*REVIEW ARTICLE*

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# Immunobiologic Aspects of the Brain and Human Gliomas

*A Review*

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TUMORS INVOLVING the central nervous system are estimated to comprise 2–5% of all mass lesions [1] and, according to results of the Third National Cancer Survey (1973–74), account for at least 3.9 deaths/100,000 population per annum in the United States, average rates being highest for white males (4.9/100,000) and lowest for nonwhite females (1.9/100,000).[2] This range is comparable to the median incidence computed for 90% of the 61 worldwide examples of 4–5 cases/100,000 age-corrected population per annum compiled by Schoenberg et al [3] and corroborated by smaller, regional studies.[4,5] This incidence rate would predict approximately 11,000–15,000 new brain tumor cases per annum in the 1970s in the United States, making malignant brain tumors more common than Hodgkin's disease.[6]

The bimodal age peaks of brain tumor occurrence in childhood and middle adult life effectively enlarge the impact of the 2.7% mortality of brain tumors with respect to all cancers.[2] Fifteen to twenty percent of all intracranial tumors occur in childhood,[7] making central nervous system tumors the second most common form of cancer after leukemia in children under 15.[8] During the middle adult years, gliomas of the cerebral hemispheres—glioblastoma multiforme comprising almost 50% of all primary intracranial masses at this time—are prevalent and rank fourth among males and eighth among females in the order of neoplastic causes for lost work years.[9]

Although the experimental induction of central nervous system tumors by a number of viral, chemical, and radioactive agents has been intensively investigated in model animal systems,[10] the cause of spontaneous primary intracranial tumors is unknown. Despite the recovery of JC and

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SV40 papovaviruses from the brains of patients with progressive multifocal leukoencephalopathy (PML) [11,12] and the simultaneous presence of oligodendroglioma and PML [13] or multifocal glioblastoma and PML,[14] a recent exhaustive analysis of cell cultures derived from 80 human brain tumors failed to demonstrate the intranuclear T antigen common to cells transformed by SV40, BK, or JC viruses in a single case.[15] These results are in direct contrast to the demonstration of SV40 T antigen in cells grown from 3/8 human meningiomas by Weiss [16] and the subsequent report of BK papovavirus DNA sequences in a cerebellar spongioblastoma by Fiori and di Mayorca.[17] As DNA–DNA hybridization techniques frequently yield false positives [15] and most of the population are infected with JC and BK viruses during childhood,[18] the case for a viral etiology of human brain tumors versus an innocuous infection of susceptible neoplastic cells is extremely weak. Similarly, RNA tumor virus associations with human gliomas are weak, with only one situation that may survive critical scrutiny. Ponten and Westermark have found a virus with the morphologic characteristics of the C-type particle and antigenic and biochemical relatedness to simian sarcoma virus associated with suspension cultured cells from a solitary intracranial tumor, the classification of which is controversial (Ponten and Westermark, manuscript in preparation).

Following the demonstration by Maltoni and Lefemine [19] that exposure of rats to vinyl chloride by inhalation resulted in the induction of significantly high numbers of encephalic neuroblastomas, Waxweiler et al [20] reported that a 4–5-fold increase in the incidence of glioblastoma multiforme occurred among workers heavily exposed to vinyl chloride. This increase was notable in that 1) glioblastomas usually comprise approximately 30–50% of intracranial tumors in adults of the age range studied here,[9] and 2) the observation of central nervous system, hepatic, lung, and lymphatic sites of increased tumor incidence in individuals exposed to vinyl chloride closely paralleled the results in experimental animal models.[19] Other chemical agents, such as acrylonitrile (vinyl cyanide), known to induce brain tumors in animals,[21] are currently under investigation for their effects upon man.

Apparent induction of a malignant astrocytoma in a human patient by prior radiotherapy of a craniopharyngioma was reported by Sogg et al [22]; subsequently, Preissig et al [23] described a malignant glioma of the cerebellum postulated to have arisen as a result of previous radiation therapy for a glomus jugulare tumor. Both studies meet the generally accepted criteria for radiation-induced neoplasia, namely, the appearance of a second and different neoplasm within the field of treatment, with a latency of at least 5 years in humans.

Several surveys have established that males are more frequently affected than females, with a male/female ratio ranging from 1.33:1 [24] to 1.5:1 [25] in children and adults over 45 years of age; for young adults the tumor incidence is essentially the same for both sexes. The high incidence of gliomas associated with congenital disorders such as von Recklinghausen's disease [26] and reports of familial and twin cases [26-29] have suggested that for a small percentage of glioma patients, genetic factors might be involved. As summarized by Isamat et al,[26] several studies have suggested that the incidence of glioma among relatives of patients with proven gliomas is significantly higher (approximately 4 times) than the theoretical random occurrence in a matched control population. The inclusion of primary brain tumors, predominantly glioblastoma multiforme, in the familial cancer aggregation designated SBLA (S, sarcoma; B, breast and brain tumor; L, leukemia, laryngeal cancer, and lung carcinoma; A, adrenal cortical carcinoma) described by Lynch et al [30] extends the circumstantial evidence implicating possible genetic factors. The etiology of "SBLA syndrome" cancers is also unknown, but the authors have postulated a "two-hit" mechanism, the first hit, germinal, involving a susceptibility "gene," the second, somatic, involving exposure, possibly intrauterine, to unspecified inductive agents.[31] Studies investigating a possible linkage with HLA antigens in glioma patients versus control subjects have been negative,[32] and although several authors have reported an increased frequency of ABO type A among glioma patients over that expected,[26,33] the association is neither exclusive nor significant.

It is evident, then, that the pathogenesis of tumors arising in the central nervous system of man is unknown. As summarized by Kleihues,[34] with the probable exception of apparent genetically determined neoplastic syndromes, "epidemiological investigations have not revealed causative environmental factors" related to tumors of the central nervous system. Thus, the probable history of human central nervous system tumors is multifactorial, potentially involving genetic, viral, chemical, and/or radioactive agents. The consequences are obvious and reflect the *in vivo* situation: extensive morphologic, antigenic, and therapeutic regimen sensitivity and prognostic diversity.

The general lack of knowledge concerning brain tumor etiology is reflected in the relatively ineffective therapeutic modalities available at the present time. There is general agreement that wide surgical resection of the tumor, when possible, is mandatory for the best prognosis [36]; the efficacy of combinations of radiation and/or chemotherapy in combination with surgery or in total combined modality studies is still being determined. As reported by Sheline,[37] and reviewed by Young and Kaplan,[8]

there is litte significant long-term extension of survival rates of postoperative glioma patients with or without radiation therapy. Recent favorable results have been obtained following substantial tumor resection and high (5000 to 6000 rads) doses of radiation therapy to the whole brain; although 1-year postoperative survival may approach 50%, the rate of survival at 2 years is less than 10%.[38] Various forms of either single agent or combined chemotherapeutic trials have been reported; the current drugs of choice being the lipid-soluble, low-ionization-potential nitrosoureas. Postoperative chemotherapy with BCNU or BCNU-5-flurouracil has resulted in limited extension of survival time [39,40]; CCNU alone [41,42] or in conjunction with radiation therapy [41–43] has not proven significantly more effective than surgery or surgery and radiation. The most effective therapy available to date for malignant gliomas is the combination of surgery, high-dose radiation therapy, and chemotherapy with BCNU [38]; this treatment is only palliative, and the average patient can expect impaired speech and mental status, paralysis, coma, and incapacitating seizures.[36] This poor quality of survival is partially caused, to an unknown degree, by the combined therapeutic measures themselves. Radiation and chemotherapy both result in general immunosuppression; cumulative drug and radiation toxicity often result in the discontinuation of experimental therapeutic protocols. Craniospinal irradiation in the therapeutic range can cause localized brain necrosis [44–46] and/or systemic effects, including perturbations of peripheral lymphocyte populations and suppression of the hypothalamic–pituitary axis with concomitant decreases in growth hormone levels and pituitary hormones in general.[46]

The cumulative toxicity of present modes of therapy and the inability to surgically resect the majority of intracranial tumors completely has led to the search for "an alternate nontoxic, noninvasive form of therapy . . . to control the last remaining tumor cells."[8] Currently, the most popular candidate is immunotherapy. Several immunotherapeutic studies of human intracranial tumors have been reported in the literature, despite a paucity of knowledge of the immunobiology of the central nervous system, normal or neoplastically transformed, or of the patients that bear these tumors. The purpose of this review is to examine the immunobiologic aspects of the brain and of human gliomas: hypothetically, 1) the nature of glioma cells as potential immunogens and 2) the nature of immune responses within the central nervous system, an "immunologically privileged site"; and practically, 3) the immune capacities and potentials of the brain tumor patient, and 4) the impact of early immunotherapeutic trials upon the current status of brain tumor therapy.

## I. Antigenic Characteristics of the Normal Human Brain Cell

### Intracellular Antigens

A great number of normal brain and nervous system antigens have been described, many of which are widely distributed throughout the vertebrate phylum. The most extensively and analytically characterized are intracellular in location; a brief outline of the most notable is presented in Table 1. The characterization of these antigens has been extensively reviewed by Bock [47]; a brief summary follows.

As is evident in Table 1, purification and biochemical characterization have been accomplished for the major intracellular, normal adult brain-associated antigens. S-100 protein, the first to be extensively characterized, is a soluble 21,300 dalton acidic protein composed of at least three 7,000 molecular weight subunits originally described by Moore [48] and located in astrocytic cytoplasm and neuronal nuclei.[58,61] S-100 has also been reported to be associated with glial cell membranes [62,63] with a polar distribution including dendritic and axonic processes.[64] The 14-3-2 protein originally described by Moore [48] and separated into two components with different electrophoretic mobilities by Bock and Dissing [65] has been demonstrated to be a dimer of two subunits ($\alpha\alpha$) of 39,000 molecular weight, designated NSE (neuron-specific enolase) [66] that behaves as a neuronal cytoplasm marker [67] and distinguishes three classes of neurons on the basis of its appearance or lack thereof during development.[47] A separate enolase, $\gamma\gamma$, is a dimer composed of the widespread 43,000-dalton subunit and the brain-specific $\alpha$ unit and has been determined to be a characteristic glial enzyme within the central nervous system.[66]

Glial fibrillary acidic protein (GFA) is also located intracellularly and was first isolated by Eng et al [49] from fibrous astrocyte-rich multiple sclerosis plaques. GFA is apparently composed of 1–7 peptides in the range of 40,500–54,000 daltons and is a definitive marker of fibrous astrocytes and their processes.[68,69] This marker is interspecies in nature and has been demonstrated to appear in developing mouse brain between 10 and 14 days postnatally, when astroglial differentiation occurs with progressive myelination.[70] Antanitus et al [68] have similarly identified GFA in primary tissue culture explants of human fetal forebrain at gestational ages between 12 and 20 weeks. The interspecies astrocyte-specific antigen NSA-1 described and characterized by Delpech et al [50,71,72] has been determined to be analogous to GFA.[73] The GFA protein, the major component of the astroglial fiber, has been postulated to provide the structural support sup-

Table 1—Normal Human "Brain-Specific" Antigens

| | Original description* | Molecular weight | Species distribution | Structure | Relative immunogenicity† | Cellular location |
|---|---|---|---|---|---|---|
| Intracellular antigens | | | | | | |
| S-100 protein | Moore[48] | 21,000–24,000 daltons | Vertebrates, including fish, birds, and reptiles | 3 × 7000 dalton subunits | Low | Astrocytic cytoplasm<br>Neuronal nuclei<br>Oligodendroglia<br>Dendritic and axonic processes |
| 14-3-2 protein brain-specific enolase | Moore[48] | 78,000 daltons | Mammals, birds | 2 × 39,000 dalton subunits | Intermediate | Neuronal cytoplasm<br>Synaptosomes |
| glial fibrillary acidic protein (GFA) | Eng et al[49]<br>Delpech and Buffe[50] | 40,000–54,000 daltons | Vertebrates, including fish and birds | 1-7 peptides | High | Astrocytic cytoplasm and processes |
| $\alpha_2$-Glycoprotein | Warecka et al[51] | NE‡ | NE; humans and rodents | —§ | NE | White matter |
| Fetal brain antigen(s) | | | | | | |
| Fetal antigens I, II, III | Kehayov[52] | NE | NE; humans | — | Low | Fetal brain cells |
| Fetal antigen | Wikstrand and Bigner[53] | NE | Humans | — | Intermediate | Fetal brain cell membranes |
| Surface antigens | | | | | | |
| D2 | Bock[47] | 139,000 | NE; humans and rodents | NE | Intermediate | Synaptosomal plasma membrane |
| MBA-2 | Martin and Martin[54] | NE | Humans, rodents, birds | — | High | Normal brain cell membranes |
| INMA | Akeson and Seeger[55] | NE | Humans, rodents | — | High | Normal brain cell membranes |
| $\theta$-type antigens | Brouet and Toben[56] | 35,000–55,000 | Humans | Stokes radius: 32.7 A | High | Normal brain cell membranes |
| Adult brain antigen | Wikstrand and Bigner[53] | NE | Humans | — | Low | Normal brain cell membranes |
| Cell-type antigens | | | | | | |
| Oligodendroglial-associated antigens | Poduslo et al[57] | NE | NE; humans, rodents, sheep | — | Intermediate | Oligodendroglia |

* Reference is to the first study reporting the antigen.

† Relative immunogenicity is noted as determined by the authors' ease in raising antiserums to the antigen in question and the titers of specific antibody obtainable.

‡ NE = not established. Under the column "Species distribution," "NE" may be followed by those species in which the marker has been identified if a species distribution study has not been done.

§ No data.

plied for myelinated central nervous system axons by the fibrous astrocytes.[74]

Warecka [75] has described $\alpha_2$-glycoprotein, an acidic protein containing neuraminic acid isolated from human brain that seems to be specific to central nervous system glial cells. $\alpha_2$-Glycoprotein is mainly present in hemispheric white matter and the spinal cord and is absent in peripheral nerves. The protein, which appears in the fetal brain at 14–18 weeks at the stage of glial myelination, appears to have fetal and adult forms that have been phylogenetically preserved, as demonstrated by homology with similarly isolated proteins from rhesus monkeys and rats.

Although organ-mince explant cultures of animal brain have been extensively investigated in studies of myelinogenesis,[76] very few studies have monitored the presence of myelin basic protein (MBP) in dissociated cell cultures of nervous system origin. Richter-Lansberg and Yavin [77] recently reported that although typical multilameller myelin membranes became microscopically apparent in rat embryo cerebral cells after 8 days in culture, electrophoretic protein pattern analysis did not reveal the existence of substantial amounts of myelin-specific proteins. This, coupled with the observation of low CNP activity, led the authors to conclude that the "myelin structures" apparent in their cultures were either biochemically undetectable or incomplete. McDermott and Smith [78] reported that two human brain tumor cell lines—HB1 derived from an oligodendroglioma and HB3 from an astrocytoma—did not produce MBP in culture as determined by radioimmunoassay; bulk-prepared human oligodendroglia did produce detectable amounts. Similarly, D. Thomas has been unable to demonstrate detectable levels of MBP in over fifteen well-characterized permanent human glioma cell lines from our laboratory by radioimmunoassay (unpublished data). The absence of MBP in dissociated cell cultures is notable and substantiates the postulate that the production of myelin by oligodendroglia might be the result of two messages received from intact axons: the first, to produce myelin, the second, to surround the axon.[79]

#### Cell Surface Antigens

Cell surface antigens of normal human brain cells have been reported, but the biochemical characterization of these moieties has, to a large degree, not been done. Isolation and characterization of mammalian brain plasma membranes by amino acid composition analysis suggested a resemblance of brain membrane proteins to the reported composition of erythrocyte ghost proteins but a lack of homology with myelin proteins.[80] Glick et al [81] reported that the glycopeptide moieties removed from the cell surface of human brain cells were composed of less complex oligo-

saccharides than those removed from human neuroblastoma cells, perhaps suggestive of a greater antigenic complexity of the tumor cells. Gregson et al [82] have successfully demonstrated that chloroform–methanol extracted gangliosides from the surface of human brain cell plasma membranes are interspecies antigenic moieties capable of inducing complement-fixing rabbit antibodies specifically reactive with rat cerebellar cells; the antigen involved appears to be glycolipid in nature.

Enumeration of normal glial-specific surface antigens is complicated by the presence of antigens shared or cross-reactive with antigens of microorganisms or other vertebrate tissues. Biberfeld [83] and Lyampert [84] have described complement-fixing, predominantly IgM antibodies present in patients infected with *Mycoplasma pneumoniae* that were highly reactive with human brain; absorption with *M pneumoniae* removed this activity. Dorling et al [85] and Kingston and Glynn [86] have reported that rabbit anti-mouse brain antibody [85] or serum from patients with Sydenham's chorea,[86] both of which were reactive with fibrous astrocytes, could be rendered devoid of astrocyte-specific activity following absorption with *Streptococcus pyogenes*, Type 24.

Phylogenetically preserved interspecies normal brain cell surface antigens have been described. Martin and Martin [54] first described the interspecies brain-specific antigen MBA-2 recognized by naturally occurring mouse IgM antibrain autoantibody; this normal tissue differentiation antigen has been detected on normal human brain, kidney, and neuroblastoma cells but not on liver, lung, muscle, spleen, or thymus. Additionally, the antigen has been found on rat, guinea pig, and chicken cells. Akeson and Seeger [55] have reported an interspecies neural membrane antigen, INMA, which is present on cultured human and murine neuroblastoma cells and on normal adult brain of both species. An analysis of cell line distribution of INMA and MBA-2 indicates that they are not identicial. A similar antigen shared by human and murine neuroblastoma and normal human brain and absent from human and murine lymphoid cells and murine brain has been reported by Casper et al.[87] Recently, Campbell et al [88] and Schachner et al [89] reported brain cell surface antigens common to murine, bovine, and human brain cells, detected by a rabbit antiserum raised against murine corpus callosum.

A large number of reported interspecies brain-associated antigens are also expressed on lymphoid cells. The original report by Reif and Allen in 1964 [90] of $\theta$(THY-1) antigen on mouse lymphocytes and brain cells was followed by demonstration that THY-1 expression in brain tissue was localized to synaptic membrane and vesicles [91]; it has subsequently been shown that this cross-reactive T-lymphocyte rat brain antigen is detected by rabbit antirat brain tubulin antiserums.[92]

Although usually confined to expression on the T cell subset of lymphocytes, Santana et al [93] have recently reported the presence of THY-1-type antigen on mouse B cells. Similarly, Golub [94] reported a cross-reactive murine brain-hematopoietic stem cell antigen. Analogous cross-reacting brain lymphoid antigens have been demonstrated on human tissues. Using a rabbit antihuman fetal brain serum, Brouet and Toben [56] have described an antigen common to a subset of human T lymphocytes (23% of peripheral blood lymphocytes and less than 1% of thymocytes) and fetal brain. Belokrylov et al [95] extended these observations, reporting that rabbit antiserums raised against mouse, rabbit, guinea pig, or human cerebral cortex gave cross-reactions on lymphocytes of thymus, lymph nodes, and spleens of animals of these species; several reports of cross-reactive antigens shared by human and murine normal brain and lymphoid tissues have since appeared.[96,97] Arndt et al,[98] using a rabbit antihuman brain serum, were able to characterize the human thymus–brain antigen at the molecular level; the approximately 55,000 dalton protein isolated by gel filtration bears the non-species-specific determinant of thymocyte–brain antigen first detected in mice and rats.[94] The murine allogeneic markers Thy-1.1 and Thy-1.2 borne by an analogous molecule isolated from murine or rat cells are not detected in human brain, however, indicating that phylogenetic divergence is present at the molecular level.[98]

Other investigators have reported specific human THY-1-type antigens that are not expressed on rodent tissues. Immunization of rabbits with human brain material has been widely successful; Whiteside [99] has described the preparation of an apparently specific anti-human T cell antiserum, following multiple immunization of rabbits with human brain homogenate. Simiarly, Stratton and Byfield [100] reported that the majority of human T cells bear an antigen(s) cross-reactive with brain, identified by a rabbit antihuman brain antiserum. Further evidence of a human THY-1-type antigen distinct from the rodent system was provided by Takada et al.[101] These investigators reported that a hyperimmune, cytotoxic rabbit antiserum raised against the human T cell line MOLT could be rendered totally nonreactive for human T cells by absorption with human brain but not with rat brain. Absorption with human brain did not remove the activity of an antiserum similarly raised against the human B cell line RPMI-1788. Similar to the report by Santana and Turk [93] implicating a THY-1-type antigen on murine B cells, a report by Bluestein and Zvaifler [102] showed that a lymphocytotoxic antibody could be isolated from the serum of patients with systemic lupus erythematosus (SLE) that does not distinguish between T and B cells of normal human peripheral blood leucocytes but is equally cytotoxic to both cell types. The lymphocytotoxic activity of this antiserum was depleted by greater than 90% by absorption with

human brain but not with mouse or rat brain. As the titer of this antibody was highest in those SLE patients with central nervous system manifestations, these data are highly suggestive of a shared human brain–lymphoid antigen not restricted only to T cells.

Both Jellinger and Denk [103] and Gupta [104] have reported the absence of normal human blood group isoantigens from normal human brain and nerve cells, as opposed to their expression in brain endothelium. The inability to detect precursor H substance in normal human brain led Gupta to hypothesize that brain tissue normally lacks species-specific isoantigens.

Detection of small amounts of antigens in tissue samples is often difficult due to the presence of contaminating normal or stromal elements; the use of well-characterized, established cell lines that may be of higher antigenicity or immunogenicity can make detection of such small amounts possible. Using a panel of intensively characterized human brain tumor cell lines,[105] Wikstrand et al [106] were able to demonstrate the expression of HLA antigens on these cultured cells both directly, by successful typing with a panel of HLA typing serums, and indirectly by demonstrating a significant diminution in reactivity of these cell lines with antihuman glioblastoma multiforme serum following removal of anti-HLA activity; this establishes that cultured human brain tumor cells have the capacity to, and do, express species-specific antigens.

Brain cells have also been demonstrated to express antigens formerly thought to be tissue-specific for other cell types; Toh et al [107] have observed positive reactivity of human astrocytes with an antiserum reactive with smooth-muscle–associated antigen by immunofluorescence. Vaheri et al [108] detected the cultured fibroblast surface antigen SFA, or fibronectin, on the fibrillary processes of cultured normal human glial cells; the antigen appears to be produced by maligant human glial cell lines but is not retained on the cell surface.

#### Human Fetal Glial Antigens

Apparent human fetal glial specific antigens have also been reported. Manasek and Cohen [109] have identified two major surface anionic glycopeptides of developing neural crest; papain and tryptic digests of these glycopeptides established that they were principally carbohydrate in nature. The authors hypothesized that independent sorting of as few as five carbohydrate moieties could create a vast number of qualitative surface differences. Kehayov et al,[52] using rabbit antiserums raised against aqueous extracts of fetal brain, have demonstrated that 8–10-week human embryonic brain contains three brain-specific antigens: 1) a phase-specific antigen(s) unique to the 8–10-week gestational stage; 2) an antigen

characteristic of embryonic and fetal brain through the 30th week of gestation, and 3) an antigen shared by adult and fetal brain. This last antigen is similar to those fetal–adult shared antigens described by Warecka and Muller [110] and by Willson et al.[111] Trouillas [112] has described a shared fetal-brain-astrocytic tumor series specificity apparently different from that elaborated by Kehayov [113]; detected by human antiserums obtained after autologous glioma extract immunization, this "carcinofoetal antigen" appears in fetal brain at approximately 2 months' gestation, remains until the eighth or ninth month, and is undetectable in adult human brain. As defined by electrophoresis, it is a fast-moving lipoprotein staining intensely with Sudan black and migrating in the $\alpha_1$ region. Finally, Dittman et al,[114] using a rabbit antiserum raised against 12–16-week-gestation human fetal brain, identified five normal human fetal brain antigens (HF1-HF5) by crossed immunoelectrophoresis; the relationship of any of these antigens to those described by Kehayov et al [113] has not been determined. Recently, results obtained in our laboratory have shown that the predominant antibody response of nonhuman primates to cultured human glioma cells is against normal-brain–associated antigens, most notably against 22-week-gestation human fetal-brain–associated antigens.[53] The amount of serologic cross-reactivity between the glioma and fetal-brain–derived cells is extensive and specific to cell lines of normal glial or gliomatous origin, as measured in antibody-mediated cytotoxic, binding, and immunodiffusion assays; whether this antigen is analogous to the 22–30-week antigen reported by Kehayov [52] is unknown. Another antigen (OFA) strongly expressed on human fetal brain of 22 weeks' gestation has also been found to be expressed by a variety of different histologic types of biopsied tumors (melanoma, breast carcinoma, various sarcomas), cultured tumor cells (melanoma, breast carcinoma, sarcoma, lymphoma, leukemia), and cultured normal cells (skin, muscle).[115]

#### Glial Cell-Type Antigens

Reports of antigens unique to various cell types of normal brain have appeared. As described above, the intracellular antigens GFA and NSA-1 appear to be specific astrocyte markers.[68,69,73–75] Similar to the description of subsets of specific surface antigens of rat neuronal cells (N1,N2,N3) and glial cells (G1,G2) by Stallcup and Cohn,[116] is the report by Poduslo et al [57] of interspecies glial cell antigens. Rabbit antiserums raised against bulk-prepared, gradient-purified cell populations enriched for human, rat, or lamb neurons or oligodendroglia specifically differentiate between these cell types, as detected by indirect membrane immunofluorescence of rat, lamb, or human cells; cross-absorption analysis verified the specific-

ity of the antineuronal and antioligodendroglial cell serums. Recently, several authors have reported that galactocerebroside, the major glycolipid in myelin, can be used as a specific cell surface marker for oligodendrocytes of rat [117] and cow [118] grown in culture. Rabbit antiserums raised against galactocerebroside bound specifically to oligodendrocytes in culture and precipitated labeled galactocerebroside in a radioimmunoprecipitation assay; both of these activities were removed by absorption with oligodendroglia or myelin. Both cell binding and precipitation titers were decreased following absorption with other galactose-containing glycolipids, such as monogalactosyl and digalactosyl diglyceride. Moieties lacking galactose (ceramide, sphinogosine) or lacking a galactose adjacent to sphingosine (glucocerebroside, lactocerebroside) fail to absorb this activity.[118] Thus, the antigalactocerebroside activity has been demonstrated to be highly specific and potentially suitable as a definitive cultured oligodendrocyte marker. Delpech et al [119] have described an antigen, NSA-3, which is present only on cells from mature primate brains; localization studies by immunofluorescence with a rabbit antiserum raised against a high molecular weight human brain protein isolated by Sepharose gel chromatography suggested the oligodendrocyte as the reactive cell.

#### Expression of Normal Human Glial Antigens by Human Glioma Cells

In 1936, Siris [120] made the original observation that rabbit antiserums prepared against aqueous extracts of normal human brain or glioblastoma tissue exhibited the same reactivity profile in complement fixation tests, namely, recognition of antigens associated with normal brain. Since then, several investigators have demonstrated the existence of organ-specific antigens in malignant brain tissue [121,122]; Delpech et al [123] prepared rabbit antiserums to normal human brain or glioblastoma multiforme tissue and, by immunofluorescence, immunodiffusion, and immunoelectrophoresis, demonstrated that very low levels of "normal brain antigens" were present in the extracts of human glioblastoma and that specific "glioblastoma" antigens were detected. Similarly, Kehayov et al [124] reported antigenic reduction of organ-specific brain antigens in human glioblastoma; Wickremesinghe and Yates [125] postulated that the loss of organ-specific antigens from glioblastoma multiforme tissue that they observed is part of a continuum; (glia-specific) antigen material is present in the benign and less anaplastic neoplastic cells and absent from the highly anaplastic cells, representing qualitative and quantitative differences between normal and neoplastic cells in the distribution of antigens in cell membranes. Dittman [114] also reported the apparent loss of the fetal-human-brain–associ-

ated antigens HF1-HF5 from extracts of glioblastomas by crossed immunoelectrophoresis; neither was the fetal tissue marker α-fetoprotein detectable. The quantitative and qualitative normal-brain–associated antigenic variability of neoplastic glial cells has been recently emphasized by Wikstrand et al,[53] who demonstrated that normal brain antigens expressed by human brain tumors are quite immunogenic. Nonhuman primate antiserums raised against glioblastoma multiforme tissue or cultured cell lines were used to demonstrate unique patterns of normal adult and fetal-brain–associated antigen expression by a large panel of cultured cell lines derived from human glioblastoma multiforme. Although the association of antigen expression and degree of anaplasia was not observed in these studies, the highly variable expression of normal-brain–associated antigens was postulated to reflect the unique characteristics of the cell population(s) that gave rise to the tumors.

As is evident in Table 2, several investigations of the expression of specific markers have yielded variable results, consistent with the observation of variable "normal brain antigenicity." Popova [136] reported an increased content of glucocerebrosides in human gliomas as compared with surrounding "normal" brain tissue; whether this represented an increase in specific moieties was not examined. Furman and Shulman [127] reported that cyclic adenosine monophosphate (AMP) levels were significantly lower in several types of brain tumors, including glioblastomas, astrocytomas, meningiomas and carcinomas metastatic to brain, as compared with normal cortex and cerebellar levels; this decrease in cyclic AMP level paralleled the abnormally low levels of adenyl cyclase found in brain tumors. The observation that the level of enzyme and product was inversely related to the degree of malignancy, coupled with previous observations of tumor growth suppression by exogenous cyclic AMP, suggested to the authors that a defect in the adenyl cyclase system may contribute to successful tumor growth. Frequently, even with biochemically well-characterized markers, conflicting results are obtained. Slagel et al [122] initially reported that as detected by microimmunodiffusion, S-100 protein was present at a concentration comparable to that of normal (human) cortex in twelve glioblastomas, one astrocytoma, and one microglioma; present at a concentration less than that of normal cortex in one oligodendroglioma; and absent or at a very low concentration in one meningioma, one medulloblastoma, and one melanoma. Conversely, by quantitative immunoelectrophoresis, Dittman et al [114] found that levels of S-100 were greatly reduced (99%) in extracts of glioblastomas and absent from extracts of meningiomas, as compared with levels demonstrated in extracts of normal brain. Haglid et al,[128] in a study of 12 human brain tumors, proposed that

Table 2—Detection of Normal Human Brain-Associated Markers in Human Brain Tumor Cells

| Marker or antigen | Sample source | Tumor cell type* | Level of marker in relation to levels in normal tissue counterpart† | Reference |
|---|---|---|---|---|
| Adenyl cyclase | Tumor tissue extracts | ASTRO | decreased | 127 |
| | | GBM | decreased | |
| | | MEN | decreased | |
| S-100 | Tumor tissue extracts | ASTRO | increased | 128 |
| | | | variable | 122 |
| | | | present | 129 |
| | | GBM | decreased | 114 |
| | | | variable | 122 |
| | | | present | 128,129 |
| | | OLIGO, MEDULLO | variable | 122 |
| | | | present | 129 |
| | | MEN | variable | 122,129 |
| | | | absent | 114 |
| | Cultured cell lines | GBM | 3/15 present | 129 |
| | | | 12/15 absent | 130 |
| | | OLIGO | 3/3 absent | 130 |
| GFA | Tumor tissue extracts | ASTRO | increased | 73,130 |
| | | GBM | increased | 114 |
| | | | present | 130 |
| | | OLIGO | absent | 73 |
| | | MEDULLO | variable | 73 |
| | | MEN | variable | 130 |
| | | | absent | 73,114 |
| | Tumor tissue sections | ASTRO | present | 131,132 |
| | | GBM | present | 132 |
| | | OLIGO | present | 131 |
| | | MEDULLO | absent | 131 |
| | | EPEND | present | 131 |
| | | | absent | 132 |
| | | SUBPEND | present | 131,132 |
| | Cultured cell lines | GBM | absent | 133 |
| | | | variable | 105 |
| $\alpha_2$-Glycoprotein | Tumor tissue extracts | ASTRO | present | 134,135 |
| | | GBM | absent | 135 |

* Tumor cell type established by histologic evaluation of primary tumor tissue used for section, assay, or culture explant. ASTRO = astrocytoma, Kernohan grades I-II; GBM = glioblastoma multiforme; OLIGO = oligodendroglioma; MEDULLO = medulloblastoma; MEN = meningioma; EPEND = ependymoma; SUBEPEND = subependymoma.

† When estimations were quantitative versus normal tissue counterpart controls, a summary "decreased" or "increased" indicates levels different from that of controls, as discussed in the text. Where the levels were the same as that of control tissue or not quantitative (where merely a qualitative assay was run), "present" indicates the presence of a marker, "absent" indicates no detectable marker. "Variable" indicates that within the population studied, no trend was observable, individual samples having elevated, identical, or decreased levels of the marker in question as compared with the control.

levels of S-100 were inversely proportional to the degree of malignancy; the investigators maintained that levels of water-soluble S-100 were significantly increased in astrocytomas of Kernohan Grades I–III, as compared with lower levels in normal gray matter, glioblastoma multiforme (Grade IV), and medulloblastoma.

The presence or absence of the astrocytic marker GFA protein in human brain tumor tissue has been extensively investigated.[73,105,114,130–133] Initially, GFA was demonstrated nonquantitatively by fixed cell immunofluorescence in astrocytomas by Uyeda et al.[137] Although there is disagreement in the literature concerning the presence or absence of GFA in oligodendrogliomas,[73,132] meningiomas,[73,114,130] medulloblastomas,[73,131] ependymomas [131,132] and the quantity in glioblastomas,[114,130,132] there seems to be general agreement concerning the following: 1) that normal human tissue GFA and that isolated from glioblastoma tissue is immunologically identical [73]; 2) that nonastrocytic tumors in general have no, or very little, GFA [130]; 3) that GFA concentration is proportional to the number of astrocytes in the tumor [130] and decreases with increasing malignancy [131]; and 4) that determination of GFA levels contributes to the classification of "problem" gliomas (primitive or highly anaplastic central nervous system tumors, mixed gliomas and sarcomas, "astroblastomas," glioma invasion and metastasis, and metastases of non-central nervous system origin).[131,132,138]

Haas [134] reported the presence of glial-specific $\alpha_2$-glycoprotein in brain tumor tissue and occasionally in the cerebrospinal fluid of patients with brain tumors; as the acidic protein was not well-characterized until 1972,[51] definitive levels of the protein in the various tumors could not be determined. In 1975, however, Warecka demonstrated a marked difference between astrocytoma and glioblastoma multiforme: $\alpha_2$-glycoprotein was always present in the astrocytomas and absent in glioblastomas demonstrably free of residual astrocytes.[135]

One of the sources of the discrepancies in the reported levels of defined markers as illustrated in Table 2 involves the investigation of cultured cells. The use of cultured cells introduces another variable that must be controlled. Several investigators have reported atypical antigen expression by cultured cells: increased GFA protein expression on short-term cultured rat glial cells [69] versus the reported increase in GFA with the age of cultured rat glial cells [139] or the disappearance of GFA between 5 and 12 passages of human glioblastoma cells [133]; the appearance of "Hodgkin's" antigen on cultured normal spleen cells but not uncultured Hodgkin's disease tissue [140]; the presence of the cultured fibroblast surface antigen fibronectin (SFA) on fibrillar processes of cultured normal human glial cells and in the surrounding medium but not on the surface of cul-

tured malignant human glial cells [108]; the presence of the fetal antigen OFA on cultured normal skin and muscle but not on tissue samples [115]; the production of nerve growth factor (NGF) by cultured human glioblastoma cells [141]; and, finally, the recent description by Thorpe and Rosenberg [142] of another fetal antigen expressed readily by short- and long-term cultivated normal adult skin cells. These observations, coupled with the more definitive study by Jerry et al [143] that demonstrated the expression of several "fetal" antigens by normal adult tissue adapted to long-term culture, have indicated that normal cells "may reexpress fetal antigens under circumstances unrelated to neoplasia but associated with either maturation arrest or rapid and excessive proliferation."[143] This echoes the caution issued by Thomas [143] concerning the prevalence of phase-specific antigens in rapidly dividing cell populations being mistaken for tumor-specific antigens.

#### Human Glioma Antigens

Descriptions of glioma-associated antigens have been surprisingly numerous, but primarily phenomenologic, and rarely confirmed in separate laboratories. Investigations based upon purposeful specific immunization protocols and controlled analysis of any observed "specific" reactivity have been rare and, in general, incomplete.

One of the first suggestions of an immunologically detectable human glioma-associated antigen was obtained by Mahaley and Day [145]; samples of glioma removed at surgery were used to induce rabbit antiglioma antiserums which after extensive absorption and fractionation were conjugated to $^{125}I$ and injected intraarterially 3–5 days prior to surgical reexploration. At surgery, samples of normal brain, tumor, and gliotic regions were sampled; concentrations of $^{125}I$-antibody were higher in the tumor than in normal brain and gliotic areas and $^{131}I$-conjugated control protein levels in 18/20 patients.[146] The degree of localization and its true specificity, however, were not significant enough to warrant continuation.

Coakham [147,148] described a surface antigen apparently specific for human astrocytomas as demonstrated by a rabbit antiastrocytoma serum; however, the serum was not definitively HLA-nonreactive, nor were the cultures used shown to be mycoplasma-free. Using indirect cultured glioma immunofluorescence, Wahlstrom et al [149] described an apparent cultured glioma cell line specificity detected by a rabbit antilyophilized human glioblastoma multiforme tissue serum; again, the possibility of cell line contamination by mycoplasma was not eliminated. Both Miyake et al [150] and Sato et al [151] have produced rabbit antiserums versus human glioma cells; absorption with normal brain material was not demonstrated

to be complete, and the broad cross-reactivity of Miyake's normal brain–absorbed serum, which was positive for lung metastases to the brain and cultured HeLa cells, renders it useless as a glial tumor-specific probe. The most convincing evidence of a human glioblastoma-specific antigen has been provided by Kehayov.[113] Rabbits were immunized with saline extracts of either normal human brain or human glioblastoma in complete Freund adjuvant followed by multiple (12–14) immunizations without adjuvant. Serums obtained after this long-term immunization were analytically absorbed and assayed by immunodiffusion and immunoelectrophoresis versus saline extracts of adult and fetal brain, non-nervous system organs, and a large panel of gliomatous and nongliomatous tumors. Antiglioblastoma serums, after absorption with human organ extracts including normal adult brain, gave one precipitin line in immunodiffusion with 13/16 glioblastomas and 9/9 astrocytomas; the serums gave no reaction with normal adult brain, meningiomas, neurinomas, or cerebral metastases of non-nervous system tumors. Adult brain-absorbed antiserums did react with extracts of human fetal brain 8–10 weeks' gestation; however, one of the apparent tumor-specific antigens recognized by this antiglioblastoma serum migrated in the $\beta$ zone, and the second in the $\alpha_2$ zone; the 8-week fetal-brain–associated antigen migrated separately. A similar but less well characterized shared astrocytic tumor series-fetal brain antigen was described by Trouillas.[112] Defined by human patient serums obtained following autologous tumor extract immunization, the tumor-fetal antigen detected was a lipoprotein of $\alpha_1$ electrophoretic mobility, distinguishable from that described by Kehayov.[113]

Complications regarding specificity introduced by the investigational use of cultured cell lines or tissue or cell extracts are more easily controlled, however, than the variables introduced by definition in clinical studies involving the outbred human species. Although several investigators have reported glial tumor-associated antigen reactivity in preparations from glial tissue detected by serum or delayed cutaneous hypersensitivity reactions of brain tumor patients,[152–157] none of these antigen preparations have been biochemically characterized, nor have the patient serums been carefully absorbed or examined for nonspecific activity.

Studies investigating the reactivity of patient serums for autologous and heterologous glial tumor cells have been more numerous but less impressive. Sheikh et al [158] and Solheid et al,[159] studying fresh or snap-frozen glial tissue and cultured glial cells, respectively, reported the detection of apparent glioma-associated antigens with patient serums by immunofluorescence; the lack of definitive analytic absorptions and the presence of high levels of activity with control antiserums effectively refute claims

of tumor-specific activity. More convincing evidence of humoral host response versus a surface specificity is provided by Boker's [160] study of 38 patient serums versus autologous tumor cells by immunofluorescence; the lack of autologous nonglial tissue controls is a severe drawback. Quindlen et al [161] have reported that weakly cytotoxic, complement-dependent antiastrocytoma antibodies obtained from glioblastoma multiforme patients are best detected in a system using a 1:1 preparation of rabbit serum as a source of complement (C′) and human cord serum as diluent, IgM from the latter inhibiting the natural antihuman xenoantibody in rabbit serum. This would potentially increase the ability to detect very weak cytotoxic reactions, but only in those situations where C′ control background lysis is a significant complication. Pfreundschuh et al [162] reported antibody activity in glioma patient serums thought to bind with at least three classes of specificities: 1) idiotypic glioma antigens; 2) antigens limited to tissue derivatives of neural crest; and 3) a broadly cross-reacting specificity present on many cultured cells of human and nonhuman origin. When the response of patient antiserums to autologous fibroblasts was tested as well, providing a control for specificity of kill, Woosley et al [163] reported that the number of significant, detectable antiautologous tumor responses was less than 20% (7/36) for anaplastic glioma patients, 25% for benign glioma patients (1/4), and approximately 7% (1/13) for meningioma patients. These authors also investigated antibody-dependent cellular cytotoxicity (ADCC) in the same controlled autologous system, and found that only 25% (4/20) tested anaplastic glioma patients had positive ADCC reactions; Martuza et al [153] found no significant positives in a patient population of 30. Martin-Achard et al,[164] in a well-controlled study of patient serum activity by ADCC assay, concluded that the high frequency of control serum activity (20%) and the ability to remove the "specific" activity from the 17% of responding patient serums by platelet absorption "did not support the concept of a specific humoral response of glioma patients to a possible common tumor-associated antigen." Hitchcock et al [165] could not produce convincing evidence of cellular immunity in a patient who had survived for 18 years with glioblastoma multiforme, assessed by skin test and lymphocyte activation tests. Using the standard Hellstrom-type patient lymphocyte–target cell cytotoxic test and cell monolayer lymphocyte absorption, Levy [166] claims to have defined two antigens on the surface of glioma cells: CGA (common glioma antigen, shared by all glial tumors, regardless of the degree of anaplasia), and GEA (glioembryonic antigen), common to anaplastic gliomas, melanomas, and fetal glial cells but absent from well-differentiated gliomas, adult glial cells, and fetal fibroblasts. Levy reported that 85% (35/41) of glioma patients demon-

strated such tumor-directed lymphocyte-mediated cytotoxicity. In a companion report,[167] the author concluded that an observed classical autologous serum blocking effect was due to antibodies directed not against CGA or GEA but against tumor cell determinants homotypic to those expressed by the tumor of the serum donor. In both these studies [166,167] control "normal" glial cultures were established from the perimeters of biopsy material that yielded "glioma" cultures, rendering a true assumption of normal glial versus transformed glial cell difficult, and compromising the specific kill control. Conversely, using the same assay system, Woosley et al [163] were unable to demonstrate significant levels of cell-mediated reactivity in greater than 25% (7/36) of glioma patients. An assumption of tumor specificity is also not applicable as an explanation of the observed cell-mediated recognition of "glioma" antigens by lymphocyte adherence inhibition assay, since the normal control, CNS trauma, and aneurysm control patients reported by Sheikh et al [168] displayed occasional significant reactivity to "normal brain" and "glioma antigens."

Finally, both Catalano et al [172] and Winters and Rich [170] have reported tissue-specific intracellular meningioma antigens; immunodiffusion and immunofluorescence, respectively, were used to assay patient serum reactivity for meningioma cell extracts and cultured cells. Serum from glioma or meningioma patients appeared to have equal reactivity for the cellular preparations [170]; as approximately only half of the patient serum tested were reactive, these antigens can hardly be considered meningioma-distinctive.

#### Viruses and Brain Tumors

The possibility of virus production and/or virus antigen expression by glial brain tumor cells has been investigated. Manuelidis and Manuelidis [171] reported that an experimentally induced murine glioblastoma line producing C-type particles exhibited a greater than 20% decrease in satellite DNA as compared with normal glial cells; a relative underreplication of satellite DNA is hypothesized to be the result of incorporation of viral DNA at satellite regions. Cuatico et al [172,173] have reported finding "viral-like characteristics" in human brain tumors and cerebrospinal fluid of brain tumor patients; these demonstrations of reverse transcriptase, 70S high-molecular-weight RNA, and their encapsulation in particles with a density of 1.17 g/ml in sucrose gradients were not adequately controlled,[172] nor was an association between the disease state and "viral-like" characteristics established. Conversely, Becker et al [174] could find no evidence of human papovavirus T antigen (shared by three types of isolated papovaviruses JC, BK, and SV40) in a series of human brain tu-

mors examined by cryostat sections and tissue culture; this series was expanded to over 80 cases, and no evidence of viral expression was found.[15]

The ability of human glioma cells to support replication of retroviruses and to express retrovirus antigens, however, has been shown in our laboratory (unpublished data). An established permanent human cell line, D54 MG, derived from a mixed glioma,[105] was infected with a xenotropic (MUX) and an amphotropic (HIX) strain of murine leukemia virus. High rates of virus replication and membrane expression of structural protein antigens p30 and gp71 of murine leukemia virus occurred. Thus, although demonstration of an endogenous glioma-associated virus and/or antigen has not been accomplished in tissue[172] or cultured cell line,[174] cultured glioma cells are capable of supporting viral replication. The only possible example of a putative RNA tumor virus associated with a human brain tumor is the investigation currently in progress in Ponten's laboratory alluded to above, in which a simian sarcoma-like virus has been found in suspension-cultured cells derived from a morphologically rare type of primary human brain tumor (Ponten and Westermark, manuscript in preparation).

## II. Immune Responses in the Brain—The Question of Immunologic Privilege

Central to any discussion of a tumor–host relationship is the issue of immune surveillance—the elimination of maligant cells as they arise by the immune system of a healthy individual. The role of immune responses in the brain-tumor–bearing host has not been extensively studied, frequently because of the dismissal of the central nervous system as an immunologically privileged site, effectively separated by the exclusive blood–brain barrier from systemic immune responses. The fallacies inherent in such an argument are numerous.

### The Brain as an Immunologically Privileged Site

The unique immunologic status of the central nervous system does essentially depend on the blood–brain barrier, or, more specifically, on the "multiple, complex membranes of the cerebral capillaries, glia, neurons, myelin, arachnoid, and choroid plexus, as well as on brain and choroidal metabolism . . . "[8] As reviewed extensively by Shuttleworth,[175] the normal brain capillary endothelium lacks the fenestrations of its counterpart in other organs, and the tight junctions form an effective molecular sieve which excludes large proteins from normal brain parenchyma. This apparent limited access to the normal brain by systemically produced immune cells or globulins as evidence of immunologic privilege is weakened by the fact that antigens within the brain evoke a systemic immune response as

effective as or more effective than those administered systemically. The brain lacks lymphatic drainage *per se*, although in certain species there is limited direct access to cervical lymphatics through drainage along the perineural lymphatics of the optic and olfactory nerves.[176] To what extent this occurs in man is not clear. The reasonable anatomic explanation for an effective barrier system has been augmented by clinical observations. First, it has been postulated that the rare metastases of brain tumors in the absence of surgical intervention [177-179] are evidence for relative immunological privilege intracerebrally, lack of metastasis being the result of efficient extracerebral immune surveillance. The failure of glioblastomas to seed extracerebrally is somewhat problematical, in that malignant cells have been detected in venous blood draining gliomas [180] and are capable of growing at peripheral autologous extracerebral sites.[181,182] The failure of metastasis, however, could be ascribed to the relatively short survival of the brain tumor patient population or to nonimmunologic barrier phenomena.[8] Second, the observation by Schneck and Penn [183] that renal transplant patients during immunosuppression have a higher incidence of brain tumors than the general population has been used as an argument for the lack of immune surveillance in the brain; whether this is, theoretically, the result of localized intracerebral immune suppression, of systemic suppression, or of blast stimulation and increased incidence of reticuloendothelial transformation associated with immunosuppressive agents is not clear.

Direct experimental evidence has been presented to support the concept of immunologic privilege, based upon a lack of direct lymphatic drainage. Both Shirai [184] and Murphy and Sturm [185] demonstrated in the 1920s that tumors transplanted to the brain would often grow although the same tumor transplanted subcutaneously was rejected. These observations were updated and extended by Medawar,[186] who definitively showed that histoincompatible skin grafts that underwent rapid rejection when implanted subcutaneously would grow indefinitely when implanted within the substance of the brain away from the ventricular system. Similar experiments were subsequently reported by Greene,[187] using heterologous donor–host intracerebral tumor transplants. Habel and Belcher [188] added quantitative data to observations of increased graft survival; they showed that the threshold dose for 100% take of transplantable tumors in syngeneic hosts was 100- to 1000-fold less for tumors injected intracerebrally, compared with the same tumor transplanted by the subcutaneous route. Recently, Morantz et al [189] ascribed the failure of systemic immunoenhancement or immunosuppression by lifetime administration of bacille Calmette Guérin (BCG) or thymectomy and antilymphocyte serum, re-

spectively, to alter the incidence of brain tumors in rats exposed intrauterinely to ethylnitrosourea versus that of controls, to the immunologic privilege of the brain. The authors concluded that efferent delay due to lack of direct lymphatic drainage and afferent block of immune cells by the blood–brain barrier effectively inhibited a productive systemic immune response and therefore supported the concept of immunologic privilege.

#### The Brain as a Partially Immunologically Privileged Site

Although the afferent arc of immune responses in the central nervous system is poorly understood, there is a considerable body of evidence demonstrating that there is no effective barrier prohibiting the access of effector cells to the brain, especially to neoplastic areas of the brain. The increased permeability of the blood–brain, or, specifically, the blood–tumor barrier, in brain tumor patients as detected by radioisotopic brain-scanning techniques has been long known and suggests the possibility of an immune cellular and/or humoral reaction intracerebrally at the site of the tumor.[8] In addition, defects in the vascular endothelium of glioma capillaries have been demonstrated by electron microscopy,[175] thus hypothetically permitting the access of sensitized lymphocytes or immunoglobulins to brain tumor cells.

A classical examination of anti-brain tumor immunity was reported by Scheinberg et al,[190–193] using a methylcholanthrene-induced ependymoblastoma of the mouse stain C57BL/6J. This intracerebrally transplantable tumor was capable of eliciting first set rejection in allogeneic hosts and a second set rejection of either subcutaneous or intracerebral tumor cell implants in syngeneic hosts; this second set reaction could also be elicited by primary subcutaneous transplant or immunization with ependymoblastoma cells plus complete Freund adjuvant.[191] These studies comprise a clear demonstration of only partial immunologic privilege; however, the rejected tumors did not undergo the same histologic series of events that would have taken place in systemic graft rejection. Rather than displaying a large number of infiltrating lymphocytes in a perivascular and diffuse pattern, the characterized systemic rejection reaction, the rejected transplanted ependymoblastomas contained only a slight degree of cellular infiltration and primarily underwent a type of hyaline degeneration.[192] That rejection of grafts in the brain may not follow the sequential events seen extraneurally has also been suggested in studies by Ridley et al[194–197] that implicate the polymorphonuclear leukocyte rather than the small lymphocyte as the primary cell associated with intracranial tumor and skin grafts during rejection. Polymorphonuclear leukocytes appeared

in intracerebrally transplanted skin grafts that were ultimately rejected prior to total necrosis of the graft. Moreover, macrophages actively infiltrated the grafts and were associated with separation of the epidermis from the dermis.

Other lines of evidence support the observations that effector cells of systemic origin can enter the brain. First, either experimental allergic encephalomyelitis (EAE) can be rapidly induced directly or lesions can be produced by passive transfer of cells.[198] The destructive lesions that occur within the central nervous system are often massive, and the cellular component seems to be predominantly the small lymphocyte. Levine [199] has shown that in lymphocyte-depleted animals treated with cyclophosphamide, fulminant EAE developed with a predominant polymorphonuclear leukocyte cellular component. Second, autochthonous tumor rejection of virally induced experimental gliomas has been observed in the brain with the typical features of the homograft rejection response [200]; diffuse and perivascular small-cell lymphocytic infiltration characterized the lesions. Third, although it occurred less frequently than in breast tumors, lymphocytic infiltration, especially perivascular accumulations of small lymphocytes, was observed in 33% of over 100 glioma cases studied by Ridley and Cavanagh [201]; similar infiltration was observed by Takeuchi and Barnard.[202] Recently Wood and Morantz [203] evaluated the lymphoreticular infiltrate of human central nervous system tumors; their conclusion was that most nervous system tumors contained "high numbers of infiltrating host cells, primarily macrophages." Although the number of cases was small, 9 glioblastomas studied had a mean macrophage content, as quantitated by EAC rosette formation, of 41% (range 5–78%), similar to that of meningiomas, while medulloblastomas displayed very little cellular infiltration.

The passage of immune cells through the "blood–brain" barrier is predictive of the success of humoral antibodies in reaching the brain. Although antibodies locally synthesized by sessile B lymphocytes within the cerebrospinal fluid space have been demonstrated as characteristic of various inflammatory diseases of the central nervous system,[204] most studies investigating the presence of antibody molecules on the surfaces of brain tumor cells have concluded that cell-bound immunoglobulins in brain tumors are the result of transudation of serum constituents through altered tumor capillary walls.[205] Tabuchi and Kirsch [206] demonstrated the presence of IgG on tumor cells of 3/9 tested glioblastoma multiforme biopsies by immunoperoxidase staining and postulated that this was presumptive evidence for an antigen–antibody complex *in vivo*; however, since Aarli et al [207] have demonstrated that normal human IgG binds to myelin sheaths, glia, and neruons via the Fc portion of the molecule and Brett and

Weller [208] have postulated that the normal astrocytic function of serum protein uptake is lost in poorly differentiated glioma cells but retained to a variable degree by reactive astrocytes, an assumption of tumor specificity by Tabuchi and Kirsch seems unwarranted.

It is evident, then, that the apparent immunologic privilege of the brain has to be altered to "partial privilege," in that 1) graft rejection, albeit retarded, does occur intracerebrally; 2) intracerebral transplants induce a systemic immune response; and 3) effectors of systemic origin enter the brain parenchyma. This "loss of privilege" is most probably the direct result of barrier compromise as a result of the typical neovascularity accompanying the neoplastic transformation and growth of gliomas or occurs on an inflammatory basis, as in EAE.

### Immune Response Capability of Brain Tumor Patients

#### General Immune Status

There are several studies that indicate that glioma patients, although usually not cachectic, wasted, or malnourished at the time of brain tumor diagnosis, are markedly immunosuppresed, particularly in cell-mediated immunologic functions.[209–215] In general, brain tumor patients have been shown to have impaired cutaneous reactivity to tuberculin, Candida, Trichophyton, mumps, and streptokinase.[209,210,213,216] *De novo* sensitization against dinitrochlorobenzene (DNCB) and keyhole-limpet hemocyanin is also suppressed,[216] a reflection of the observed subnormal response of brain tumor patients' lymphocytes to mitogens and allogeneic cells in vitro.[209,210,214,215] The most thorough investigations of the general anergy of brain tumor patients have been performed through the collaborative efforts of Brooks and colleagues at the University of Kentucky and Mahaley and colleagues at the University of North Carolina. These investigators have demonstrated that 1) brain tumor patients have a consistently lower peripheral blood count throughout the course of disease [213]; 2) this decrease was apparently in the class of T cells responsible for cell-mediated responses to DNCB [212]; and 3) while the B cell and Fc receptor lymphocyte populations in the peripheral blood of brain tumor patients remain unaltered, the population of complement receptor lymphocytes may be increased.[216] The specificity of these peripheral lymphoid cell alterations to glioma patients remains unclear, however, because of the lack of suitable neurologic disease controls in this study.

The general humoral immune status of brain tumor patients has not been extensively studied; although "blocking" serum factors have been postulated to account for depressed cell-mediated activity, Wahlstrom [217]

found no suppressive effect of patient serum on the *in vitro* response of lymphocytes from normal controls and brain tumor patients to purified protein derivative (PPD). Conversely, Young and Kaplan [218] reported the detection of a plasma factor(s) in the serum of brain tumor patients that inhibited the capacity of lymphocytes from normal controls and brain tumor patients to form T cell rosettes. Since preincubation of normal lymphocytes in "inhibitory plasma" did not affect the ability of these cells to undergo blast transformation in the presence of the mitogen phytohemagglutinin, the nature of this factor is unclear. Mahaley et al [213] analyzed the serum tetanus and influenza antibody titers of glioblastoma patients; a decline in the ability to respond to booster injection paralleled postoperative clinical deterioration. Although overall immunoglobulin levels were normal, elevated levels of IgM were detected in glioblastoma patients preoperatively and declined with the course of the disease. Elevation of IgM has also been reported in meningioma patients.[219] The elevated levels of IgM reported in these patients may not be specifically related to the underlying neoplastic condition. In neither study was it demonstrated that the observed "elevated levels" were the result of a true increase in IgM of high molecular weight versus the nonspecific increase in IgM of low molecular weight found in patients with a variety of diseases including amyloidosis, systemic lupus erythematosus, and chronic infections.[220,221]

### Specific Cell-Mediated Immunity of Brain Tumor Patients

As was reviewed above in "Human Glioma Antigens,"[152-157] the reliable detection of specific cell-mediated immunity to "glioma-associated" antigens has been severely curtailed by the lack of a truly glioma-specific antigenic preparation for use in *in vitro* analyses of lymphocyte stimulation or *in vivo* analyses of patient reactivity. Nonetheless, several investigators, using antigen preparations that have not been biochemically characterized or shown to be free of normal brain-associated activity, have claimed to have detected specific cell-mediated "anti-glioma" activity by a variety of assays including delayed cutaneous hypersensitivity,[154,222,223] Hellstrom microcytotoxicity assays,[166,167,224] blast transformation,[225,226] and lymphocyte adherence inhibition.[168] The only study in which the antigen preparations used for *in vivo* testing were analyzed by polyacrylamide gel electrophoresis revealed that the "glioma-specific" antigenically active fractions in tests of cutaneous delayed hypersensitivity contained specificities shared with normal white matter, thereby eliminating an assumption of true gliomatous specificity.[165]

The cell-mediated reactivity reported by these authors has been postulated to represent host response to tumor and to be the detectable coun-

terpart of the lymphocytic infiltration observed in human brain tumors.[201,202] Albright et al,[227] however, have reported that even an intracerebral response in glioma patients is likely to be inadequate. In a study designed to test the potential of intratumoral inoculation of purified protein derivative (PPD) in BCG-immunized patients to induce a localized, bystander immune response, these authors demonstrated that in none of the 10 patients intracerebrally inoculated was the extent of inflammation more than moderate, or the inflammatory infiltrate capable of encompassing the peripheral advancing margins of the tumor. This relative lack of response occurred in all patients, irrespective of the degree of response to intradermal PPD. These observations, coupled with Wahlstrom's demonstration that peripheral lymphocytes from glioma patients react equally well with cultured autologous tumor cells or adult and fetal glial cells by cytotoxic or blastogenic assays,[217,228] suggest that the cell-mediated response of the glioma patient may be neither specific nor effective.

### Specific Humoral Immunity of Brain Tumor Patients

The "specific" humoral reactivity of patient serums has been reviewed above in "Human Glioma Antigens"; in general, several authors have claimed the detection of specific antiglioma patient serums by cytotoxicity,[152,155,161,163] antibody-dependent cell-mediated cytotoxicity,[163,164] immunofluoresence,[158–160] and hemadsorption assays.[162] The problems inherent in such investigations with patient serums are numerous and require stringent controls for specificity, including autologous nongliomatous targets and paired allogeneic gliomatous and nongliomatous targets. Few, if any, studies have been so rigorously controlled; the group maintaining that approximately 80% of brain tumor patients demonstrate significant humoral cytotoxic reactivity against allogeneic astrocytoma cells [152,161] have recently reexamined the specificity and reliability of the microcytoxicity assay used, which even under ideal conditions did not control for tumor-associated versus tumor-specific activity; their conclusion is that an assumption of a tumor-specific humoral response is not warranted.[229] Martin-Achard et al,[164,230] in an extensive, well-controlled study of brain tumor patient serums by antibody-dependent cellular cytotoxicity, concluded that the ability to absorb out "positive" patient serum reactivity with nongliomatous absorbents, primarily platelets, and the high degree of presumably antinormal brain activity detected in control normal serums precluded the concept of a common (brain) tumor-associated antigen as detected by patient serums.

## III. Status and Prospects of Immunodiagnosis, Immunomonitoring, and Immunotherapy of Human Brain Tumors

The exquisite specificity characteristically exhibited by the immunologic reaction between antigen and receptor of either antibody or cell membrane reactive site has been the motivating force behind efforts to develop immunodiagnostic and immunotherapeutic methods. Any immunologic approach to diagnosis, disease status monitoring, or therapy requires 1) the existence of a specific, unique, and characteristic biochemical marker or antigen against which 2) highly specific reagents, presumably antibodies or cells with antibody-like receptors, can be produced. Although these prerequisites are common to all three procedures—diagnosis, monitoring, and therapy—the former two frequently can be accomplished through *in vitro* assay, thereby eliminating the necessity for a biologically tolerable immune reagent, which is inherently necessary in passive immunotherapeutic regimens.

### Markers of General Use in Monitoring Central Nervous System Neoplasia

Although there has been little clinical proof of the value of immunotherapy and immunoprevention, there are several situations, among which nervous system tumors provide some of the best examples, for the use of tumor-associated moieties for diagnosis and following the course and size of tumors. Several nonspecific markers, not necessarily immunologically detectable, have been studied in brain tumor patients. Weiss et al [231] have reported that the levels of acute-phase proteins ($\alpha_1$ acid glycoprotein, $\alpha_1$ anti-trypsin, haptoglobin, C-reactive protein) were significantly increased in glioma and nonneural solid tumor patients and were related to immune status as well as to the extent of tumor growth. Most investigators, however, have taken advantage of the unique opportunity afforded by the central nervous system—the cerebrospinal fluid (CSF) compartment—to attempt to detect markers of diagnostic or prognostic value. Initially, evaluation of the CSF was largely confined to cytological analysis, with widely variable diagnostic success [232,234]; but as summarized by Balhuizen et al,[235] the percentage of correct positive diagnoses based on preoperative cytologic examinations of CSF samples is low, approximately 15–20%, and thus of little diagnostic value. Noncellular markers in the CSF have proven to be variably reliable; Houck et al [236] reported that the ratio of fucose to hexose in the CSF glycoproteins in patients with primary brain tumor was increased as compared with normal control subjects, although the range of levels detected overlapped with that of neuro-

logic disease control subjects, rendering the assessment of fucose: hexose ratios nonspecific with regard to brain tumors. Buckell et al [237] concluded that lactate dehydrogenase (LDH) levels and isoenzyme distributions in the cerebral cyst fluids of brain tumors could be used as an index of malignancy, the amount of the $LDH_5$ isoenzyme being increased in fluids from more malignant tumors relative to more benign tumors. These values are not reflected, however, in the corresponding plasma or CSF, a situation that severely limits the use of LDH as a monitoring marker. A higher degree of utility is possible in the determination of polyamines in cases of medulloblastoma as reported by Marton et al [238]; lumbar CSF fluid showed an elevation of CSF putrescine levels that was significant as compared with that of normal control subjects and showed a positive correlation with clinical course in 15/16 patients. As monitoring of medulloblastoma patients by radionuclide and computerized tomography screening is complicated by the intracerebral location and seeding pattern of the tumor, the potential of this CSF screen, if its apparent specificity continues, is great. Similarly, Paoletti et al [239] have investigated the possibility of the use of desmosterol as a biochemical marker of glioma growth. Short-term administration of triparanol, a drug that blocks the conversion of desmosterol to cholesterol, to patients with a suspected malignancy, followed by determination of desmosterol and cholesterol levels in the CSF, results in detectable differences in the desmosterol/cholesterol ratios of brain tumor patients versus control subjects. The assay is not clearly predictive; 15% of observed results are not definable, and the false-negative rate is approximately 7%.[240] Seidenfeld and Marton [241] have recently collated the results of recent studies of putative CNS tumor markers and have calculated the relative sensitivity and specificity of each marker and combinations thereof. The authors have concluded that at the present time desmosterol and polyamine levels can be of predictive value in monitoring reoccurrence and therapy progress, and that multiple marker tests utilizing those assays with high specificity and sensitivity have the best predictive value for therapy. The recent successful demonstration by Carson et al [242] that polyacrylamide gel electrophoresis of CSF samples from patients with multiple sclerosis followed by resolution of individual components by radioimmunoassay for myelin basic protein is of diagnostic value, however, is an indication that specific immunologic evaluation of CSF is a realistic goal.

#### The Use of Defined Normal Brain Antigens in the Diagnosis of Human Brain Tumors

The detection of normal brain-associated antigens in human brain tumor material is referenced in Table 2. Reports of the detection of adenyl

cyclase, S-100, GFA protein, or $\alpha_2$-glycoprotein levels in human brain tumors has been extensive.[73,114,122,127-135] However, all of these markers are detected in extracts, cultured cell lines, or tissue sections of biopsy tissue; and although potentially valuable as an aid to histologic diagnosis or grading, they are not useful for the monitoring of disease progress or therapy. The lack of agreement between laboratories concerning the amount of a given normal brain-associated antigen, if any, present on various tumor cell types [114,122,128,129,131,132] precludes a systematic grading system. There appears to be general agreement that the number of GFA protein–positive cells declines with increasing malignancy in the astrocytic series [73,130,131,138,243,244]; for these tumors, the presence or absence of GFA might serve as an index of neoplastic differentiation. For nonastrocytic brain tumors, however, the utility of this marker is questionable.[73,114,130-132] The variability in antigenic expression encountered was summarized by Wickremesinghe and Yates in a general analysis of the presence of organ-specific antigens on a broad panel of human central nervous system tumors by immunodiffusion: ". . . there are qualitative *and* quantitative differences between normal and neoplastic cells in the distribution of (a given) specific antigen in cell membranes."[125]

#### "Specific" Human Tumor-Associated Antigens in the Diagnosis of Human Brain Tumors

The lack of demonstrably brain tumor-specific antigens (Section I) has precluded large-scale investigations of the use of specific brain tumor markers; however, the use of questionably specific moieties has been reported. Mori et al [245] claimed that the detection of elevated levels of "astroprotein," a cerebroprotein of the acidic 10B fraction originally described by Bogoch [246] by radioimmunoassay in the CSF or tumor cystic fluid was of diagnostic significance in 58% of glioma patients and 19–23% of patients with nongliomatous brain tumors and miscellaneous intracranial disorders, none neoplastic. The nonspecificity of this ill-defined moiety renders it clinically useless as a tumor-specific probe. Bogoch and Bogoch [247] have extended studies of two glioma-associated antigens—"astrocytin" and "malignin." Immobilized preparations of the latter antigen were used to detect "anti-malignin" antibody successfully in 92.5% of brain cancer patients and 86.6% of non-CNS cancer patients. The authors further contend that detection of anti-malignin antibody was of diagnostic value in detecting unsuspected cancer in 5 normal control volunteers; antibody levels were elevated in long-term survivors, leading authors to postulate that determination of anti-malignin serum titers would be of prognostic value. Because neither the methodology employed nor the specific results obtained have ever been published in sufficient detail for inde-

pendent analysis or reproduction in other laboratories, it is difficult to assess the value of these observations.

#### Immunotherapy of Human Brain Tumors

Any rational approach to "active" immunotherapy relies on the existence of an immune system that if not already activated, is inducible; moreover, the existence of tumor specific antigens is necessary for both "active" and "passive" immunotherapy. The additional requirement for biologically tolerable immunostimulators or agents used in patient treatment has contributed to the relatively slow progress in the development of immunotherapeutic regimens. Nonetheless, much work has been done with relevant animal models, and in recent years, several immunotherapeutic trials with human patients have begun. The immunotherapy of human brain tumors has been reviewed [8,216,248]; this section will briefly outline the rationale behind immunotherapy in general and review the immunotherapeutic trials with human brain tumor patients to date.

#### Immunotherapeutic Designs—Rationale and Classification

The autochthonous antitumor response is composed of several individual biologic reactions, some of which are beneficial and some detrimental to the host. The rational goal of any immunotherapeutic regimen, then, must be to stimulate or enhance those responses of value to the host, while having little or no augmentative effect on deleterious responses. At the present time, it would appear most beneficial to stimulate cell-mediated responses and suppress humoral immunity.[249] However, the observation that in some situations humoral immunity may be beneficial [250] emphasizes the need for dual specificity of immunotherapeutic systems: tumor specificity and immune response specificity.

The approaches to human cancer immunotherapy can be summarized under three headings. *Nonspecific immunotherapy* consists of methods that are designed to stimulate, augment, or boost the often subnormal immune capacities of the cancer patient nonspecifically by means of various adjuvants. The adjuvants frequently used include bacille Calmette Guérin (BCG), the methanol extraction residue of BCG (MER), Corynebacterium parvum, the chemical antihelminthic levamisole, and polynucleotides. *Active specific immunotherapy* refers to the specific immunization of the patient with treated tumor cells, "tumor antigen" preparations, or cross-reacting antigens (viral or bacterial) in an attempt to specifically or selectively augment functional immune reactions. *Passive*, or *adoptive, immunotherapy* involves the administration of immune cells, serum, or parts thereof from a specifically immunized donor in an effort to transfer specific immunity to the null-reactive or weakly reactive cancer patient.

All three types of immunotherapy—nonspecific, active specific, and passive, or adoptive—have been utilized in therapeutic trials with brain tumor patients; these studies are summarized in Table 3. Evaluation of the results of these trials is severely hampered by the multi-regimen therapy received by most glioma patients. Before entry into clinical immunotherapy protocols, the majority of patients have had surgery—biopsy, partial, or total resection, and additional radiation and steroid therapy and/or chemotherapy in an attempt by their physicians to deliver the best conventional care. Consequently, many immunotherapy subjects are clinically deteriorating, relapsed, or moribund, and the complexity of the therapeutic regimens employed makes critical analysis difficult.

Nonspecific immunotherapy has not been extensively investigated; Miki et al [251] attempted to stimulate the immune response of 45 primary brain tumor patients whose reactions to PPD were negative by intradermal inoculations of BCG; of the 65.3% (17/26) glioma patients whose PPD skin test was positive following BCG inoculation, the survival rate at 3 years after surgery was greater than 50%, as compared with non-BCG-immunized *historical* control subjects, whose 3-year survival rate was approximately 12%. BCG-inoculated patients whose PPD reaction remained negative had a rate of survival at 3 years that was comparable to that of the uninoculated controls. This demonstration of a correlation between immune capability and longer average survival is consistent with the observation that a relationship does exist between the degree of lymphocytic infiltration of a tumor and the long-term prognosis of the patient.[257,258] The study reported by Selker et al [252] utilizing systemic administration of Corynebacterium parvum is small and incomplete; no conclusions can be reached concerning benefit, due to the short duration of the observation period.

Active specific immunotherapy has been the most extensively studied, beginning with the initial studies by W. H. Bloom in 1960.[182] This study, however, as were others to follow, was difficult to interpret because of the lack of adequate and corroborative classification of the tumors being studied and separation into astrocytoma, anaplastic astrocytoma, and glioblastoma groups. Untreated glioblastoma survival is usually only 6 months, whereas astrocytoma patients frequently may survive for 3 to 5 years. Unless an independent pathologist also classifies the patient material, as is routinely performed for the United States Brain Tumor Study Group, great variations in survival times, and hence, interpretation of therapeutic modalities, may be introduced into a given study. These questions can be raised about the original study by W. H. Bloom in 1960, and about the patient series reported by Trouillas in 1973.[112] The study by Trouillas utilized 65 patients with malignant gliomas. These patients were divided

Table 3—Summary of Immunotherapeutic Trials

| Type of immunotherapy | Tumor* | Prior treatment† |
|---|---|---|
| Nonspecific | "Primary malignant brain tumors" (45) | Surg, Rad, Chem |
| | Primary brain tumors (6) | Surg |
| | "Glioblastoma" (49) | None |
| | "Glioblastoma" (6) | |
| Active specific | Astrocytoma Grades III and IV (27) | Surg + rad |
| | "Malignant gliomas" (38) | Surg + rad<br>Surg |
| | "Malignant gliomas" (10) | Surg<br>Surg + chem |
| | "Brain tumors" (5) | Surg<br>Surg + chem |
| Passive | Brain tumors of children (10) | Surg, Rad, Chem |
| | Glioblastoma multiforme (18) | Surg, Rad, Chem |

* Tumor type diagnoses frequently neither available nor consistent; the reader is referred to number in parentheses represents number of patients treated with the immunotherapeutic

† Prior treatment is extremely variable from study to study; again, the reader is referred to therapeutic regimens were compared, eg, surgery + immunotherapy versus surgery + entering immunotherapy protocols had variable therapeutic histories, including one, two, or

‡ Immunotherapy administered. A brief description is given. The reader is referred to the

§ Objective performance is a summary based upon the author's conclusions; all studies speculative.

into 4 treatment groups; surgery, surgery plus radiotherapy, surgery plus immunotherapy, and surgery plus radiation plus immunotherapy. In this study, immunotherapy consisted of viable autologous tumor cells combined with Freund complete adjuvant. Immunizations were given 5–7 days apart for a total of 4–10 inoculations. Of the 28 patients receiving

in Human Central Nervous System Cancer

| Immunotherapy administered‡ | Objective performance versus standard therapy§ | Investigators |
|---|---|---|
| 0.8 mg BCG ID 1–8 times | Extension of survival ($P < .05$) | Miki et al, 1976[251] |
| Systemic *Corynebacterium parvum* | Not significant; increased intracerebral pressure | Selker et al, 1978[252] |
| Intracarotid administration of *Clostridum butyricum* | 16/49 patients died during treatment; 33/49 no improvement | Heppner and Mose, 1978[253] |
| Heterologous non-neurological tumor cell lysate intratumorally and intravenously | No detectable extension of survival time | Gomez et al, 1978[254] |
| Irradiated (15,000 rad) autologous tumor tissue | Negative; at 30 mo, 27/27 immunized pts dead; 7/35 non-immunized alive | Bloom, 1973[255] |
| Viable autologous tumor cells + CFA | Best median survival in immunotherapy combined group; not significant | Trouillas, 1973[112] |
| BCG + autologous tumor cells treated with neuraminidase and mitomycin C; intratumoral PPD | Negative; no effect on median survival time | Albright et al, 1977[227] |
| BCG + autologous tumor cells treated with neuraminidase and mitomycin C; intratumoral PPD | Negative; no effect on median survival time | Ommaya, 1976[248] |
| Intratumoral transfusion of allogeneic bone marrow cells | Suitable control population not available; significance of long-term survivors unknown | Takakura, 1975[256] |
| Intratumoral transfusion of peripheral blood autologous lymphocytes | No significant change in 11/18 patients<br>Improvement in 7/18 patients | Young and Kaplan, 1978[8] |

each study for a detailed list of the tumors constituting any given "brain tumor" group. The protocol.
each study. Surg = surgery; Rad = radiation; Chem = chemotherapy. Where distinct radiation + immunotherapy, prior treatment is listed as "surg" or "surg + rad." Where patients three previous regimens, the prior treatment is listed as "{Surg, Rad, Chem}."
original study for details.
share suitable standard treatment control problems; the conclusions reached are primarily

therapy, 25 developed cutaneous hypersensitivity responses to autologous tumor cells following immunotherapy. From Trouillas' work, the median survivals obtained were 5.5 months for surgery, 7.5 months for surgery plus radiation, 7.4 months for surgery plus immunotherapy, and 10.1 for surgery plus radiation plus immunotherapy. Unfortunately, while these

statistics appear impressive, the lack of control for pathologic variation among the patients prevents any definite conclusions being drawn from this work. Lack of proper classification was not a problem in a similar trial reported by H. J. G. Bloom,[255] but totally conflicting results were obtained. Bloom randomized 62 patients with malignant gliomas into two treatment groups; the first group received surgery plus radiation, while the second group received surgery, irradiation, and immunization with irradiated autologous tumor cells. Comparison of survival time for these two groups demonstrated no significant difference. Unfortunately, of the 27 patients receiving autologous tumor cells, only 10 received more than one inoculation. In addition, the cells administered had been irradiated with 15,000 rads, a dose that has been subsequently shown to significantly decrease the antigenicity of tumor cells.[259] In contrast to Trouillas' study, skin testing of these patients revealed that none developed delayed cutaneous hypersensitivity responses to autologous tumor cells following therapy. More recent studies by Albright et al [227] and Ommaya,[248] using active immunization with neuraminidase and mitomycin C-treated autologous cells with the adjuvant BCG, followed by intratumoral PPD, have been unimpressive; Albright et al [227] observed no effect upon disease progression in the 10 glioma patients receiving immunotherapy, and all 5 of Ommaya's treated patients developed reoccurrences within 12 months.[248] No evidence of the induction of allergic encephalomyelitis (EAE) was reported in the latter two studies; however, Brooks and Roszman[216] recently reviewed the studies of Bloom [255] and Trouillas[112] and found a possible case of EAE in Bloom's immunotherapy group and a single occurrence of EAE in the immunotherapy group reported by Trouillas. The induction of EAE, most probably resulting from an immune response to cross-reactive moieties between brain and the immunizing autologous tumor tissue, is an inherent danger of active specific immunization with tumor tissue. Specifically, utilizing an immunization protocol involving doses of human glioblastoma multiforme in Freund adjuvant comparable to the doses used by Bloom and Trouillas, Bigner and Wikstrand [260] have demonstrated the ability to induce lethal, acute EAE in nonhuman primates (Macaca fascicularis) with human glioblastoma tissue containing undetectable levels of myelin basic protein (MBP) by radioimmunoassay. Conversely, these same authors have shown that cultured human glioblastoma multiforme cells, in total cumulative doses greater than $16 \times 10^9$ cells, are incapable of inducing even microscopically detectable EAE, whether or not adjuvants, including complete Freund, incomplete Freund, or bacille Calmette Guérin cell wall preparation (BCG-CWS), are incorporated into the immunization schedule. This observation is in line with the report by

Richter-Landsberg and Yavin [77] that undetectable levels or antigenically incomplete forms of MBP were elaborated by rat embryo cerebral cells in culture despite the appearance of multilamellar myelin membranes; similarly, D. G. Thomas has been unable to find detectable levels of MBP in this laboratory's over fifteen well-characterized human glial brain tumor cell lines by radioimmunoassay (unpublished data). The apparent lack of antigenic MBP in cultured cells [77] and the inability of cultured human glial brain tumor-derived cells, alive or lethally irradiated and incorporated with adjuvant,[261] to induce EAE in nonhuman primates have encouraged premature immunization trials. The most responsible protocols involve the immunization of the patient with HLA-mismatched, lethally irradiated cultured brain tumor cells, as in the successful non-EAE-inducing immunization regimens reported by Wikstrand and Bigner.[261] The observation in that model system, however, has been that the serums produced by primates in response to allogeneic, irradiated, or unirradiated human glial brain tumor cells are primarily directed against normal brain-associated antigens present on the cultured human glial brain tumor cells.[126]

Three groups have reported attempts to adoptively transfer competent lymphocytes either directly to the tumor cavity via intratumoral reservoirs inserted following surgical resection of the tumor [8,256] or to the subarachnoid space.[262] Takakura et al,[256] in a study of childhood brain tumors, including several cases of medulloblastoma and a few gliomas and astrocytomas, claimed significant extensions of survival time following the intratumoral administration of ABO and Rh-matched allogeneic bone marrow cells. Again, as was the case in the earlier active immunization studies,[112,182] the lack of independent pathological classification, the small number of patients, the lack of simultaneous control groups, and the long-term survival of conventionally treated medulloblastoma patients [46] make the interpretation of this data difficult. Similar problems mar the study of adult gliomblastoma multiforme patients receiving autologous peripheral blood leukocyte intratumoral infusions reported by Young et al.[8] The unchanged status of 8/18 patients following leukocyte infusion and the relatively short observation time of these patients, many of whom had received large amounts (6000 rads) of radiation therapy, complicates analysis. Neuwelt et al [262] utilized multiple direct injections of autologous lymphocytes into the subarachnoid space of patients with glioma; in the 4 patients studied, no toxicity was observed, but in only 1 patient was there post mortem evidence of lymphoid cell migration beyond the subarachnoid space to the tumor bed. None of these authors reported any instances of EAE; Young et al have looked extensively for clinically undetectable le-

sions in autopsy specimens by detailed light- and electron-microscopic review of the brain, peripheral nerve, and spinal cord.[8]

#### Overview and Prospectus

At this point it would be valuable to assess 1) the current state of affairs with regard to the brain tumor problem; 2) the major areas of weakness and needed research; 3) specific areas of research in progress, and 4) prospects for the future.

##### Status of Brain Tumor Research

It is apparent that the human brain tumor problem—characterized by bimodal age peak distribution, sex ratio differences, and variable predominance of tumor type with age—is a multivariate problem. No single etiology or pathogenetic mechanism can or probably will be identified, a situation that underlines the basic lack of knowledge about gliomas and their biology. Dismal as this may seem, recent progress has been made in several areas, and some pieces of the puzzle, if not placed, are in hand. First, only recently have the first etiologic associations been established for human gliomas, definitely in a few cases of radiation-induced tumors, and possibly in some cases of chemical carcinogens. Not only will recognition of causes of human gliomas be important for future prevention, but for their influence on the immunogenicity of gliomas. Second, our knowledge of basic markers and biochemistry of normal central nervous system cells has increased tremendously in past years. Procedures for isolating enriched or purified cell populations (oligodendroglia, neurons) and specifically identifying them by quantitative and qualitative markers have been developed, allowing investigation of the evolution and development of the tissues of the central nervous system. The expression—aberrant or normal—of some of these markers in neoplastic cells can now be determined, elucidating functional pathways in both normal and neoplastic cells. Against this defined backdrop of normal marker expression, it is now possible to investigate any potential differences in neoplastic cells—a goal recognizable for years in many systems, but only recently in neurooncology. Concomitantly, our knowledge of the immune capacity and reactivity of the brain tumor patient has been extended. The previous assumption of qualitative immune privilege of the brain has been extensively modified to the point of extinction, and the capacity for, and demonstration of, intracranial immune responses only quantitatively different from the rest of the body has occurred. The knowledge of the relative anergy of, and the need for an augmented immune response in, the brain tumor patient will be instrumental in formulating future immunotherapeutic

protocols. The immunotherapeutic attempts to date, although premature, have been instructive in defining which therapies may be well tolerated and which appear to be of minimal or no benefit. The field, then, can be properly summarized to be in a state of readiness for directed research based upon current basic brain tumor biology.

In summary, several facts are now clearly established about immunity and human gliomas.

1) The cause of at least some gliomas can be recognized.
2) Glioma patients at the time of diagnosis can be stratified in terms of immune responsiveness, from those who are severely depressed to those who are relatively intact.
3) Glioma cells both within and among gliomas have wide qualitative and quantitative ranges of expression of normal adult and fetal brain antigens.
4) The "immune privilege" of the brain compared to other organ sites is best described in quantitative terms relative to differences in rates of antigenic recognition and the afferent limb of the immune response than in an absolute sense.

### Areas of Needed Research

There are areas of basic brain tumor biology that need intensified investigation. The etiology of brain tumors in experimental animal models and in spontaneous human kindreds must be examined through basic virologic, environmental carcinogen, and physiologic techniques, coupled with cytogenetic analysis of the effects of possible etiologic agents. Are there detectable agents and cytogenetically detectable effects thereof? The basic immunobiologic questions still remain: Are there brain-tumor–distinctive—in a qualitative or quantitative sense—substances that can be used to monitor tumor size or as adjuncts in diagnosis? Is there a relationship between tumor size and antigenicity? What are the mechanisms involved leading to peripheral immunosuppression in many glioma patients long before they have large tumors or are systemically ill? Can an effective "cleaning up" immune response be induced in the relatively anergic brain tumor patient as an adjunct to surgery, radiation, and chemotherapy? Can such a response exist without the threat of EAE? From a tumor biological standpoint, what is the nature of neoplastic transformation in the central nervous system? Is the standard glioblastoma the result of uniclonal or multiclonal transformation of "stem cells," differentiated cells, or a heterogeneous mixture of cell types? Is there a relationship between aneuploidy and tumorigenicity or aneuploidy and antigenicity?

Research in Progress and Future Directions

Many of the basic questions identified above are currently being investigated. Investigation of the effects of chemical carcinogens on human populations are being compiled by Maltoni and colleagues in Italy and Wagoner et al in the United States. Although the shotgun search for viruses as etiologic agents has been nonproductive, improved knowledge and techniques in brain cell biology will make their detection, if present, possible. Basic morphologic studies of cultured human brain tumor cells are well established in Ponten's and Westermark's groups in Sweden and in our laboratory in the United States where a new program of karyotypic and cytogenetic analysis has begun. The identification of fetal antigen recapitulation in brain tumor cells has oriented current immunologic research, and the departure from the traditional immunologic approach through use of the hybridoma—monoclonal antibody technique in this and other laboratories should ultimately resolve questions of brain tumor antigenicity not approachable by less avid reagents. In the area of defining human glioma-associated antigens, we predict that the monoclonal antibody methodology will revolutionize the search for the characterization of tumor-associated antigens to the same extent that cell culture techniques revolutionized virology and led to the isolation of such long suspected agents as poliovirus.

Most investigators in the field would agree with Ridley's conclusion that "the present position in regard to gliomas, as with other solid cancers, is that immunotherapy on its own is not yet feasible."[263] Immunotherapy trials to date have been "discouraging" [264] or, at best premature and inconclusive.[8,216,248,265] Although the production of acceptable clinical data may be possible by the simplification and rigid control of clinical therapeutic protocols,[8] basic work must be accomplished in animal and *in vitro* model systems so that rational expectations regarding the future of immunobiology as applied to human brain tumor diagnosis, monitoring, or therapy will be generated.

There are basic efforts and approaches to this problem in progress. The use of the athymic nude mouse bearing transplants of human brain tumors [266] to study the differential chemotherapeutic sensitivity to various agents is undergoing investigation, the goal being to select suitable chemotherapy on an individual tumor basis. A similar approach is being investigated *in vitro*, using cultured cells in monolayer [267] or clonal agarose culture [268] in a screen of human astrocytoma cells for drug sensitivity by scintillation autofluorography.[269] The role that immunobiologic mechanisms will eventually play in brain tumor biology, whether of diagnostic, monitoring, or therapeutic application, will need to be defined through animal, *in vitro*, and controlled clinical trials.

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