Anaplastic Human Gliomas Grown in Athymic Mice

Morphology and Glial Fibrillary Acidic Protein Expression

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The morphologic and biochemical characteristics of human surgical biopsy specimens taken from 17 patients with anaplastic human gliomas and of athymic mouse-grown tumors derived from them were examined. Fourteen were categorized as glioblastoma multiforme, one as an anaplastic astrocytoma, one as a recurrent glioblastoma multiforme, and one as a gliosarcoma. Fifteen of 17 tumors stained positively immunohistochemically for glial fibrillary acidic protein (GFAP), a glial-specific marker. When portions of the 17 surgical biopsy specimens were injected into the flank subcutaneous space of athymic mice, 16 produced tumors; different portions of a single biopsy specimen were used to establish three separate tumor lines; *in toto*, 18 tumor lines were established. Mouse-borne tumors contained various proportions of fibrillary and protoplasmic astrocytes, gemistocytes, small anaplastic cells, and multinucleated giant cells. Some were more homogeneous than the human tumors from which they were derived, while others contained a mixed population similar to that of the original biopsy specimen. Of these initial 18 tumors, 16 were stained for GFAP and 14 contained from fewer than 5% to almost 100% GFAP-expressing cells. Ten of the tumor lines were studied in serial passage, several demonstrating increased cellularity with increased passage. GFAP expression was followed through serial passage, and 7 of 10 tumor lines continued to express it, often in reduced amounts, and 2 of 10 ceased expression, one (the gliosarcoma) never having expressed it. These data demonstrate that while athymic mouse-borne human anaplastic gliomas retained some features of the human tumors from which they were derived, they varied from one another morphologically. These mouse-borne tumors also continued to evolve, often changing their levels of GFAP and demonstrating increased cellularity with passage. (Am J Pathol 1981, 105:316–327)

THE ATHYMIC NATURE of the nude mouse was first reported by Pantelouris[1] in 1968, and the first transplantation of neoplastic human tissue followed shortly.[2] Since then, a multitude of human tumors has been transplanted to athymic mice, with a variety of success. But one characteristic of this technique continues to be evident. The tumors grown in this manner express a substantial degree of similarity to the cellular, histologic, and biochemical characteristics of the original biopsy specimens from which they were derived. For example, the first tumor transplanted to athymic mice was a mucoid-producing colonic carcinoma; it continued mucoid production in the mouse.[2] Hormone-producing tumors have been propagated in athymic mice, and production of such hormones as adrenocorticotropic hormone,[3,4] antidiuretic hormone, β-melanocyte-stimulating hormone,[3] erythropoietin,[5] and human chorionic gonadotropin (hCG)[6] have been demonstrated in the mouse-carried tumors. The hCG-producing tumor was still secreting this hormone after four serial passages in the mice, and the erythropoietin-secreting tumor after five passages. Other biologic markers, including melanin production,[7] carcinoembryonic antigen,[8] and α-fetoprotein[6,8] have continued to be expressed during serial passage. Mouse-borne tumors also can be stable with regard to their chromosome content.[9,10] This

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stability and the continued expression of differentiated characteristics suggest that the microenvironment within the mouse may not be dissimilar to the original setting in man. If this is true, then the athymic mouse–human tumor system may constitute the best available *in vivo* system for the study of human tumors, especially when chemotherapeutic sensitivities, heterogeneity of cell type, or growth kinetics is the subject of interest. In this study, we evaluated and compared architectural features and glial fibrillary acidic protein (GFAP; a glial-specific protein) expression in anaplastic human gliomas and in the tumors derived from them by serial passage in athymic mice.

Materials and Methods

Initiation of Athymic Mouse Tumors

Biopsies of human brain tumors obtained at surgery were carried to the laboratory in sterile 4 C serum-free Richter's zinc option medium (Gibco). The tissue was minced and injected into the right-flank subcutaneous space of congenitally athymic "nude" mice (nu/nu BALB/c). Both male and female mice were used. Tumor volume was monitored once or twice each week by measuring the width (a) and length (b), then applying the formula $a^2\ b/2$ to get an accurate estimate of the tumor weight.[11,12] The athymic mouse-propagated tumors were passed serially in the same manner in which the human surgical biopsy specimens were originally introduced into the first set of athymic mice. When the tumors reached dimensions of approximately 1 cu cm, the mice were killed and the tumors excised. Part of the tissue was passed into other mice, and part was retained for histologic and immunohistochemical analyses.

Peroxidase-Antiperoxidase Staining for Glial Fibrillary Acidic Protein

Tumors obtained at surgery and tumors grown in athymic mice were fixed in 10% formalin and then embedded in paraffin by conventional techniques.

The tumor biopsy specimens and mouse-grown tumors were stained for GFAP by a well-described peroxidase-antiperoxidase technique.[13,14] Eight-micron sections were rehydrated through a graded alcohol series, and any endogenous peroxidases were neutralized by a 30-minute incubation in an absolute methanol–0.3% hydrogen peroxide solution. In order to reduce nonspecific binding of the secondary antibody, all slides were incubated with 10% normal goat serum for 10 minutes, washed three times in phosphate buffer, and then incubated overnight at 4 C with rabbit anti-human GFAP serum (1:500 in 10% normal goat serum). The antiserum was prepared by injection into rabbits of GFAP isolated from human fibrous astrocyte-rich multiple sclerosis plaques by preparative polyacrylamide gel electrophoresis in Dr. Eng's laboratory.[13] To insure that the staining procedure was working properly and that each tissue section was capable of being stained successfully, a rabbit anti-human serum generated against a human glioma-derived cell culture line (U-251 MG) was used as a technique positive control. Phosphate-buffered saline and normal rabbit serum were used as primary antiserum negative controls, and sections of normal human brain were included as positive controls for the anti-human GFAP serum. After three washes, the slides were incubated with goat anti-rabbit IgG serum (1:40) for 30 minutes, then washed and incubated with rabbit peroxidase-antiperoxidase (1:100, Sternberger and Meyer, Inc.) for another 30 minutes. After they were washed, the slides were subjected to a 7-minute incubation with diaminobenzidine solution (5 mg diaminobenzidine in 10 ml 0.05 M Tris-HCl, pH 7.6, 5 ul 30% H_2O_2). After the peroxidase-diaminobenzidine reaction was stopped by washing with deionized H_2O, the slides were counterstained with hematoxylin or methyl green for 15 seconds and then dehydrated through a graded alcohol series to xylene and mounted.

Evaluation of Histologic and Immunohistochemical Data

Hematoxylin and eosin sections of all surgical biopsy blocks were coded and evaluated by one of us (SHB). Each tumor was categorized according to the World Health Organization Classification, and then each surgical biopsy and each athymic mouse-propagated tumor were evaluated for characteristics including necrosis, pseudopalisading, mitotic figures, vascular proliferation, and individual cellular morphology. Necrosis was considered to be absent (−), equivocal (±), present without pseudopalisading (+0), or present with pseudopalisading (++). We determined the number of mitotic figures from the most cellular area of each tumor by counting the number of mitotic figures in ten high power (400×) fields. Endothelial cell proliferation was judged positive (+) when reduplication of the endothelial cell layer and formation of glomeruloid vascular tufts were observed, negative (−) when these changes were absent, and intermediate (±) when only abnormally plump endothelial cells were seen. Morphologically neoplastic cell types were divided into six groups: 1) stellate and bipolar cells were called "fibrillary astrocytes"; 2) small round or fusiform

cells with little cytoplasm were called "small anaplastic cells"; 3) plump, round cells with abundant eosinophilic cytoplasm were called "gemistocytes"; 4) polygonal cells with round nuclei and pink or clear cytoplasm were called "protoplasmic astrocytes"; 5) large, densely packed spindle-shaped cells were called "anaplastic spindle cells"; and 6) large, bizarre, multinucleated cells were called "multinucleated giant cells."

Tissue sections from human biopsies, the first passage of these tumors grown in the athymic mice, and tumors serially passed that were stained for GFAP were coded and evaluated by two of us (SHB and TRJ). Cases were considered positive for GFAP if morphologically neoplastic cells contained the reaction product and the percentage of GFAP-positive tumor cells was subjectively evaluated.

Results

Morphologic Study of Human Surgical Biopsy Specimens

A total of 17 human brain tumor biopsy specimens was obtained at surgery for use in this study; 14 were classified as glioblastoma multiforme, one as an ana-

Table 1—Cell Types and Percentage of GFAP-Positive Tumor Cells Found in the Anaplastic Human Gliomas and the First-Passage Athymic Mouse-Borne Tumors Derived From Them

Tumor number	Tumor diagnosis	Cell types	% GFAP-positive cells	First passage	Cell types	% GFAP-positive cells
91	GBM	FIB ASTRO GEM SM ANA	75-100	91	SM ANA	<5
112-115	GBM	FIB ASTRO PROTO ASTRO	75-100	112	FIB ASTRO PROTO ASTRO	5-25
				114	PROTO ASTRO	5-25
				115	PROTO ASTRO	5-25
120	GBM	MNG CELLS GEM	50-75	120	PROTO ASTRO	ND
132	GBM	MNG CELLS, FIB ASTRO, GEM, ANA SPL	25-50	132	ANA SPL	50-75
137	AA	FIB ASTRO	75-100	137	FIB ASTRO	ND
142	GBM	FIB ASTRO PROTO ASTRO	5-25	142	FIB ASTRO	75-100
155	GBM	FIB ASTRO PROTO ASTRO	25-50*	155	FIB ASTRO PROTO ASTRO	0
175	RGS	FIB ASTRO ANA SPL	0	175	ANA SPL	0
183	GBM	FIB ASTRO GEM	75-100	183	FIB ASTRO GEM	75-100
241	GBM	GEM SM ANA	5-25*	241	SM ANA	<5
294	GBM	FIB ASTRO GEM SM ANA	50-75	294	FIB ASTRO GEM SM ANA	25-50
338	GBM	FIB ASTRO GEM	25-50*	338	FIB ASTRO	50-75
341	GBM	FIB ASTRO PROTO ASTRO	75-100	000	NA	NA
350	GBM	FIB ASTRO PROTO ASTRO GEM	75-100	350	FIB ASTRO	25-50
391	GBM	FIB ASTRO PROTO ASTRO MNG CELLS	25-50	391	FIB ASTRO SM ANA MNG CELLS	25-50
456	GBM	FIB ASTRO PROTO ASTRO MNG CELLS	25-50	456	FIB ASTRO	25-50
457	RGBM	FIB ASTRO	UI	457	FIB ASTRO	25-50

* These three tumors displayed a more diffuse GFAP staining pattern and lacked the usual perinuclear cytoplasmic staining seen in other tumors. While more difficult to interpret, we feel that these tumors are indeed positive.

FIB ASTRO = fibrillary astrocyte; PROTO ASTRO = protoplasmic astrocyte; GEM = gemistocyte; ANA SPL = anaplastic spindle cell; SM ANA = small anaplastic cell; MNG CELL = multinucleated giant cell; GBM = glioblastoma multiforme; AA = anaplastic astrocytoma; RGBM = recurrent glioblastoma multiforme; RGS = recurrent gliosarcoma; NA = not applicable; ND = not done; UI = uninterpretable.

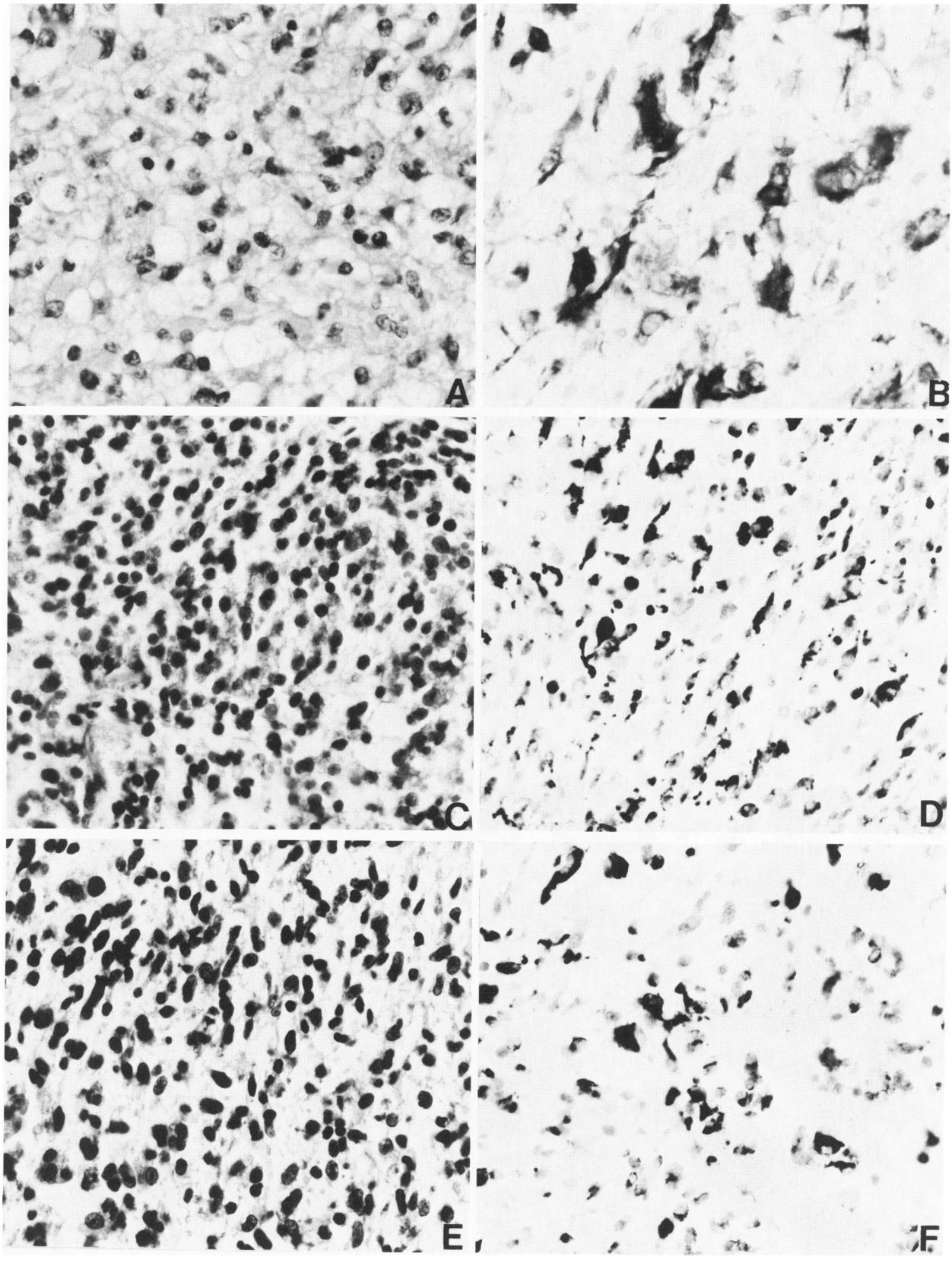

Figure 1—Anaplastic human glioma 112 and the athymic mouse-borne tumors derived from it. **A**—The original human surgical biopsy contains both fibrillary and protoplasmic astrocytes. (H&E, × 425) **B**—Peroxidase-antiperoxidase staining of GFAP reveals that the human surgical biopsy contains cytoplasm-rich GFAP-positive cells. (Hematoxylin counterstain, × 425) **C**—Transplantation of tumor 112 into athymic mice resulted in an increase in cellularity without a change in cell type; the tumor still consists of fibrillary and protoplasmic astrocytes. (H&E, × 425) **D**—Peroxidase-antiperoxidase staining of GFAP demonstrates areas rich in cells containing GFAP, while the tumor as a whole contained 5% to 25% GFAP-positive cells. (Hematoxylin counterstain, × 425) **E**—After four passages in the athymic mouse, the tumor has retained its original cell types and is still highly cellular. (H&E, × 425) **F**—Between 25% and 50% of the cells are GFAP-positive after four passages. (Hematoxylin counterstain, × 425) (With a photographic reduction of 4%)

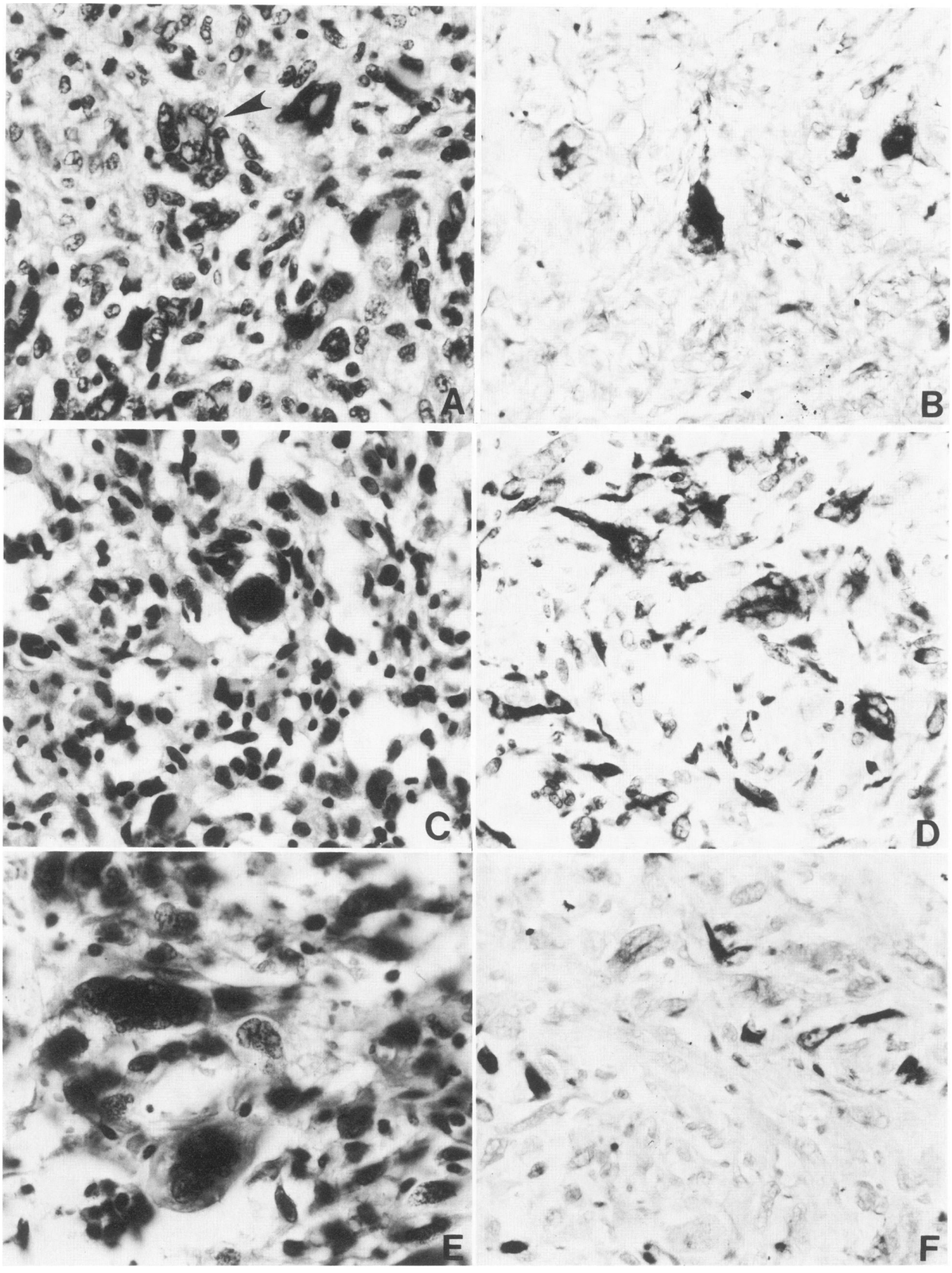

Figure 2—Anaplastic human glioma 391 and the athymic mouse-borne tumors derived from it. **A**—The original human surgical biopsy specimen contains a mixed population of cell types, including fibrillary and protoplasmic astrocytes and multinucleated giant cells (*arrow*). (H&E, × 425) **B**—Fewer than 50% of the cells in the surgical biopsy specimen are GFAP-positive when stained by the peroxidase-antiperoxidase method; the multinucleated giant cells are usually GFAP-positive (Hematoxylin counterstain, × 425) **C**—After transplantation to the athymic mouse, the tumor remains heterogeneous, with prominent multinucleated giant cells. (H&E, × 425) **D**—GFAP staining reveals that fewer than 50% of the cells are GFAP-positive. (Hematoxylin counterstain, × 425) **E**—At passage level three, morphology remains unchanged from the first passage. (H&E, × 425) **F**—GFAP-positive cells are seen less frequently (fewer than 25% of the cells) by the third mouse passage. (Hematoxylin counterstain, × 425) (With a photographic reduction of 3%)

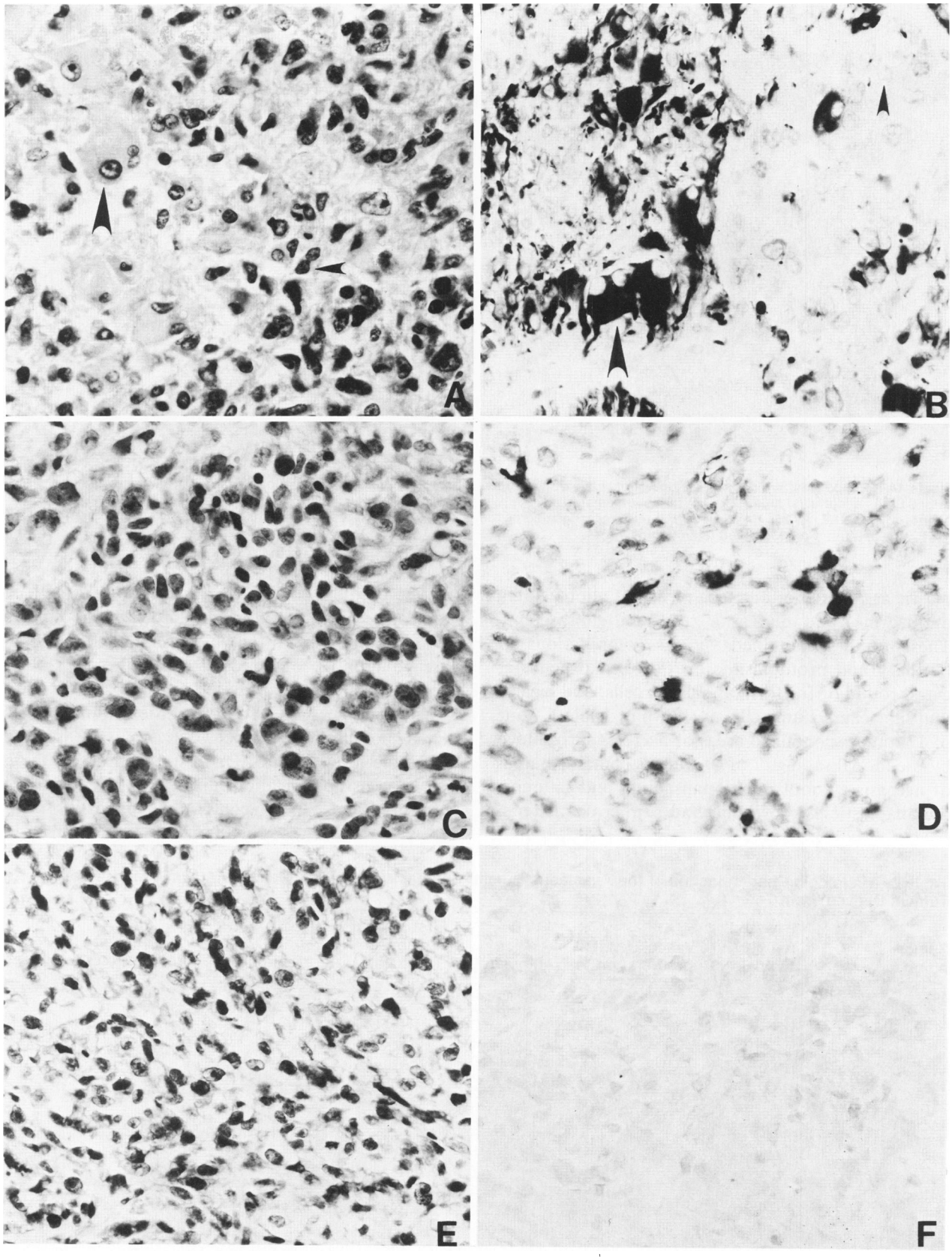

Figure 3—Anaplastic human glioma 91 and the athymic mouse-borne tumors derived from it. **A**—The original human surgical biopsy specimen contains a mixed population, including gemistocytes (*large arrow*) and small anaplastic cells (*small arrow*). (H&E, × 425) **B**—When stained for GFAP by the peroxidase-antiperoxidase technique, many gemistocytes (*large arrow*) stain intensely positive, while the majority of small anaplastic cells are GFAP-negative (*small arrow*). (Hematoxylin counterstain, × 425) **C**—Transplantation of tumor 91 to the athymic mouse has resulted in a homogeneous population of small anaplastic cells. (H&E, × 425) **D**—When stained for GFAP, fewer than 5% of the cells throughout the tumor were GFAP-positive. (Hematoxylin counterstain, × 425) **E**—After three passages in the athymic mice, the tumor still contains only small anaplastic cells and is highly cellular. (H&E, × 425) **F**—By the third passage in the athymic mice, all cells were GFAP-negative. (Hematoxylin counterstain, × 425) (With a photographic reduction of 3%)

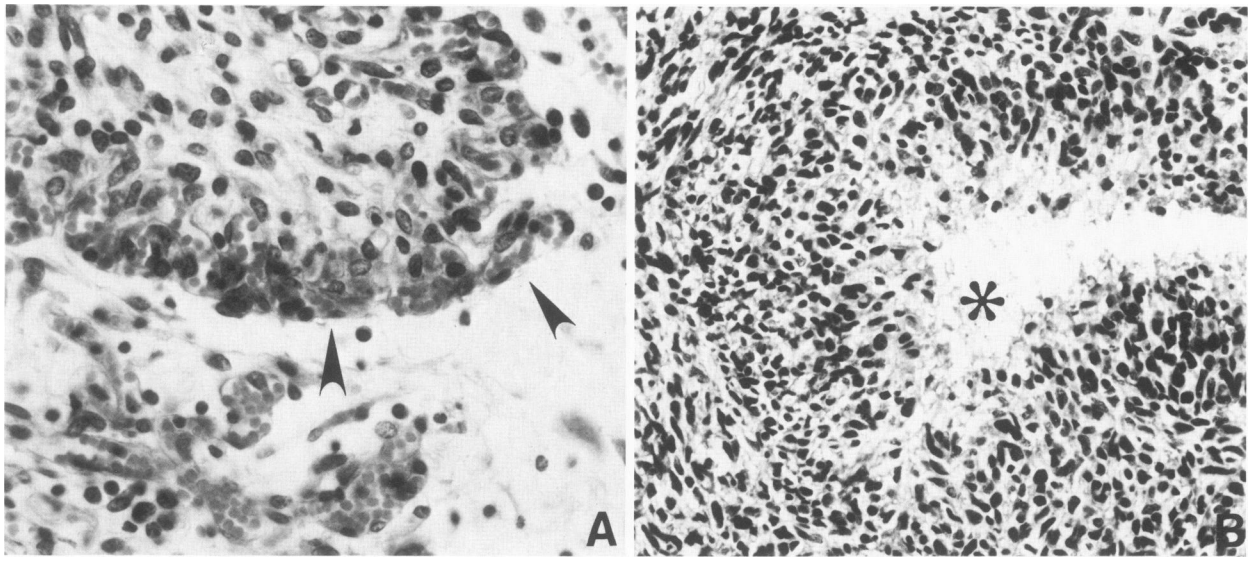

Figure 4A—Tumor 114 (third passage) is one of only two athymic mouse-borne tumors containing abnormal, proliferating vascular elements. Although the endothelial linings are not reduplicated and only slightly plump endothelial cells are seen, a rich plexus of capillaries has developed (*arrows*). (H&E, × 425) **B**—This first-passage mouse-borne tumor (241) demonstrates pseudopalisading around an area of necrosis (*). (H&E, × 425) (With a photographic reduction of 4%)

plastic astrocytoma, one as a recurrent glioblastoma multiforme, and one as a recurrent gliosarcoma. These tumors contained various combinations of fibrillary and protoplasmic astrocytes and gemistocytes, round or fusiform anaplastic cells, and bizarre multinucleated giant cells, as shown in Table 1. Nine of 17 tumors contained areas of necrosis surrounded by pseudopalisading, 7 of 17 tumors contained areas of necrosis without pseudopalisading, and 1 tumor, the anaplastic astrocytoma, had neither characteristic. Eleven of 17 tumors contained examples of true vascular cell proliferation, while four of the tumors revealed only slightly atypical endothelium. The average number of mitoses per high-power field varied from fewer than one to as many as six.

When stained for GFAP, 15 of the 17 tumors contained populations of neoplastic cells expressing detectable levels of GFAP. In these 15 cases, there was no consistent relationship between cellular configuration and GFAP content; examples of both

Table 2—Histologic Characteristics of the Anaplastic Human Gliomas and the First-Passage Athymic Mouse-Borne Tumors Derived From Them

Tumor number	Necrosis	Vascular cell proliferation	Mitoses	First-passage mouse tumors	Necrosis	Vascular cell proliferation	Mitoses
91	+0	+	3-4	91	+0	–	3-4
112-115	+0	±	1	112	±	–	<1
				114	±	–	<1
				115	±	–	<1
120	+0	+	1	120	–	–	1
132	+0	±	5-6	132	±	–	5-6
137	–	–	<1	137	–	–	<1
142	+ +	+	<1	142	±	–	1-2
155	+ +	±	<1	155	–	–	<1
175	+0	+	2-3	175	–	–	3-4
183	+ +	±	<1	183	+ +	–	1-2
241	+0	+	5-6	241	+ +	–	5-6
294	+ +	+	3-4	294	+ +	–	3
338	+0	+	<1	338	–	±	<1
341	+ +	+	<1	000	NA	NA	NA
350	+ +	+	<1	350	–	–	2-4
391	+ +	–	1	391	–	–	4-5
456	+ +	+	2-4	456	+ +	–	<1
457	+ +	+	1	457	+ +	–	4-6

Table 3—Morphologic Characteristics and GFAP Expression in the Serially Passed Athymic Mouse-Borne Anaplastic Human Gliomas

Tumor number	Passage level	Cell types	Necrosis	Vascular cell proliferation	Mitoses	% GFAP-positive tumor cells
91	1	SM ANA	+0	–	3-4	<5
91	3	SM ANA	+ +	–	4-6	0
91	5	SM ANA	–	–	2-3	0
112	1	FIB ASTRO PROTO ASTRO	±	–	1	5-25
112	4	FIB ASTRO PROTO ASTRO	+ +	–	3	25-50
114	1	FIB ASTRO PROTO ASTRO	±	–	1	5-25
114	3	FIB ASTRO PROTO ASTRO	–	±	2-3	5-25
115	1	FIB ASTRO PROTO ASTRO	±	–	1	<5
115	3	FIB ASTRO PROTO ASTRO	–	–	<1	<5
120	1	PROTO ASTRO	–	–	1	ND
120	2	FIB ASTRO PROTO ASTRO	–	–	1	50-75
120	3	FIB ASTRO PROTO ASTRO	+0	–	2	25-50
132	1	ANA SPL	±	–	5-6	50-75
132	2	ANA SPL	+0	–	6	75-100
132	3	ANA SPL	+0	–	2	75-100
175	1	ANA SPL	–	–	3-4	0
175	6	ANA SPL	–	–	2	0
241	1	SM ANA	+ +	–	5-6	<5
241	3	SM ANA	+ +	–	4-6	5-25
241	4	SM ANA	+ +	–	6	<5
391	1	FIB ASTRO SM ANA, MNG CELL	–	–	4-5	25-50
391	3	ANA SPL, FIB ASTRO, SM ANA, MNG CELL	–	–	2	5-25
456	1	FIB ASTRO	+ +	–	1	25-50
456	4	ANA SPL, SM ANA, FIB ASTRO	–	–	3-4	<5
456	5	ANA SPL MNG CELLS	–	–	4-6	0
456	6	ANA SPL	+0	–	5	0

FIB ASTRO = fibrillary astrocyte; PROTO ASTRO = protoplasmic astrocyte; ANA SPL = anaplastic spindle cell; SM ANA = small anaplastic cell; MNG CELL = multinucleated giant cell.

GFAP-positive and GFAP-negative cells of each of the six cellular configurations could be found. One of the 17 tumors was judged uninterpretable for GFAP expression due to extensive necrosis. The percentage of tumor cells judged GFAP-positive varied from fewer than 5% to between 75% and 100%.

Morphologic Study of Initial Mouse-Propagated Tumors

Sixteen of 17 biopsied anaplastic human gliomas produced subcutaneous tumors in athymic mice. In the 34 mice in which tumor growth occurred, the latency periods from transplantation to a volume of 500 cu mm varied between 24 and 364 days. With one biopsy, four different portions of the tumor were transplanted into the mice, and three separate tumor lines (112, 114, and 115) were established. Therefore, from 16 surgical biopsies, 18 athymic mouse propagated tumors were initiated. The predominant cellular morphologic characteristics of the initial tumors in the mice are shown in Table 1. Compared with the human biopsy material, these tumors contained the same morphologic cell types but were

usually more homogeneous. Eleven of 18 cases showed only fibrillary and protoplasmic astrocytes (Figure 1). Four of 18 cases showed a homogeneous population of round, fusiform or spindle shaped anaplastic cells. Three of 18 showed a mixture of cell types (Figures 2 and 3). Five of 18 first-passage mouse-carried tumors demonstrated necrosis and pseudopalisading (Figure 4), 1 of 18 showed necrosis without pseudopalisading, 5 of 18 were judged equivocal, and 7 of 18 judged negative of demonstrating necrosis alone. Only one first-passage mouse-grown tumor contained any endothelial proliferation, and this represented thickening of the endothelial cells without reduplication of the endothelial cell lining of the vessels. As in the original biopsy specimens, mitotic figures varied from fewer than one per 400× field to five or six (Table 2).

Of the 18 first passage athymic mouse-grown tumors generated from the 16 surgical biopsies, 16 were tested for GFAP expression and 14 contained GFAP-positive cell populations. As with the original biopsy specimens, the percentage of GFAP-positive tumor cells varied widely. Although both GFAP-positive and negative examples of each cell type could be found, fewer than 5% of small anaplastic cells expressed GFAP in the initial mouse-borne tumors.

Morphologic Study of Serially Passed Tumors

Ten of the tumor lines were studied in serial passage. Nine of these tumors originally displayed cellular homogeneity, and eight maintained their individual cellular structure, although several did become more densely cellular with passage. One of the nine homogeneous tumors initially contained a GFAP-positive populaton of fibrillary and protoplasmic astrocytes, which was gradually replaced by a homogeneous population of GFAP-negative anaplastic spindle cells (456). The tenth tumor was initially heterogeneous and retained the same mixture of cell types with serial passage (Figure 2). None of these tumors initially showed endothelial cell proliferation. One, however, showed this feature in the third passage (Figure 4). In nine of ten tumors, there was no relationship between the presence of necrosis in the different animal passages, but one tumor did retain a prominent pattern of necrosis with pseudopalisading. Mitoses were easily found in most fields; their numbers varied from fewer than one to six per high-power field.

Ten of the athymic mouse-propagated tumor lines were studied for GFAP expression in serial passage beyond the first passage. Seven of ten continued to express detectable amounts of GFAP (Figure 1), three of the seven expressing lesser amounts with serial passage (Figure 2). Two of ten ceased GFAP expression (Figure 3), and one (derived from the gliosarcoma) never expressed GFAP (Table 3).

Discussion

Transplantation of human anaplastic gliomas to athymic mice results in an extremely high percentage of actively growing tumors. In this study, 16 of 17 (94.1%) of the gliomas transplanted to the mice survived to produce growing tumor. The time required to reach 500 cu mm ranged from 24 to 364 days. Although in initial implantation, tumor growth occurred in only half the mice (34/68), with serial passage, the successful "take" rate increased from 50% to 80.8%, and doubling time ranged from 3 to 19.1 days in exponentially growing tumors. Once a tumor had been successfully transplanted to a second animal generation, it was never lost due to failure to grow.[15]

Although human anaplastic gliomas adapt well to growth in athymic mice, the number of studies detailing the growth of anaplastic gliomas in athymic mice is small. In 1977, Rana et al[16] injected one human glioblastoma into athymic mice. The tumor grew in all three mice into which it was injected and retained similar morphologic characteristics to the biopsy to the extent that multinucleated giant cells and pseudopalisading around areas of necrosis were noted. Reid et al[17] and Wara et al[18] have also described successful growth of individual glioblastoma in athymic mice. Reid et al[17] reported an increase in cell density with passage, and Wara et al[18] reported that one of two glioblastomas studied became more "sarcomatous" with increased serial passage in the mouse. Shapiro et al[19] placed seven anaplastic gliomas into athymic mice. All seven initially grew, but only two became established, to become serially transplanted tumor lines. Both these tumor lines came from gliosarcomas. In our group of transplantable human gliomas, we have tumors expressing a variety of histologic phenotypes; several of our cases are similar to the few described in the literature, suggesting that the dissimilar cases in the literature are simply examples of the tumor types possible within a wide spectrum of phenotypes. Like the tumor reported by Rana et al,[16] 391 (Figure 2) in our study retained with passage a pleomorphic cell population that included multinucleated giant cells. Five other of our transplanted tumors continued to demonstrate necrosis with pseudopalisading (Figure 4). We also noted increased cellularity in many of the transplanted tumors, as compared with the biopsy specimens, and an almost total absence of vascular abnormalities in the mouse-borne tumors. Eleven of

16 of the transplanted tumors also demonstrated a reduction in the number of cellular subpopulations after transplantation and a relative increase in the number of small anaplastic cells.

GFAP is presently the best differentiation marker for cells of astroglial origin.[20] In normal and reactive tissues, it is astrocyte-specific, while in human central nervous system tumors GFAP is considered generally glioma-specific.[13,14] Two techniques for the detection of GFAP in normal, reactive, and neoplastic tissues have been used. Studies employing immunoelectrophoresis have evaluated the presence of GFAP in CNS tumor homogenates,[21-24] but electrophoresis fails to distinguish between neoplastic and nonneoplastic tissues. Because the tumor tissue tested is dissected only on the basis of gross examination, any amount of normal, reactive, or only partially infiltrated tissue may be included with the tumor tissue analyzed. The use of the peroxidase-antiperoxidase technique allowed the localization of GFAP in the cells of the tumors. Duffy et al and Deck et al[25,26] found that this technique permitted them to differentiate gliomas from sarcomas, while van der Meulen et al[27] found GFAP-positive neoplastic cells in 25 of 38 Kernohan Grade III and IV astrocytomas and postulated an inverse relationship between GFAP positivity and clinical malignancy. In this study, on the other hand, we found all glioblastomas to contain GFAP positive tumor cells; this discrepancy may reflect a sampling problem. In our study not every block from each tumor was GFAP-positive. Eng and Rubinstein[13] and Velasco et al[28] both found a variety of neoplastic cell types positive for GFAP; in their studies, as in ours, they found that the small anaplastic cells present in some astroglial tumors were generally negative.

Two studies have examined GFAP expression in mouse-borne human gliomas, but in both cases the tumors were initiated with glioma-derived tissue culture cells.[29,30] Because these tumors have experienced an additional selective step, it is difficult to evaluate them in comparison with the tumors established directly from surgical biopsy specimens. Both papers reported, however, that the transplanted tumors continued to express GFAP in the mouse. Yamashita et al[30] saw GFAP positivity maintained through the fourth passage, while Maunoury et al[29] followed one tumor to the thirteenth passage, where it was still GFAP-positive; another, however, discontinued GFAP expression at the ninth passage. The GFAP data in our study (Table 1) show that the tumors tended to decrease, (8) rather than maintain (4) or increase, (3) their levels of GFAP expression after growth in the athymic mice. Table 3 suggests that increased passage in the mouse correlates with a decrease in GFAP expression. Of the five tumors studied at passage level four or greater, four contained fewer than 5% to 0% GFAP-positive cells. Tumor 132, however, contained 75–100% GFAP-positive cells after three passages and also had a very long doubling time.[15] It is of interest that in the quantitative study done by Jacque et al,[21] the lowest GFAP levels in glial tumors were found in glioblastoma. GFAP content correlated inversely with increased anaplasia.

The use of athymic mouse-borne human anaplastic gliomas as models for the study of the biologic characteristics of these tumors is increasing. The expanding interest in this disease model requires an evaluation of its characteristics and of its tendency to change or progress with serial passage. Heterogeneity of both cell type and biochemically or immunologically detected characteristics within and between tumors is clearly a characteristic of glioblastoma.[31-33] The maintenance of morphologic characteristics in the mouse-propagated tumors suggests that the mouse is capable of supporting many of the morphologic cell types seen in the original biopsy specimen. On the other hand, morphologic (or measurable biochemical) dissimilarities between the original biopsy specimen and the initial mouse-borne tumors may be the result of one or more factors. For example, cell populations present in the sections of the surgical biopsy may not be representative of those cell populations present in the tumor tissue that was actually injected into the mouse. Also, undefined selective forces within the mouse may encourage or discourage the growth of any given cell subpopulation or the expression of any given characteristic. One must recall that the athymic mouse is not totally immunoincompetent and that the subcutaneous space of the mouse is different in a multitude of ways from the substance of the human brain. As Scherer[34] has pointed out, the architecture of the preexisting environment, whether brain or subcutaneous tissue, will have a significant effect on the appearance and behavior of a tumor. Necrosis, for example, may be less a primary characteristic of a particular tumor than a characteristic dependent on the size of the mouse-borne tumor and the quality of its blood supply. The vascular tissue of these tumors is assumed to be, though not experimentally proven, of mouse in origin. If this is the case, the difference in species may also be the reason for the general absence of vascular cell proliferation, a characteristic so common in the original biopsy specimens.

The growth rate,[15] histologic data, and immunochemical data show that human anaplastic gliomas

react to heterotransplantation in a heterogeneous manner. Latency periods for initial tumor growth in the mice varied from 24 to 364 days, while histologic characteristics including number and type of cellular subpopulations, degree of cellularity, and necrosis were retained, changed, or deleted in an unpredictable way with transplantation. A study of GFAP expression emphasizes this point. Some tumor lines decreased GFAP expression, while others maintained or increased GFAP levels after heterotransplantation.

Our overall interpretation is that these data demonstrate that transplantation of anaplastic human gliomas into athymic mice results in 1) continued tumor progression, which proceeds in a unique manner for each tumor; and 2) the generation of diverse and dissimilar tumors, as judged by growth rate, morphologic data, and GFAP expression data.

Most previous studies of anaplastic human glioma progression in humans have been limited to those cases where a low-grade astrocytoma evolves into a more anaplastic tumor, such as a glioblastoma multiforme. Increases in patient survival time caused by radiation and chemotherapy regimens now offer, however, the opportunity to study surgical biopsies at more than one point in a single tumor's natural history. It may be useful to determine to what extent the evolution of human anaplastic gliomas in man resembles, or differs from, the evolution of the same tumors grown in athymic mice, and whether in either situation tumor cell selection plays a role in influencing tumor progression.

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