Proliferative Potential of Human Astrocytes

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Abstract
Although a number of studies have demonstrated proliferation of nonneoplastic astrocytes in experimental animal models, the proliferative potential of human astrocytes has not been well defined. Using double-label immunohistochemistry, we identified proliferating cells with the proliferation marker MIB-1 and astrocytes with glial fibrillary acidic protein staining in human biopsy and autopsy tissue. MIB-1 labeling of astrocytes was monitored in a variety of conditions containing significant numbers of reactive astrocytes, including infections, arteriovenous malformations, demyelinating lesions, metastatic tumors, and long-standing gliosis. Twenty-nine of a total of 54 cases showed no evidence of astrocyte-specific MIB-1 labeling despite prominent reactive changes. An average proliferation rate of 0.9% was present in the remaining 25 cases. Labeling indices were highest in infectious conditions and acute demyelinating lesions. We also examined astrocyte proliferation in 5 cases of progressive multifocal leukoencephalopathy. Astrocytic labeling indices were notably elevated in these cases, with an average labeling index of 5.8%. We conclude that low, but appreciable, astrocytic proliferation may occur in nonneoplastic human astrocytes. These findings have implications for astrocyte function in the normal and disease states and for the diagnostic distinction between reactive lesions and low-grade astrocytic neoplasms.

Key Words: Astrocyte, Brain neoplasms, Glial fibrillary acidic protein (GFAP), MIB-1, Proliferation.

INTRODUCTION
Astrocytic gliosis, characterized by an increase in the number of astrocytes with immunoreactivity for glial fibrillary acidic protein (GFAP), is a typical feature of many pathologic conditions of the central nervous system (CNS). The apparent increase in the number of astrocytes is commonly thought to reflect reactive changes, including induction of GFAP expression, rather than proliferation of astrocytes (1). However, astrocytic proliferation has been well documented in experimental animals, such as rodent models of traumatic or ischemic injury. Proliferating cells have been labeled by incorporation of $^{3}$H-thymidine or bromodeoxyuridine (BrdU) into replicating DNA and astrocytes identified on the basis of morphology (2) or anti-GFAP immunohistochemistry (3–8). In all of these studies, proliferating astrocytes were identified in the area of injury. In some studies, the degree of astrocytic proliferation appeared modest with 1% to 2% of astrocytes proliferating (3), although in other studies up to 46% of astrocytes showed evidence of DNA replication (7). Differences in the amount and timing of the nucleotide analog administered likely account for some of the variability in the labeling indices (4). Astrocytes typically represent about 10% to 20% of all proliferating cells in such lesions (2, 6).

A smaller number of studies have addressed astrocyte proliferation in the nonhuman primate brain. In the absence of injury, estimates of the number of GFAP-immunoreactive astrocytes that can incorporate BrdU vary widely from study to study (9–12). Following ischemia (13) or infection (14), astrocytes in macaque monkeys are capable of proliferation and comprise 5% to 15% of BrdU-labeled cells in an ischemia model (13).

In contrast to the relatively large amount of data available in experimental animal studies, few investigators have reported on the proliferative potential of human astrocytes. Human astrocytes have long been suspected of at least some proliferative potential based on the appearance of binucleate astrocytes in disorders like Creutzfeldt-Jakob disease (15), although cell fusion provides an alternative explanation for these infrequent forms (16, 17). There is, however, additional evidence for astrocyte proliferation in humans. Analysis of brains from 5 cancer patients who received BrdU for diagnostic purposes showed that 18% of BrdU-positive cells in the dentate gyrus expressed GFAP (18). The cell proliferation marker Ki-67, expressed during the G1, S, G2, and M phases of the cell cycle (19), has been used to suggest astrocytic proliferation in multiple sclerosis lesions. Ki-67-immunoreactive astrocytes have been reported in acute plaques from a total of 6 patients in 2 separate studies (20, 21). Labeling indices of about 3% were typical, although variability from 1% to 34% was seen in 1 study (20).

Clear definition of the proliferative potential of human astrocytes has important implications for understanding the function of the nervous system under normal conditions and in disease states. Previously thought to provide the uninteresting “glue” holding the brain together, glia (derived from the Greek root for glue) are now known to perform a number of important functions (22). Astrocyte regulation of extracellular...
glutamate concentration and ammonia detoxification are well established. Newer roles in the regulation of synaptic activity, synaptogenesis, and neurogenesis are now emerging. Glias also have key functions in the context of pathologic insults to the nervous system. Astrocytes play an important role in the development of cytotoxic brain edema. Following localized injury, a brisk astrocyte response forms a glial scar that may inhibit neural regeneration (23). In addition to the mechanical barrier posed by the scar, reactive astrocytes also upregulate and secrete proteoglycans, molecules that have been implicated as inhibitors of axonal extension. Alternatively, trophic factors released from astrocytes at the site of injury may provide a suitable substrate to aid regeneration by facilitating neurite extension and neuronal survival (24).

Determining the potential for astrocytes to proliferate in nonneoplastic conditions is also relevant diagnostically. Evaluation of cell proliferation is a key component in the diagnosis and prognostic assessment of glial neoplasms. Markers like MIB-1 (recognizes Ki-67 in paraffin sections) are commonly used to determine the extent of glial proliferation in tumors, particularly low-grade lesions. The extent of astrocytic proliferation that can occur in nonneoplastic, reactive brain lesions is thus important to determine.

Using the MIB-1 proliferation marker together with double-label anti-GFAP immunohistochemistry, we demonstrate that human astrocytes can proliferate in response to a variety of insults. However, their proliferative potential appears modest, both in pediatric and adult populations, suggesting a limit to the potential for newly formed astrocytes to contribute to alterations in astrocyte function. These data also demonstrate that, although high levels of astrocytic proliferation similar to those seen in high-grade glial tumors are unlikely to occur in reactive conditions, lower levels compatible with the proliferation rates of low-grade gliomas are not uncommon. The presence of modest astrocyte proliferation does not thus distinguish a reactive condition from a low-grade astrocytic neoplasm.

**MATERIALS AND METHODS**

**Cases**

We examined a total of 59 cases chosen to represent a variety of conditions characterized by brisk reactive gliosis. All cases showed significant astrocytic hypertrophy as determined by staining with hematoxylin and eosin and GFAP immunohistochemistry. Pediatric patients represented 21 of the 59 cases analyzed. Twenty-eight patients were female. Cases were divided into the following categories: infectious, demyelinating, nonneuroglial neoplasms, vascular malformations, long-standing gliosis, other, and progressive multifocal leukoencephalopathy (PML). The infectious category included 7 bacterial abscesses, 1 case of cryptococcosis, 2 cases of cysticercosis, and 4 cases of encephalitis of presumed viral etiology. All of the demyelinating lesions were acute and were characterized by active demyelination with relative preservation of axons and significant infiltration by chronic inflammatory cells, including numerous macrophages. Cases included 4 female and 2 male patients. The nonneuroglial neoplasms included 4 cases of metastatic carcinoma and a single case each of metastatic melanoma, lymphoma, and hemangiblastoma. The “other” category included gliotic brain surrounding two encephaloceles and one Rathke’s cleft cyst. Long-standing gliosis was secondary to mesial temporal sclerosis in 3 cases, a remote infarct in 2 cases, and 1 case of Pick’s disease.

**Immunohistochemistry**

Double-label immunohistochemistry was carried out on 4-μm paraffin sections using a rabbit polyclonal antibody to GFAP at 1:12,000 (Dako, Carpinteria, CA) and the MIB-1 mouse monoclonal antibody at 1:200 (Dako). Antigen retrieval using microwave treatment in citrate buffer was performed prior to antibody incubation. Secondary antibodies coupled to alkaline phosphatase (anti-rabbit; Dako) or horse-radish peroxidase (anti-mouse; Dako) were used. Labeling indices were calculated as the number of GFAP and MIB-1 double-labeled astrocytes divided by the total number of GFAP-positive astrocytes. At least 100 GFAP-immunoreactive astrocytes were counted in the area of highest astrocyte-specific proliferation. In every case, the entire glass slide was examined for the presence of proliferating astrocytes. Only cells with clearly visible nuclei were counted (25).

**RESULTS**

To investigate the proliferative potential of human astrocytes, we identified biopsy and autopsy specimens that contained either focal lesions or sampled diffuse pathologic conditions characterized by prominent reactive gliosis. Reactive astrocytes were identified initially by the presence of enlarged cell bodies containing abundant eosinophilic cytoplasm and hypertrophic processes (Figs. 1–3). The astrocytic nature of these cells was confirmed by GFAP immunostaining, and the degree of astrocytic proliferation documented by double-label immunohistochemistry for GFAP and MIB-1 (Figs. 1–3). A total of 59 cases were examined, and lesions were grouped according to etiology: infectious, demyelinating, nonneuroglial neoplasms, vascular malformations, long-standing gliosis, benign cysts (other), and PML (Table). PML cases were distinguished from other infectious and demyelinating lesions because of their high proliferation rate.

Overall, astrocytic proliferation was quite modest. Half the cases (29 of 59) showed no evidence of MIB-1 labeling in astrocytes, despite the fact that all cases had numerous astrocytes with histologic and immunohistochemical evidence of reactive changes. There was no appreciable difference between adult and pediatric cases among the various categories with the exception of nonneuroglial neoplasms. There was a low rate of astrocytic proliferation adjacent to these tumors in the 8 adult cases (0.3%), but no evidence of astrocytic proliferation surrounding nonneuroglial tumors in the 4 pediatric cases. Failure to detect proliferating astrocytes is unlikely to reflect technical failure of the MIB-1 immunostain because chronic inflammatory cells either infiltrating brain parenchyma or in the intravascular compartment consistently showed MIB-1 immunoreactivity (e.g. Fig. 1F, arrowhead) even in cases where no proliferating astrocytes were identified.

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FIGURE 1. (A, B) Low power view of hematoxylin and eosin-stained sections showing lesions representative of those containing proliferating astrocytes. (A) Arteriovenous malformation showing chronic inflammation and reactive astrocytosis. (B) Abscess with severe acute and chronic inflammation. (C) Higher power view of a hematoxylin and eosin-stained section of the region surrounding an abscess showing typical reactive astrocytes (arrow) with abundant eosinophilic cytoplasm and prominent processes. (D–F) GFAP (red) and MIB-1 (brown) double-label immunohistochemistry. (D) Typical infrequent double labeling of reactive astrocytes in an arteriovenous malformation. A number of reactive astrocytes are present, but only one is positive for MIB-1 (arrow). (E) More frequent double labeling in the inflammatory zone surrounding an abscess (arrows). (F) Double-labeled astrocyte adjacent to a blood vessel (arrow) in an arteriovenous malformation. Note the MIB-1-positive cell in the perivascular inflammatory infiltrate (arrowhead). (G–I) Long-standing gliosis in a case of chronic encephalitis shows numerous reactive astrocytes visible on hematoxylin and eosin staining (G, H). (I) No MIB-1-positive astrocytes are observed in cases of long-standing gliosis. (J) An enlarged astrocyte with bizarre nuclear morphology, a so-called Creutzfeldt cell, can be seen on hematoxylin and eosin staining (arrow). (K, L) Nuclear material in Creutzfeldt cells is invariably immunoreactive for MIB-1 (arrows). Scale bar in (A, B) = 100 μm. Scale bar in (C–F), (H–L) = 25 μm. Scale bar in (G) = 50 μm.
The degree of astrocytic proliferation typically paralleled the intensity of acute and chronic inflammation. A more modest degree of inflammation (Fig. 1A, D) generally correlated with less astrocytic proliferation, while astrocytes surrounded by intense acute and chronic inflammation more often showed MIB-1 immunoreactivity (Fig. 1B, E, F). Thus, in all cases of acute and organizing abscesses, astrocytes adjacent to the lesion showed proliferation (1.2%, n = 8). In contrast, infectious and noninfectious lesions characterized by a more modest degree of inflammation in the surrounding brain parenchyma showed fewer proliferating astrocytes. This trend was particularly evident in cases of long-standing gliosis. Although all cases showed abundant reactive astrocytes (Fig. 1G, H), no MIB-1-labeled astrocytes were identified (Table; Fig. 1I).

A distinctive form of reactive astrocyte, the Creutzfeldt cell, was present in many of our cases. Originally described in multiple sclerosis lesions, these cells show bizarre morphology and nuclear fragmentation that can be seen on hematoxylin and eosin staining (Fig. 1J). We found that the nuclear fragments in Creutzfeldt cells were invariably positive for MIB-1 (Figs. 1K, L, and 2D).

A relatively high average proliferation rate (1.1%) was seen in biopsies from patients with acute demyelinating lesions (Fig. 2; Table). Proliferating astrocytes were identified in 5 of 6 cases. All cases showed typical histopathology with areas of chronic inflammation (Fig. 2A), selective demyelination as demonstrated with stains for myelin and axons, and the presence of numerous macrophages (Fig. 2). Many proliferating astrocytes in these cases displayed characteristic reactive morphology (Fig. 2B). In addition, a number of binucleate (Fig. 2C) and Creutzfeldt (Fig. 2D) cells were also seen. Binucleate astrocytes and astrocytes containing several nuclear fragments were invariably immunoreactive for MIB-1.

The highest average proliferation rate (5.8%) was observed in PML (Table). The single highest proliferation rate in an individual case (13.0%) was also seen in PML. The diagnosis of PML was suggested in demyelinating lesions by the presence of typical viral cytopathic changes in oligodendroglial cells (Fig. 3A). All cases showed moderately enlarged astrocytes with irregular nuclei and hypertrophic eosinophilic processes (Fig. 3B), although bizarre giant astrocytes were not identified in every case. The diagnosis of PML was verified by a positive immunostaining with antibodies to polyoma virus (Fig. 3C). Polyoma virus immunostain identified not only infected oligodendroglia (Fig. 3C, arrow) but also cells with more irregular nuclei consistent with astrocytes (Fig. 3C, arrowhead). All cases of PML contained MIB-1-immunoreactive...
astrocytes (Fig. 3D, arrow). MIB-1 immunostaining also identified cells with enlarged, rounded nuclei and scant cytoplasm consistent with infected oligodendroglia (Fig. 3D, arrowhead).

**DISCUSSION**

We demonstrate that proliferation of human astrocytes is a common response to a variety of insults. In the cases examined, the degree of proliferation correlated with the extent of acute and chronic inflammation in the adjacent brain parenchyma with the highest overall rates of proliferation seen adjacent to acute and organizing abscesses (1.2%) and in demyelinating lesions (1.1%). Almost all of these cases contained proliferating astrocytes. However, even in these selected subgroups, overall astrocyte proliferation was modest, representing only about 1% of total astrocytes. Proliferation indices were even lower in the remainder of the cases (Table), despite prominent histologic and immunohistochemical evidence of reactive changes. Indeed, only half of the cases showed any MIB-1-immunoreactive astrocytes.

Our findings significantly extend previous analyses of astrocyte proliferation in humans. Astrocytic proliferation has been examined in two previous studies of multiple sclerosis lesions (20, 21). Examination of acute plaques from a total of 6 patients in 2 separate studies suggested that approximately 3% of astrocytes were proliferating, although the variability among lesions was high. In a study of brains from 5 cancer patients who were administered BrdU for diagnostic purposes, 18% of BrdU-positive cells in the dentate gyrus expressed...
GFAP (18). These findings support the potential of human astrocytes to proliferate but provide only limited information on the degree of astrocytic proliferation possible in the human nervous system. Our study suggests that human astrocytes typically show a restricted capacity to proliferate.

The generally low rates of astrocytic proliferation we document in human astrocytes outside the context of polyoma viral infection contrast with results from experimental animal models. In murine models of ischemia and trauma, significant astrocytic proliferation is typically present (3–8), with up to 46% of astrocytes showing evidence of DNA replication (7). A variety of factors may have contributed to the generally lower levels of astrocyte proliferation seen in our study. The technique used to identify proliferating astrocytes (administration of a nucleotide analog vs. immunohistochemistry for MIB-1), the age of the lesion, the distance from the injury site, and species-specific astrocyte biology may all contribute to the measured rates of astrocyte proliferation. Although extensive comparative analyses of human astrocytes to astrocytes in other species have not been carried out, there do appear to be important differences. For example, a large proliferating population of subventricular zone astrocytes lines the lateral ventricles in humans but not in other primates or in rodents (26).

The extent to which human astrocytes can proliferate in response to injury may have important implications for neuronal regeneration and repair. Following injury that significantly impairs the blood-brain barrier, astrocytes play a key role in excluding non-CNS molecules from the brain parenchyma and limiting neurodegeneration (27). Astrocytes may also aid in the response to injury through their documented role in providing growth and neurotrophic factors. However, astrocytes, in particular through their participation in the glial scar, most likely inhibit regeneration as well (23). Whatever the aggregate role of astrocytes in CNS injury, our data suggest that reactive resident astrocytes, rather than a substantial newly formed population of cells, subserve these functions.

MIB-1 immunoreactivity was invariably present in an unusual form of reactive astrocyte, the Creutzfeldt cell (Figs. 1K, L, and 2D). These bizarre cells were originally identified in multiple sclerosis lesions and are commonly considered reactive rather than proliferative in nature. Our findings strongly suggest that the cell cycle machinery has become activated in these astrocytes. However, the actual proliferative potential of Creutzfeldt cells is unclear. The extreme nuclear fragmentation characteristic of these forms may not be compatible with successful completion of cytokinesis. Instead, Creutzfeldt cells may be degenerating. The degree to which cell cycle activation may presage cell death rather than cell proliferation remains an open question.

The highest rates of astrocytic proliferation were in cases of PML (Table). The high proliferation rates may reflect the ability of JC virus to upregulate cell cycle regulatory proteins. Cultured human astrocytes infected with JC virus upregulate cyclin A and cyclin B1 (28). Astrocytes from cases of PML also show increased expression of cyclin A and cyclin B1 as well as Ki-67 expression (29).

Determination of proliferation indices is often part of the clinical evaluation of neuroglial tumors. In this study, we demonstrate that there is a modest amount of astrocytic proliferation in nonneoplastic conditions that should be considered when differentiating tumors from reactive conditions. Although the proliferation rates we document are not high enough to overlap with those typical in high-grade glial tumors, the rates are similar to those seen more typically in low-grade gliomas (25, 30, 31). Thus, a modest degree of astrocytic proliferation will not necessarily distinguish a low-grade glioma and a reactive condition. However, outside the context of PML, a substantially elevated MIB-1 index in astrocytic cells does suggest the diagnosis of a tumor. In this regard, it is important to note that our indices represent astrocyte-specific proliferation rates. In the case of lesions with substantial proliferation of nonastrocytic cells, the total MIB-1 labeling indices would be significantly higher.

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