (Cappel, Cochranville, PA) in place of 2% BSA. Primary and secondary antibodies were 1:50 mouse monoclonal anti-FVIIIrAg (Dako) and 1:100 biotinylated horse anti-mouse (Vector). Controls for vascular markers consisted of substituting an irrelevant antibody for UEA I lectin or normal mouse serum for FVIIIrAg. All controls performed were negative for glucose transporter and vascular markers.

Grading of Stained Sections

Endothelial markers were used to assess the vascular density of each specimen, allowing an estimate of the fraction of vessels positive for the glucose transporter. These markers have been shown to identify vessels in astrocytic tumors as efficiently as those of normal brain [16, 17]. For anaplastic astrocytomas and glioblastomas, only the highest-grade areas were considered, since histological diagnosis is based on the highest-grade area observed.

Tumors were placed into three categories with regard to staining by antibody to the glucose transporter: "positive" when all observed vessels were stained (1 anaplastic astrocytoma and 1 glioblastoma with only small, focal areas of negative vessels were included in this category); "mixed" when significant numbers of stained vessels and unstained vessels were seen; and "negative" when all observed vessels were unstained (2 anaplastic astrocytomas and 1 glioblastoma with only small, focal areas of positive vessels were included in this category). The intensity of staining relative to that found in normal brain tissue was noted for positive and mixed tumors.

Imaging Studies

For each specimen, preoperative imaging studies were assessed for the presence or absence of contrast enhancement as a marker of the permeability barrier. Magnetic resonance (MR) scans and computed tomograms (CT) were considered equivalent, and in no case where both were available did

Characteristics of Tumors Studied

Tumor	n	Age (yr; mean ± SEM)	Sex (M/F)	Side (R/L)	Lobe
Low-grade astrocytoma	10	50 ± 4	4/6	2/8	5 T, 4 F, 1 P
Anaplastic astrocytoma	10	44 ± 4	7/3	3/7	3 T, 3 F, 2 P, 1 F/P, 1 deep
Glioblastoma multiforme	10	59 ± 6	6/4	3/7	5 T, 2 P, 1 F, 1 P/O, 1 deep
Metastases	3	56 ± 4	0/3	2/1	1 T, 1 F, 1 F/P

T = temporal; F = frontal; P = parietal; O = occipital.

results disagree. If actual films were unavailable, official final reports were used (6 cases). Only noncontrast imaging was performed preoperatively in 1 case of low-grade astrocytoma and 1 anaplastic astrocytoma.

Results

Tumor Specimens

The Table lists characteristics of the specimens studied. Specimens of glioblastoma and metastasis were from older patients, as expected. The high incidence of temporal lobe tumors reflects the selection of lobectomy specimens.

Expression of Glucose Transporter

All grades of astrocytic tumors were studied to assess how variations in the state of differentiation and relative density of astroglial cells affected glucose transporter expression. Only the highest-grade areas of anaplastic astrocytomas and glioblastomas were considered, since histological diagnosis is based on the highest-grade area observed. In addition, a small sample of carcinomas metastatic to brain was studied to assess the effects of nonneural malignant cells on glucose transporter expression by brain-derived vessels.

Vessels in low-grade astrocytomas stained positively for glucose transporter in 9 specimens (Fig 1A and B) and with a mixed pattern in 1. Anaplastic astrocytoma vessels stained positively in 8 specimens (Fig 1C and D), with negative staining in 2. Glioblastoma multiforme vessels stained positively in 2 specimens, negatively in 6 (Fig 1E and F), and with a mixed pattern in 2. Glucose transporter was not detected in the vessels of any of the 3 metastatic tumors we examined (Fig 1G and H). In no case were tumor cells stained by glucose transporter antiserum. The intensity of staining relative to that of normal brain tissue (Fig 2) was decreased in the 1 low-grade astrocytoma with a mixed staining pattern, 2 positive anaplastic astrocytomas, 1 glioblastoma with mixed staining, and both positive glioblastomas. Therefore, despite 2 "positive" specimens, no glioblastoma stained with the completeness and intensity of low-grade astrocytomas or the majority of anaplastic astrocytomas. These results are summarized in Figure 3. Because of the heterogeneous morphology of glial tumor vessels, attention was paid to the staining characteristics of specific foci of vascular proliferation (coiled masses of small vessels, often with enlarged endothelial cells [14]). Although the incidence of vascular proliferation did increase with increasing grade, this abnormal morphology never accounted for the majority of vessels. The staining of these proliferative vessels by glucose transporter antiserum was consistent with that of the tumor in toto (being either positive, negative, or mixed) in all but 1 specimen.

Relationship of Glucose Transporter Expression to the Permeability Barrier

The presence of contrast enhancement on preoperative imaging studies was taken as evidence of loss of the permeability barrier that normally limits interendothelial diffusion. Variations in vascular permeability among the grades of tumors allowed us to assess the relationship between permeability and transporter expression.

Two of 9 low-grade astrocytomas demonstrated contrast enhancement preoperatively. Seven of 9 anaplastic astrocytomas showed enhancement. All 10 glioblastomas and all 3 metastases showed contrast enhancement.

The frequency of glucose transporter positivity in relation to that of contrast enhancement for each grade of glial tumor is represented in Figure 4. Only specimens for which imaging studies were available were included in the analysis. Low-grade astrocytomas and glioblastomas demonstrated an inverse relationship between transporter expression and contrast enhancement. However, most anaplastic astrocytomas were transporter-positive despite an abnormally high vascular permeability evidenced by contrast-enhanced imaging. This association occurred in 6 of 9 anaplastic astrocytomas. Only 1 of 9 low-grade astrocytomas and 2 of 10 glioblastomas stained positively and showed contrast enhancement. Remaining tumors were either transporter-positive and nonenhancing (7 low-grade astrocytomas and 1 anaplastic astrocytoma), transporter-negative and enhancing (1 anaplastic astrocytoma, 6 glioblastomas, and 3 metastases), or mixed and enhancing (1 low-grade astrocytoma and 2 glioblastomas).

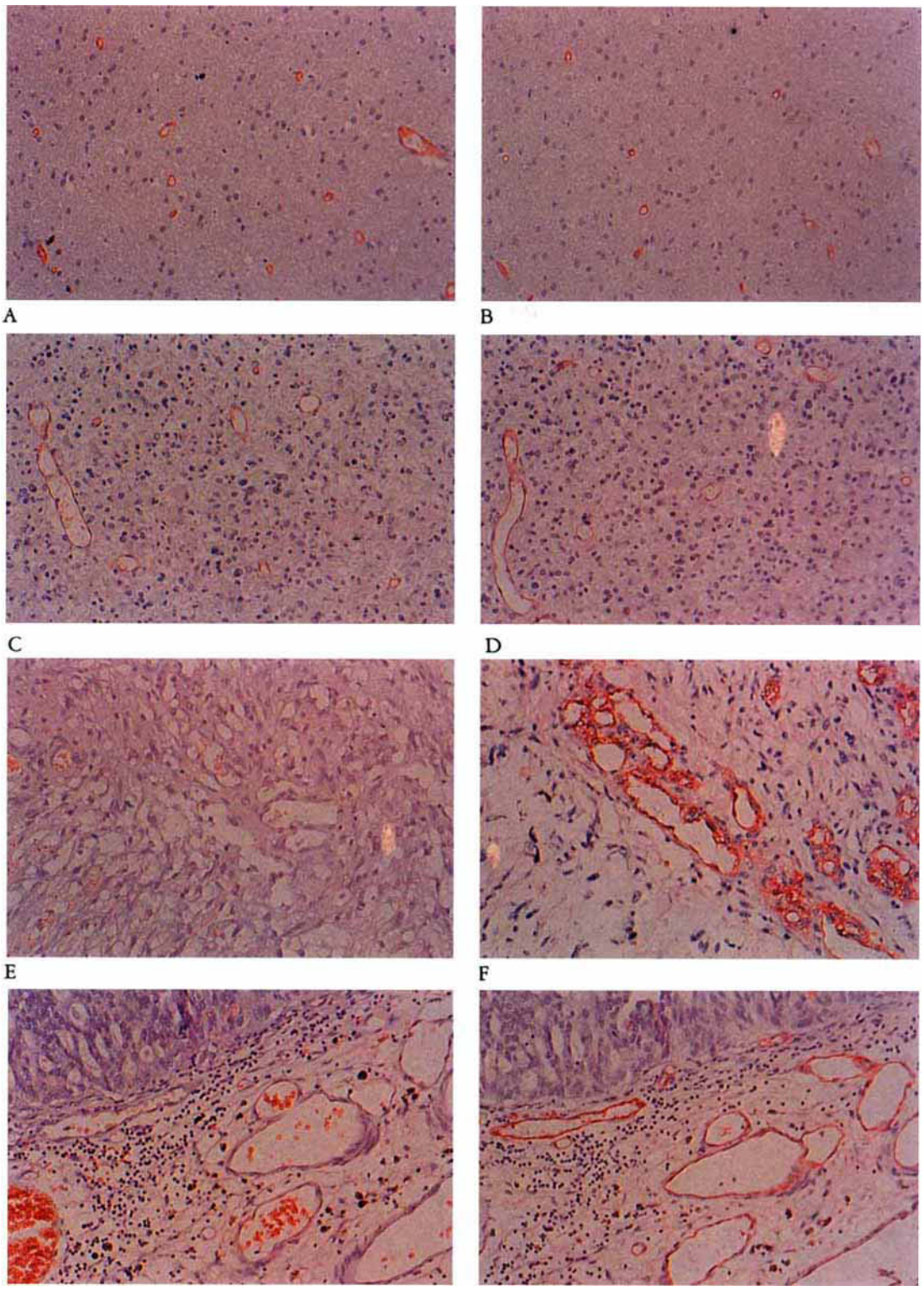

Fig 1. Tumors stained with glucose transporter antiserum (A, C, E, and G) or endothelial markers Ulex europaeus *I (UEA I) (B and D) or anti–Factor VIII-related antigen (FVIIIrAg) (F and H). (A and B) Low-grade astrocytoma. (C and D) Anaplastic astrocytoma. (E and F) Glioblastoma multiforme. (G and H) Metastasis. Positive staining is evidenced by a red to brown reaction product. Vessels of the low-grade astrocytoma and anaplastic astrocytoma stain positively, while metastasis and glioblastoma vessels are negative for glucose transporter. UEA I and FVIIIrAg stain all vessels. As expected, erythrocytes are also stained by glucose transporter antiserum. (Hematoxylin counterstain, × 25 before 425% enlargement.)*

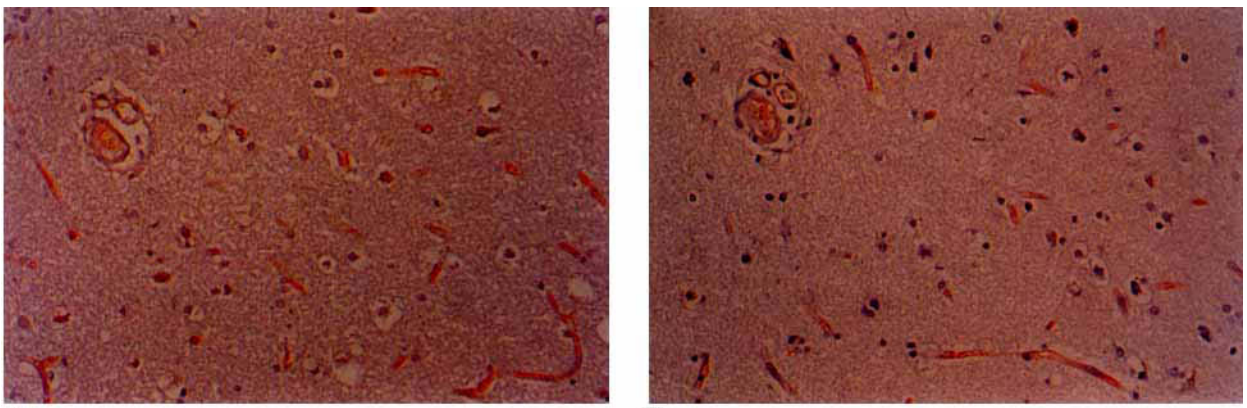

Fig 2. Normal brain stained with (A) glucose transporter antiserum or (B) the endothelial marker Ulex europaeus *I. All vessels express high levels of glucose transporter. (Hematoxylin counterstain, × 25 before 480% enlargement.)*

One anaplastic astrocytoma was both transporter-negative and nonenhancing.

Discussion

The blood-brain barrier is more than a simple restriction of permeability. Specialized enzymes and transport systems regulate the entry of substances into the brain [2]. These proteins form a biochemical component of the barrier, which is necessary for normal brain function. In pathological conditions, barrier function is usually disturbed but not eliminated [18]. However, most assessments of blood-brain barrier function include only permeability studies, both in the laboratory and in the clinic.

We assessed a biochemical property of the blood-brain barrier in human astrocytic neoplasms, a pathological condition in which the permeability barrier has been extensively studied. Using immunohistochemical techniques, we showed that blood vessels of such tumors may express apparently normal glucose transporter levels. This high expression was seen in low-grade and anaplastic astrocytomas, but the expression of transporter decreased significantly in glioblastoma multiforme.

We also investigated the relationship between glucose transporter expression and contrast enhancement, the clinical measure of barrier permeability. It has been shown that the vessels of normal, nonenhancing cerebrum express high glucose transporter levels [4, 5]. If the expression of transporter and permeability restrictions are strictly linked, one would expect vessels of nonenhancing tumors to be transporter-positive. This association held for 8 of 9 such tumors. Conversely, vessels of enhancing tumors would be expected to be transporter-negative or to stain with a mixed pattern. This was only true for 13 of 22 enhancing tumors. Anaplastic astrocytomas accounted for the majority of tumors that were transporter-positive despite contrast enhancement (see Fig 4). In fact, as anaplasia progressed, permeability increased before transporter expression decreased. The expression of glucose transporter independently of permeability restrictions suggests that these two barrier components may be induced or maintained by separate mechanisms.

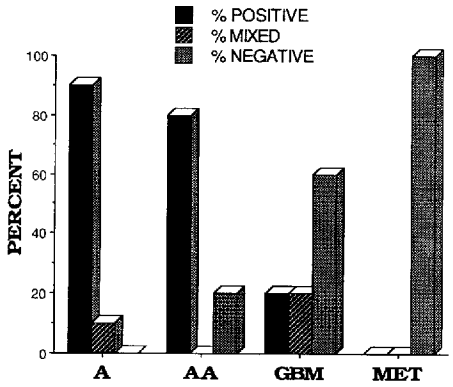

Fig 3. Frequency of glucose transporter expression as a function of tumor type. Positive: essentially all observed vessels are stained by glucose transporter antiserum; mixed: significant numbers of stained vessels and unstained vessels are observed; negative: essentially all vessels are unstained (see Materials and Methods). The majority of low-grade astrocytomas (A) and anaplastic astrocytomas (AA) are transporter-positive. In glioblastomas (GBM) the frequency distribution shifts to a predominance of transporter-negative tumors. No metastasis (MET) expressed transporter. (n = 10 for A, AA, and GBM; n = 3 for MET.)

Similar observations have been made in other systems. In muscle, capillary endothelial cells express higher levels of both the muscle and brain-type glucose transporter than do surrounding myocytes [19]. This high expression occurs despite the ready permeability of these vessels to horseradish peroxidase [20]. In the developing rat brain, levels of glucose transporter pro-

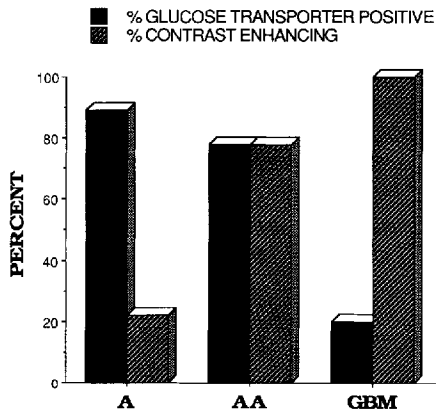

Fig 4. Frequency of glucose transporter positivity and contrast enhancement as a function of tumor type. Essentially all vessels of normal cerebrum (nonenhancing by definition) are known to be transporter-positive. Low-grade astrocytomas and glioblastomas demonstrate an inverse relationship between transporter expression and contrast enhancement. The majority of anaplastic astrocytomas are transporter-positive despite increased vascular permeability. A = low-grade astrocytoma (n = 9); AA = anaplastic astrocytoma (n = 9); GBM = glioblastoma multiforme (n = 10).

tein and messenger RNA (mRNA) both decline transiently just after birth, a time when permeability restrictions are increasing [21]. Hence, in normal muscle and developing rat brain, microvascular glucose transporter expression is regulated independently of vessel permeability.

The heterogeneity of astrocytic neoplasms makes it possible that sampling error accounts for the identification of transporter-positive yet contrast-enhancing tumors. Pathological specimens staining positively might be derived from nonenhancing regions within an enhancing tumor. However, studies correlating CT and MR images with histological findings consistently show that in lesions demonstrating contrast enhancement, solid tumor was found only in enhancing areas and not in nonenhancing regions [22–24]. The latter areas contained only isolated tumor cells infiltrating intact parenchyma. Since this study assessed only specimens of solid tumor tissue and excluded infiltrated border zones, it is very likely that transporter-positive specimens from enhancing lesions represented areas of increased vascular permeability. It is possible but unlikely that rare negative vessels, undetected in our specimens, account for enhancement (e.g., by allowing escape of contrast, which diffuses over a large area). Our use of two vascular markers to detect all vessels within a specimen should minimize this error.

Normal brain parenchyma can induce many biochemical and structural features of the blood-brain barrier, even in vessels that normally do not express such a barrier [25, 26]. Of the many brain cells potentially responsible, attention has long been focused on the astrocyte [27]. Astrocytic foot processes ensheathe 99% of brain capillaries, sharing a common basement membrane to form an astrocyte-endothelial complex [28]. Developmentally, this astrocytic investment is temporally associated with capillary maturation [29] and the appearance of biochemical barrier components [30, 31]. Janzer and Raff recently showed that pure astrocytes transplanted to the chorioallantois are vascularized by vessels impermeable to Evans blue [10]. Endothelial cells cocultured with astrocytes demonstrate increases in the number and complexity of tight junctions [11]. Astrocytes also produce platelet-derived growth factor and fibroblast growth factor [32, 33], both of which are known to induce the glucose transporter in fibroblasts [34]. C6 glioma cells, which express astrocytic properties [35, 36], are capable of inducing gamma-glutamyl transpeptidase [37] and polarization of amino acid transport [38] in cultured endothelium, both of which are barrier-associated properties. Of particular interest, endothelial cells increase their glucose uptake in the presence of either C6 cells or normal astrocytes, but not oligodendrocytes [13].

Tumors caricature properties of normal tissue, and despite their altered behavior, unmask normal biological processes [39]. In astrocytic tumors, vessels are bathed in an environment abnormally enriched in astroglial-derived cells, and relatively depleted of other cell types. Our observation that low-grade astrocytomas express normal transporter levels, along with the known inductive capacity of astrocytes, suggests that astrocytes induce glucose transporter expression in human brain. Ultrastructural studies revealed an essentially normal capillary ensheathement by neoplastic cells in these well-differentiated tumors [8, 9], preserving the intimate astrocytic-endothelial relationship. Expression of transporter was maintained in more anaplastic tumors and was consistently absent only in glioblastoma multiforme, whose vessels are incompletely invested by extremely dedifferentiated cells [7, 9]. Although this study does not exclude the possibility that nonastroglial cells persisting in these tumors are the actual inducers, this is unlikely because of the extremely low density of such cells relative to normal and the disruption of their normal cytoarchitectural relationships. It is also unlikely that glucose transporter induced by another cell type is merely retained by vessels growing into astrocytomas. Metastatic tumors did not express transporter despite a brain-derived vascular supply, and multiple reports of peripheral tissue transplants into brain consistently failed to demonstrate vessels retaining barrier-associated properties [25, 26]. However, it is difficult to prove that specific inductive influences occur simply by studying brain tumor sections. Therefore, we are currently investigating an in vitro model of neural angiogenesis to assess

more definitively the role of astroglial cells in glucose transporter induction [40].

Our findings may have implications for the therapy and prognosis of astroglial neoplasms. It is unknown whether glucose flux into tumors with permeable vessels is significantly increased by transporter expression. If, despite contrast enhancement, the presence of specialized transporters signifies that glucose diffusion into parenchyma is restricted, then entry of chemotherapeutic agents might also be limited. In such tumors, novel systems for drug delivery may be more efficacious [41]. The ability of a tumor to induce high transporter levels may indicate a retention of differentiated properties associated with lower relative malignancy and improved prognosis. If so, the presence of glucose transporter by immunostaining may provide a means of subclassifying astroglial tumors relevant to patient survival.

This work was supported by National Institutes of Health research grant NS-27229 (L. R. D., P. I.). C. Guerin is a National Research Service Awardee (CA-09574). J. Laterra and H. Brem are Clinical Investigator Development Awardees of the National Institute of Neurological Disorders and Stroke (NS-01329 and NS-010508).

The authors thank Wes Gage and George Pettis for expert technical assistance.

References

1. Reese TS, Karnovsky MJ. Fine structural localization of a blood-brain barrier to exogenous peroxidase. J Cell Biol 1967;34: 207–217
2. Goldstein GW, Betz AL. The blood brain barrier. Sci Am 1986;254:74–83
3. Crone C. Facilitated transfer of glucose from blood into brain tissue. J Physiol (Lond) 1965;181:103–113
4. Kalaria RN, Gravina SA, Schmidley JW, et al. The glucose transporter of the human brain and blood-brain barrier. Ann Neurol 1988;24:757–764
5. Gerhart DZ, LeVasseur RJ, Broderius MA, Drewes LR. Glucose transporter localization in brain using light and electron immunocytochemistry. J Neurosci Res 1989;22:464–472
6. Mangiardi JR, Yodice P. Metabolism of the malignant astrocytoma. Neurosurgery 1990;26:1–19
7. Long DM. Capillary ultrastructure and the blood-brain barrier in human malignant brain tumors. J Neurosurg 1970;32:127–144
8. Long DM. Capillary ultrastructure and the blood-brain barrier. Human brain tumors. In: Comptes rendus. Proceedings of the Sixth International Congress on Neuropathology. Paris: Masson Editeur, 1970:994–996
9. Weller RO, Foy M, Cox S. The development and ultrastructure of the microvasculature in malignant gliomas. Neuropathol Appl Neurobiol 1977;3:307–322
10. Janzer RC, Raff MC. Astrocytes induce blood-brain barrier properties in endothelial cells. Nature 1987;325:253–257
11. Tao-Cheng JH, Brightman MW. Development of membrane interactions between brain endothelial cells and astrocytes in vitro. Int J Dev Neurosci 1988;6:25–37
12. Beck DW, Roberts RL, Olson JL. Glial cells influence membrane associated enzyme activity at the blood-brain barrier. Brain Res 1986;381:131–137
13. Maxwell K, Berliner JA, Cancilla PA. Stimulation of glucose analogue uptake by cerebral microvessel endothelial cells by a product released by astrocytes. J Neuropathol Exp Neurol 1989;48:69–80
14. Burger PC, Vogel FS. Surgical pathology of the nervous system and its coverings. New York: John Wiley, 1982:226–266
15. Nelson JS, Tsukada Y, Schoenfeld D, et al. Necrosis as a prognostic criterion in malignant supratentorial, astrocytic gliomas. Cancer 1983;52:550–554
16. McComb RD, Jones TR, Pizzo SV, Bigner DD. Immunohistochemical detection of factor VIII/von Willebrand factor in hyperplastic endothelial cells in glioblastoma multiforme and mixed glioma-sarcoma. J Neuropathol Exp Neurol 1982;41: 479–489
17. Weber T, Seitz RJ, Liebert UG, et al. Affinity cytochemistry of vascular endothelia in brain tumors by biotinylated Ulex europaeus type I lectin (UEA I). Acta Neuropathol (Berl) 1985;67:128–135
18. Stewart PA, Hayakawa K, Hayakawa E, et al. A quantitative study of blood-brain barrier permeability ultrastructure in a new rat glioma model. Acta Neuropathol (Berl) 1985;67:96–102
19. Vilaro S, Palacin M, Pilch PF, et al. Expression of an insulin regulatable glucose carrier in muscle and fat endothelial cells. Nature 1989;342:798–800
20. Karnovsky MJ. The ultrastructural basis of capillary permeability studied with peroxidase as a tracer. J Cell Biol 1967;35:213–236
21. Sivitz W, DeSautel S, Walker PS, Pessin JE. Regulation of the glucose transporter in developing rat brain. Endocrinology 1989;124:1875–1880
22. Kelly PJ, Daumas-Duport C, Kispert DB, et al. Imaging-based stereotaxic serial biopsies in untreated intracranial glial neoplasms. J Neurosurg 1987;66:865–874
23. Earnest F, Kelly PJ, Scheithauer BW, et al. Cerebral astrocytomas: histopathologic correlation of MR and CT contrast enhancement with stereotactic biopsy. Radiology 1988;166:823–827
24. Burger PC, Heinz ER, Shibata T, Kleihues P. Topographic anatomy and CT correlations in the untreated glioblastoma multiforme. J Neurosurg 1988;68:698–704
25. Stewart PA, Wiley MJ. Developing nervous tissue induces formation of blood-brain barrier characteristics in invading endothelial cells: a study using quail-chick transplantation chimeras. Dev Biol 1981;84:183–192
26. Svendgaard NA, Bjorklund A, Hardebo JE, Stenevi U. Axonal degeneration associated with a defective blood-brain barrier in cerebral implants. Nature 1975;255:334–337
27. Davson H, Olendorf WH. Transport in the central nervous system. Proc R Soc Med 1967;60:326–329
28. Wolff JR, Bar T. Development and adult variations of the pericapillary glial sheath in the cortex of rat. In: Cervos-Navarro J, ed. The cerebral vessel wall. New York: Raven Press, 1976:7–13
29. Bar T, Wolff JR. The formation of capillary basement membranes during the internal vascularization of the rat's cerebral cortex. Z Zellforsch 1972;133:231–248
30. Risau W, Hallman R, Albrecht U. Differentiation dependent expression of proteins in brain endothelium during development of the blood-brain barrier. Dev Biol 1986;117:537–545
31. Laterra J, Stewart PA, Goldstein GW. Development of the blood-brain barrier. In: Polin RA, Fox WW, eds. Neonatal and fetal medicine-physiology and pathophysiology. Philadelphia: WB Saunders 1991 (in press)
32. Richardson WD, Pringle N, Mosely MJ, et al. A role for platelet-derived growth factor in normal gliogenesis in the central nervous system. Cell 1988;53:309–319
33. Ferrara N, Ousley F, Gospodarowicz D. Bovine brain astrocytes

express basic fibroblast growth factor, a neurotropic and angiogenic mitogen. Brain Res 1988;462:223–232
34. Hiraki Y, Rosen OM, Birnbaum MJ. Growth factors rapidly induce expression of the glucose transporter gene. J Biol Chem 1988;263:13655–13662
35. Bissel MG, Rubinstein LJ, Bignami A, Herman MM. Characteristics of the rat C6 glioma maintained in organ culture systems. Production of glial fibrillary acidic protein in the absence of gliofibrillogenesis. Brain Res 1974;82:77–89
36. Kumar S, Holmes E, Scully S, et al. The hormonal regulation of gene expression of glial markers: glutamine synthetase and glycerol phosphate dehydrogenase in primary cultures of rat brain and in C6 cell line. J Neurosci Res 1986;16:251–264
37. DeBault LE, Cancilla PA. Gamma-glutamyl transpeptidase in isolated brain endothelial cells: induction by glial cells *in vitro*. Science 1980;207:653–655
38. Beck DW, Vinters HV, Hart MN, Cancilla PA. Glial cells influence the polarity of the blood-brain barrier. J Neuropathol Exp Neurol 1984;43:219–224
39. Pierce GB, Shikes R, Fink LM. Cancer: a problem of developmental biology. Englewood Cliffs, NJ: Prentice-Hall, 1978:27–48
40. Laterra J, Guerin C, Goldstein GW. Astrocytes induce neural microvascular endothelial cells to form capillary-like structures *in vitro*. J Cell Physiol 1990;144:204–215
41. Yang MB, Tamargo RJ, Brem H. Controlled delivery of 1,3-bis(2-chloroethyl)-1-nitrosourea from ethylene-vinyl acetate copolymer. Cancer Res 1989;49:5103–5107