Masaji Murakami, Yukitaka Ushio, Yoshimasa Morino, Takao Ohta, and Yasuhiko Matsukado.
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Pages 181–185.

The labels to Figures 6–10 were inadvertently omitted.

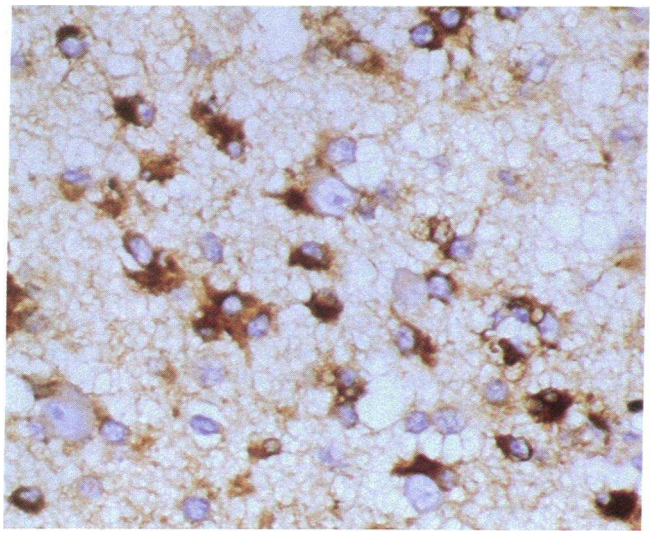


Figure 5. Apo E localization in low grade astrocytoma (× 520). Intensely immunoreactive apo E is seen around the nucleus of most astrocytoma cells. Light counterstain with hematoxylin.

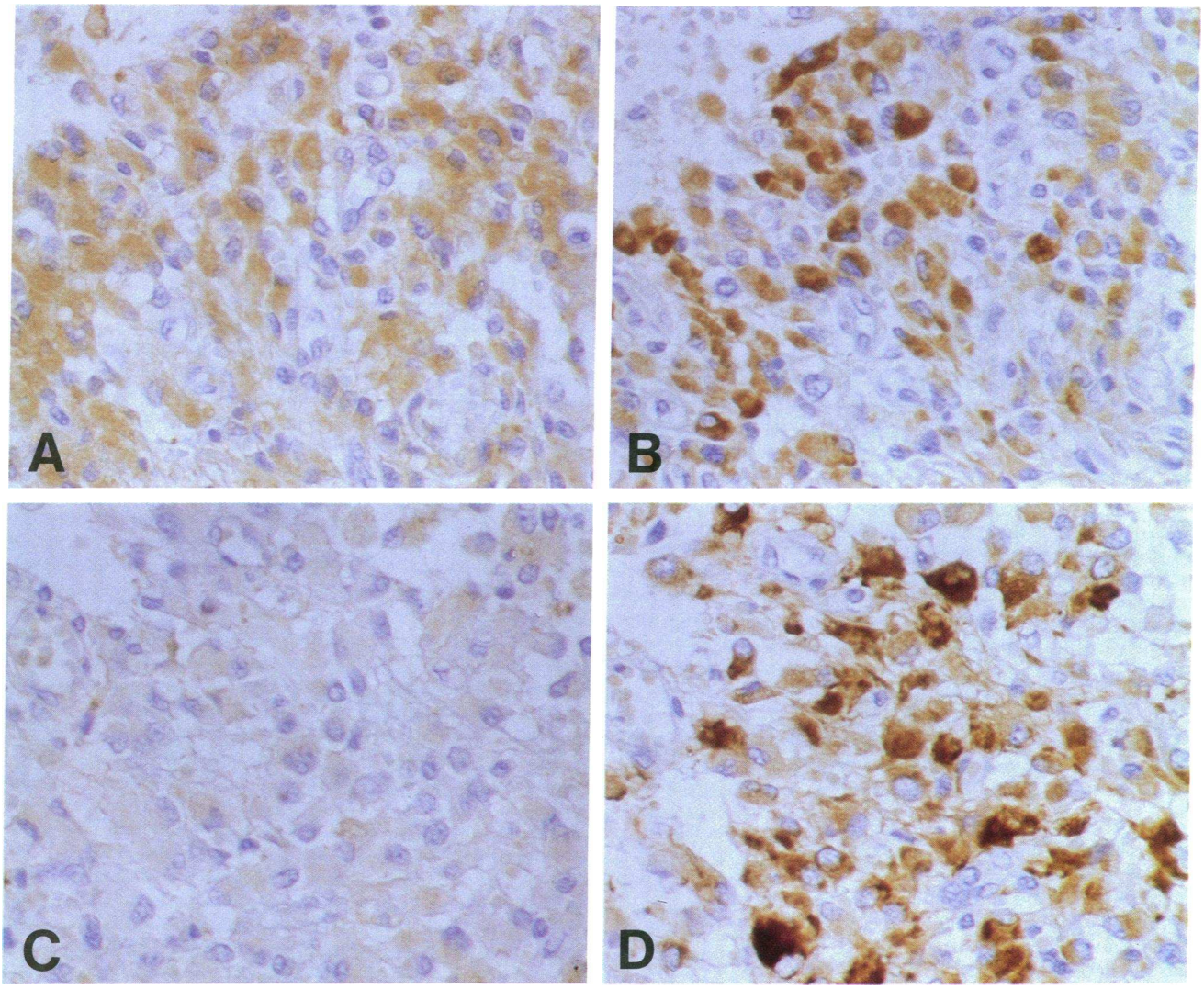


Figure 6. The difference between apo E and GFAP immunorectivity in high grade astrocytoma (× 520). Perikaryal immunostaining is seen in the tumor cells, with a moderate degree of reaction for apo E (*A*) and a strong reaction for GFAP (*B*) in the same fields of serial sections. No immunoreaction for apo E (*C*) is observed in some areas with a strong immunoreaction for GFAP (*D*) in the serial sections. Light counterstain with hematoxylin was used.

Table II. Correlations between Tumor Grading and Apo E or GFAP Immunoreactivity in 18 Astrocytomas

	+++	++	+	–
Apo E				
Grade 1, 2 cases	1	1		
Grade 2, 9 cases	3	4	2	
Grade 3, 7 cases		1	6	
GFAP				
Grade 1, 2 cases	1	1		
Grade 2, 9 cases	4	3	2	
Grade 3, 7 cases	3	4		

A much more negative correlation was observed between tumor grading and immunoreactivity in apo E than in GFAP. Immunoreactivity is expressed according to the intensity and the number of positive cells, as follows: +++, strong, more than 60%; ++, moderate, 20–60%; +, weak, less than 20%; –, negative, 0%.

specific anti-apo E antibody at 1:3,000. In addition, the most appropriate dilution of the monospecific anti-apo E antibody to use was 1:60,000, thereby indicating that apo E is fixation sensitive (data not shown).

Secretion of apo E from cultured glioma cells. To investigate whether apo E was secreted from the astrocytic tumor cells into the cultured medium, the following study was performed (Fig. 12). Upon primary culture of the low grade astrocytoma, a significant amount of apo E was detected at the basal value in medium. The secretion of apo E from the cells was enhanced 1.8–2.0-fold by β-methasone, db-cAMP, or LDL. The mechanism is unknown. In contrast, no apo E secretion was observed with the glioblastoma cell line (KMG-5), even in the presence of various agents. In addition, the immunofluorescence study using cultured cells indicated that the primary culture of the astrocytoma contained ~ 80% GFAP positive cells and the cell line contained ~ 5% or less (data not shown). To examine whether apo E detected in the medium is due to tumor cell destruction, we measured lactate dehydrogenase activity of the culture medium. The lack of lactate dehy-

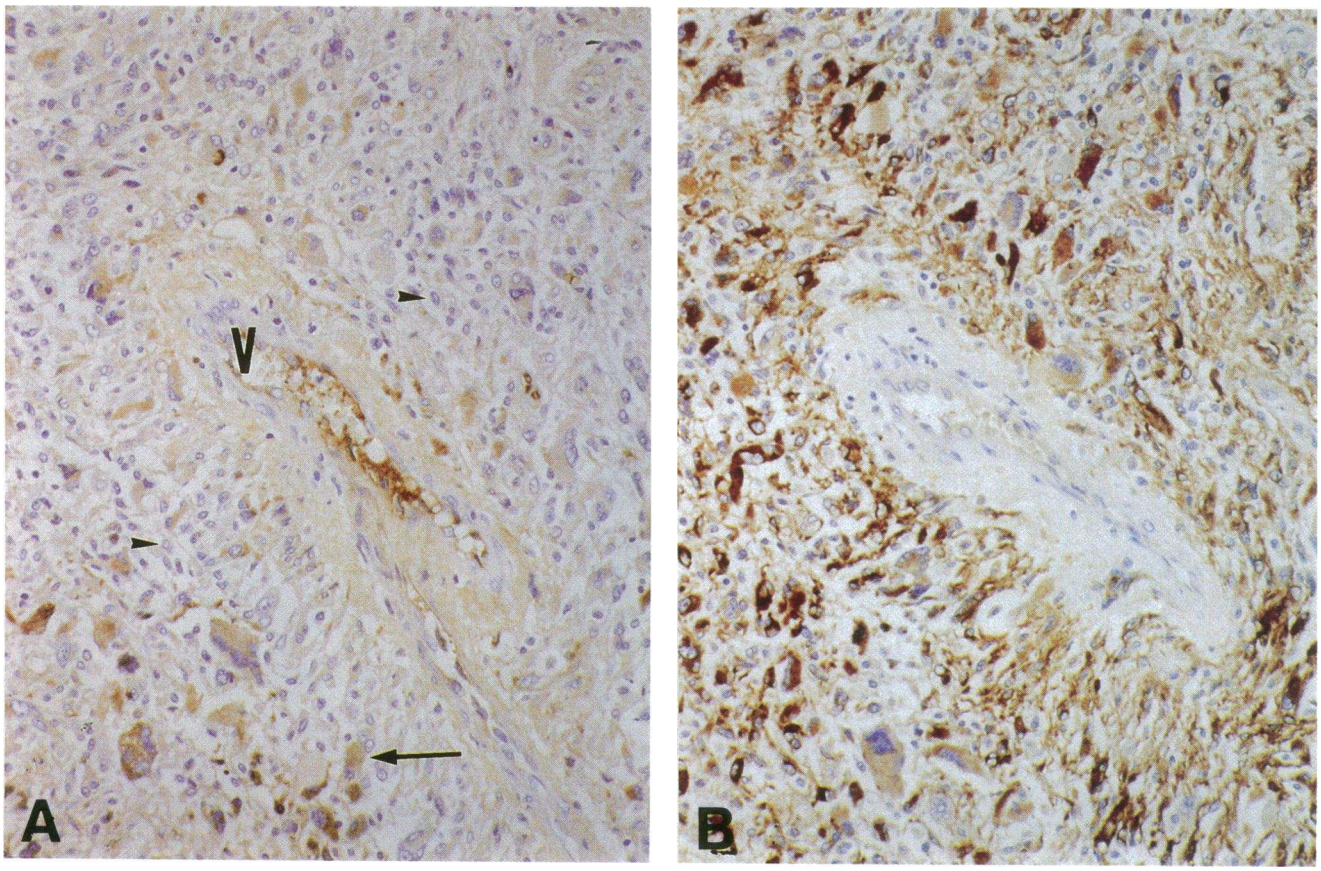
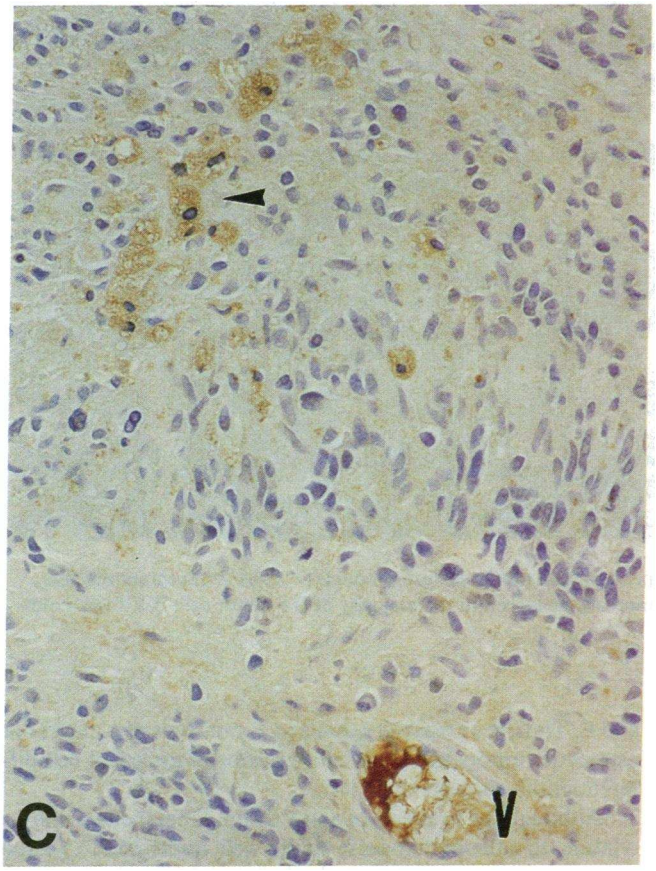
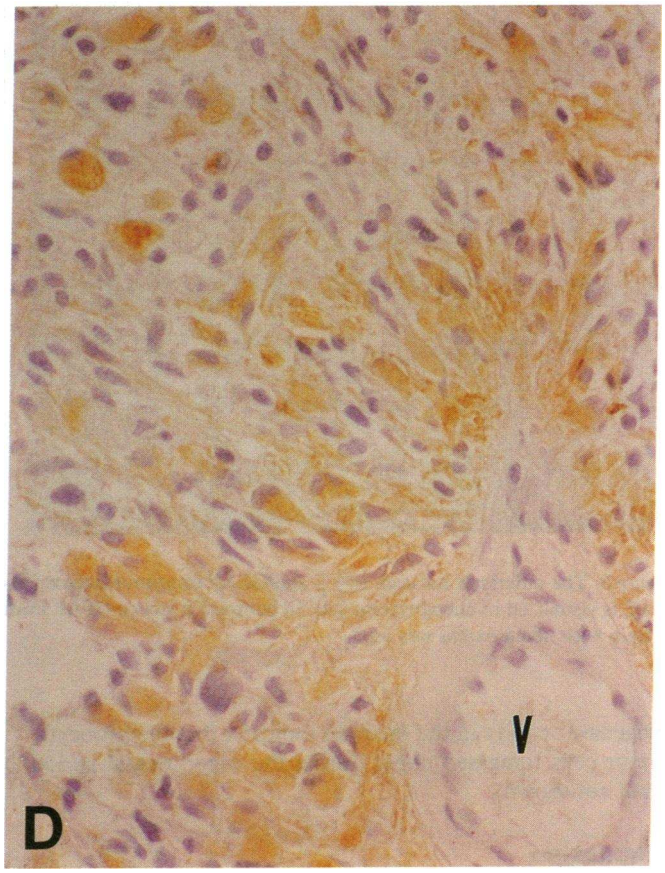


Figure 7. The difference between apo E and GFAP immunoreactivity in glioblastoma. In case 1 (*A* and *B*), the cells with a gemistocytic or bizarre form (*arrow*) are moderately to intensely reacted for apo E and the cells with large and multinucleated forms are weakly reacted for apo E, and the small, round components show very little or no immunostaining for apo E (*arrowheads*). In addition, serum components within the blood vessel (*V*) show a strong reaction for apo E (*A*, × 260). In contrast, a higher GFAP immunoreactivity is detected in several varieties of the cells in the same field as *A* in serial sections (*B*, × 260). In case 2 (*C–E*), only a few of the tumor cells show moderate immunoreaction for apo E (*arrowhead*), although serum components within the blood vessel (*V*) demonstrate a strong reaction for apo E (*C*, × 420). In contrast, a high number of the tumor cells especially in the perivascular areas reveal GFAP immunoreaction to a moderate degree (*D*, × 520). None of the cells are stained positively using human macrophage-specific antibody (*E*, × 520). Light counterstain with hematoxylin was used.





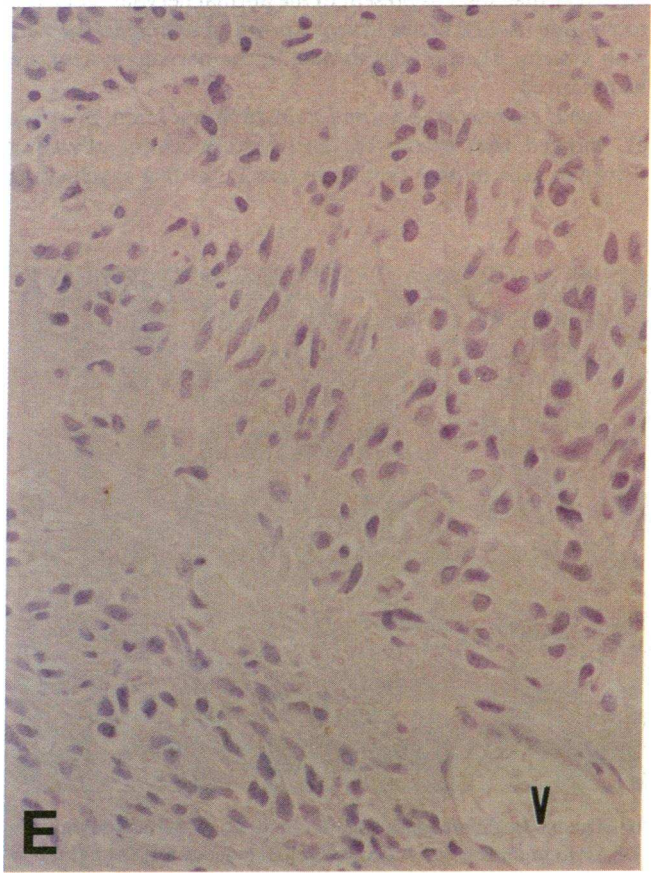


Figure 7 (Continued)

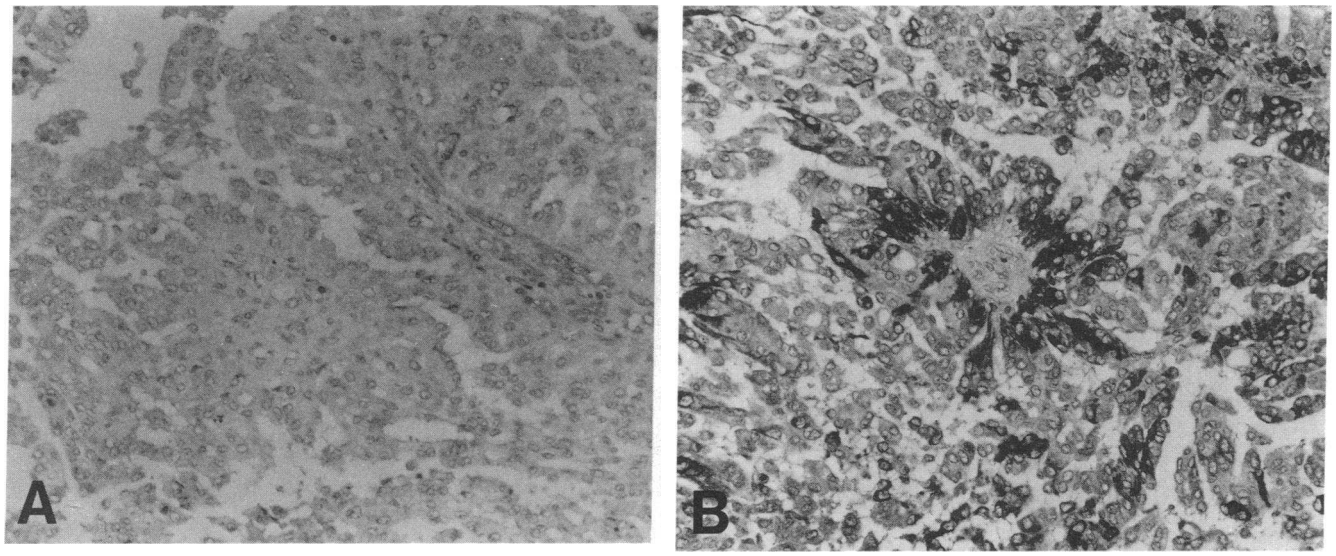


Figure 8. The difference between apo E and GFAP immunoreactivity in ependymoma (dark reaction product) (× 150). No immunoreactive apo E is visualized in ependymoma (*A*). In contrast, a strong GFAP immunoreaction is seen in the majority of the tumor cells (*B*). Light counterstain with hematoxylin was used.

drogenase activity in the medium indicates that the astrocytic tumor cells remained intact during the experimental period (data not shown).

Discussion

We obtained evidence that all types of astrocytomas and glioblastomas exhibit an immunoreaction for apo E, with a negative correlation between the intensity of immunoreactivity and the degree of anaplasia. The astrocytic elements are stained in the case of the medulloblastoma with glial differentiation. In addition, well differentiated cultured astrocytoma cells secrete apo E. Thus, the possibility that apo E can serve as a marker of astrocytic tumors as well as an indicator of astroglial cell differentiation warrants attention.

Although the function of apo E in lipid metabolism and its relation to atherosclerosis is fairly well understood (3), the discovery that apo E is present in the normal nervous tissue is a recent one (9–11), hence little is known of its role in the nervous system. The findings to date in the nervous system are summarized.

Apo E expression in normal state. In the rat brain, Boyles et al. showed that astrocytes and certain nonmyelinating Schwann cells in vivo synthesize apo E but that myelinating

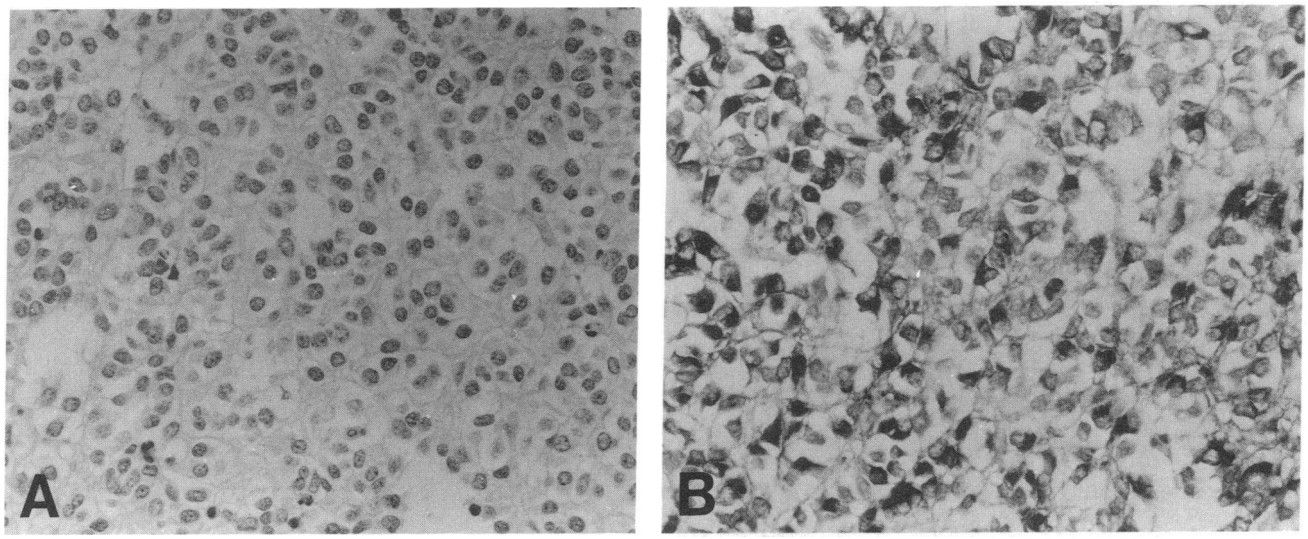


Figure 9. The difference between apo E and GFAP immunoreactivity in the oligodendroglioma (dark reaction product) (× 400). No immunoreactive apo E is observed in oligodendroglioma (*A*). In contrast, a strong GFAP immunoreaction is demonstrated in about half the number of tumor cells (*B*). Light counterstain with hematoxylin was used.

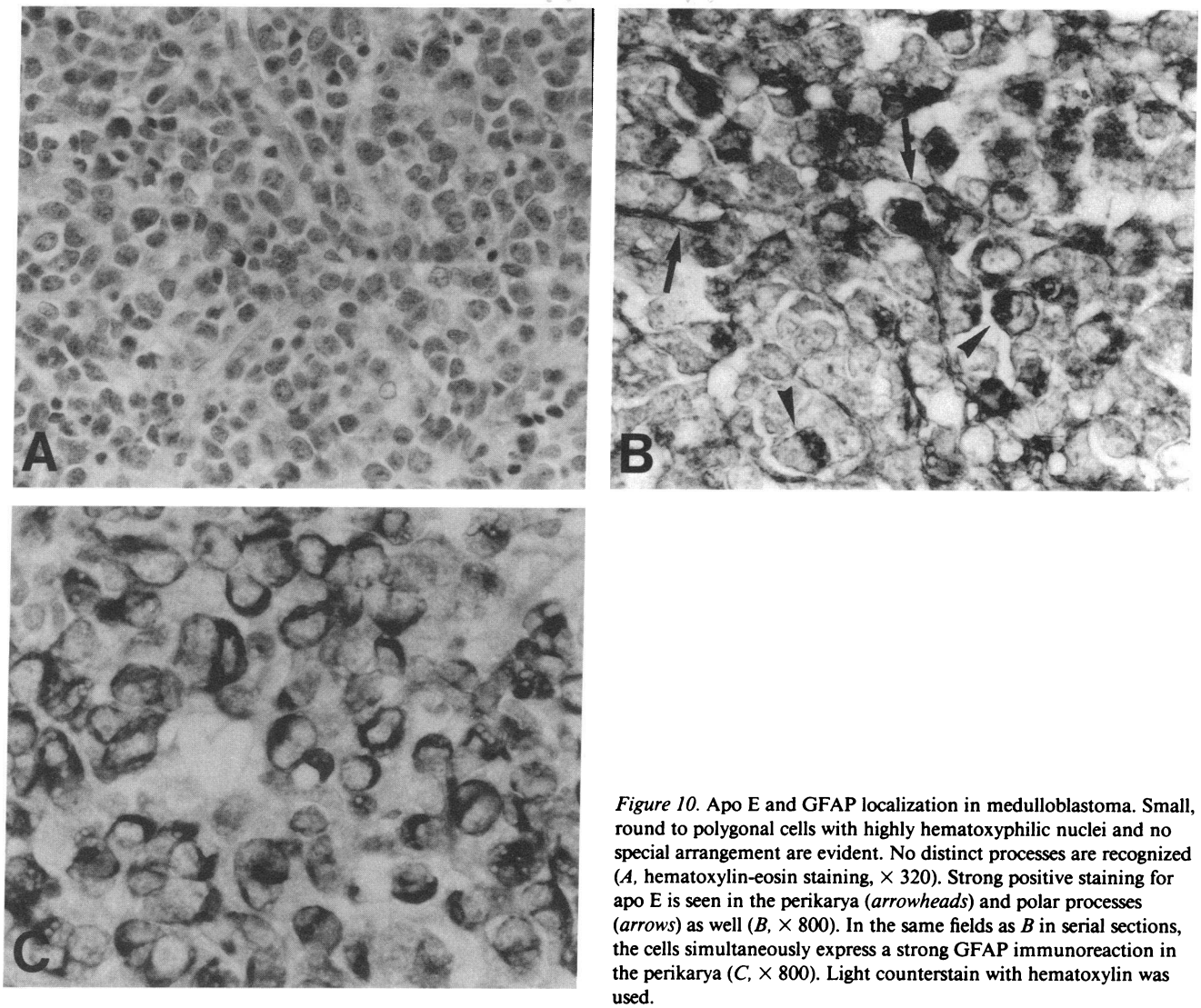


Figure 10. Apo E and GFAP localization in medulloblastoma. Small, round to polygonal cells with highly hematoxyphilic nuclei and no special arrangement are evident. No distinct processes are recognized (*A*, hematoxylin-eosin staining, × 320). Strong positive staining for apo E is seen in the perikarya (*arrowheads*) and polar processes (*arrows*) as well (*B*, × 800). In the same fields as *B* in serial sections, the cells simultaneously express a strong GFAP immunoreaction in the perikarya (*C*, × 800). Light counterstain with hematoxylin was used.

Schwann cells and oligodendrocytes do not produce this protein (11).

Apo E expression in nerve injury. After peripheral nerve injury, apo E was found to be significantly increased in the degenerating portion of the nerve (29–33). Invading macrophages are the major source of this protein (33). After central nerve injury, apo E was found to increase in the brain tissue (32). However, macrophages apparently did not play the dominant role at the onset of degenerative events, rather reactive astrocytes appeared to contribute to the total amount of the protein in the damaged nerve early in the history of the lesion, with macrophages contributing significantly only some weeks later (33).

Apo E expression in neoplasms. Apo E was found to be synthesized by human neurofibrosarcoma-derived cell culture, but not by cells from benign neurofibroma (12). However, these cases are rare and the authors did not clearly establish that immunocytochemical localization of apo E is a useful approach for identification or staging of human glial neoplasms.

The present study focuses on the usefulness of apo E for diagnosis. Our results indicate that apo E localization in human brain tumors may be clinically relevant and diagnostically useful.

As macrophages are present in some neoplasms, and secrete copious quantities of apo E (34), the question arises as to whether apo E-reactive macrophages are present in the specimen used. To ascertain this critical point, human macrophage-specific antibody (28) was used for cell identification. The finding that none of the cells were stained positively in case 2 of glioblastoma would argue against the apo E-reactive cells being macrophages (Fig. 7).

The apo E reactivity identified in neoplastic astrocytic cells appears to be synthesized by these cells. The possibility that apo E from the plasma enters these tumors where the blood-brain barrier no longer exists can be ruled out. If such were true, most apo E immunoreactive cells would be expected to be found in regions closest to the blood vessels. This is not the case. Preliminary electron microscopic immunohistochemical investigations have shown that apo E is present in the Golgi apparatus of astrocytoma cells (unpublished observation). In addition, the apparently higher molecular mass of apo E from tissue extracts of a human glioma (~ 37,000 D), compared with 34,000 D for plasma apo E, would argue against the point