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Quantitative Aspects of Reactive Gliosis: A Review*

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Recent studies of gliosis in a variety of animal models are reviewed. The models include brain injury, neurotoxic damage, genetic diseases and inflammatory demyelination. These studies show that reactive gliosis is not a stereotypic response, but varies widely in duration, degree of hyperplasia, and time course of expression of GFAP immunostaining, content and mRNA. We conclude that there are different biological mechanisms for induction and maintenance of reactive gliosis, which, depending on the kind of tissue damage, result in different expressions of the gliotic response.

KEY WORDS: Gliosis, astrocytes, GFAP, brain damage, demyelination.

INTRODUCTION

Gliosis is a universal event in the central nervous system (CNS) following tissue damage of any kind (reviewed in 1-3). It is characterized by the presence of large numbers of reactive astrocytes, distinguished from normal astrocytes by their larger size, longer thicker processes, and increased staining of glial filaments. In earlier days such astrocytes were demonstrated with special stains, such as the Cajal gold-sublimate or the Holzer stain, which were fairly specific for glial filaments, but during the past twenty years these have been supplanted by the immunohistochemical detection of glial fibrillary acidic protein (GFAP), the major protein of astrocyte intermediate filaments and a specific marker for astrocytes (4-6).

The determination of reactive gliosis is usually based on the appearance of immunostained sections. Since the morphological expression of reactive gliosis is similar, independent of the injury, there has been a tendency to assume that the reaction is stereotypic. Recent quantitative studies of this phenomenon show that this is not the case and that different types of CNS damage induce a wide variety of astrocyte responses. These data have led to new insights into this common pathological process.

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Brain Injury

Stab wounds of the rodent cerebrum are the most common and best defined model of reactive gliosis (7-20). There is fairly good agreement among the studies as to the time course and nature of the gliotic reaction; what differences there are may be attributed to the extent of damage caused by the lesion.

Reactive astrocytes can first be detected in the vicinity of the wound within 3 h post-operation (11,13,18). A similar very early appearance of reactive glia has been seen following cryogenic lesions (21). Some investigators report that this early reaction is followed by a period of 6-24 h in which the numbers of GFAP+ astrocytes away from the vicinity of the wound actually decrease (11,18), although they are present adjacent to the lesion. The gliotic reaction then spreads throughout the ipsilateral cortex and into subcortical regions, reaching maxi-

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mum intensity at 3-7 d post-operation. The contralateral hemisphere is rarely affected, although spread of gliosis to the contralateral hemisphere has been observed in young rats (1-month-old) with large lesions and in animals injured with laser irradiation (22,23). The glial reaction then gradually resolves, attaining a nearly normal appearance by 3 weeks (11,13,18). The cortex and hippocampus have more reactive cells than the corpus callosum, and deeper subcortical regions resolve more slowly than cortex (11,13,18). Sham-operated controls, in which the skull has been abraded but not opened, mount a stronger gliotic response in the first 24 h than do the lesioned animals (18).

GFAP and GFAP mRNA. The amount of GFAP in the operated hemisphere roughly parallels the intensity of the GFAP immunocytochemical staining pattern (18). There is no statistically significant increase of GFAP content relative to intact animals until 3 d post operation. The amount of GFAP approximately doubles by 5-7 d and then declines to normal by 21 d (18). The expression of mRNA for GFAP follows quite a different time course (19,20). We found that GFAP message levels increase to 2-fold control values at 6 h, and 5-fold at 12 h. Thereafter they remain at 3.5 - 6-fold until 5 d and decline to nearly normal at 21 d (19). A very similar time course, with somewhat higher GFAP mRNA levels, has also been reported, and in these animals elevated levels of GFAP mRNA are also found in the contralateral cortex (20). This rapid increase in GFAP mRNA precedes a significant increase in GFAP by about 2 d, and the decrease of message is more precipitate than the slow decline of GFAP content (19).

Astrocyte Proliferation. A common finding is that most astrocytes in the normal cortex are negative or only weakly stained for GFAP in aldehyde-fixed sections. (*This may be artefactual since by merely changing the fixative, GFAP immunostaining can be readily demonstrated in cortical protoplasmic astrocytes and abolished in white matter astrocytes (24). This is a good example of an often-disregarded principle of histochemistry that negative results do not prove absence and must be interpreted cautiously. It is probable that all astrocytes have a finite level of glial filaments, but, depending on the conditions, that level may be below the threshold of detection.*) Therefore the great increase in the numbers of GFAP+ cells following injury has led to the natural assumption that gliosis involves significant astrocyte proliferation. This has been a point of considerable contention, but careful [^{3}H-]thymidine-labeling studies now show that although some reactive astrocytes are generated by cell division, most are not (7,9,13-17), and many of the [^{3}H-]thymidine-labeled cells appear not to be astrocytes (13,14,25,26). The actual numbers of [^{3}H-]thymidine-labeled astrocytes vary considerably among the studies because of differences in labeling protocols and amount of trauma.

Cavanagh's demonstration of mitiotic figures in astrocytes after lesioning was the first proof of astrocyte hyperplasia, but quantitation by this technique is difficult (7). A limited study using ^{3}H-thymidine pulse labeling showed only a few labeled astrocytes (9). A more thorough pulse-labeling study showed that there were no labeled astrocytes 48 h post-lesion, and that 3-5% of astrocytes in the vicinity of the lesion were labeled at 72-96 h (13). However after cumulative labeling for 6 d, 17% of the reactive astrocytes near the wound were labeled. Concerned that some of the GFAP-negative cells might also be astrocytes, Miyake et al. (14) repeated these experiments, identifying astrocytes by immunostaining for S-100 protein. They found a [^{3}H-]thymidine labeling index of 1.2% 48 h post-lesion and 10.7% after cumulative labeling for 6 d. Thus, for reasons that are not clear, these two markers gave somewhat different results. With a different labeling protocol ([^{3}H-]thymidine injections 8 h apart and sacrificing 1 h later), Topp et al. (17) found that the peak labeling index in the cortex for yound adults was 20% at 3-4 d post-lesion, whereas hippocampal astrocytes were maximally labeled at 2 d post-lesion. Aged animals exhibited the greatest astrocyte labeling index (40%) in cortex at 3 d post-lesion. All of these data refer to a zone adjacent to the lesion; reactive astrocytes more than 1-2 mm distant have much lower labeling indices.

Many of the GFAP- cells that label with [^{3}H-]thymidine can be identified as inflammatory cells, microglia and endothelial cells (12,13,14,25,26). The concern that some GFAP- cells could be astrocytes that have not yet begun to express GFAP seems well founded. Takamiya et al. (15) pulse-labeled 1 month old rats at 3 d post-lesion and sacrificed them 6 h, 24 h and 48 h later. The labeling index at 6 h was 3%, but increased to 15% at 24 and 48 h. Another study in 1 month-old rats showed that only a few astrocytes incorporated thymidine after pulse-labeling during the period 2 h to 16 days post-lesion. However, in animals labeled 2 d post-lesion and sacrificed 40 d later 17% of the astrocytes in region of the wound were heavily labeled (16). These data show that, at least in young animals, there are GFAP- cells that take up thymidine at 2-3 d post-lesion and later become GFAP+. These could either by immature astrocytes, astrocyte precursors; or merely normal protoplasmic astrocytes that have not yet expressed detectable levels of GFAP.

A number of investigators have noted that some

reactive astrocytes at the edge of the wound stain for vimentin as well as for GFAP (10,15,22,27,28). Since vimentin is present in immature astrocytes and ceases to be expressed in most as differentiation proceeds (29-32), it is tempting to speculate that the vimentin+ cells may be those undergoing division. Such studies, however, have not been done. It has been stressed that in animals with ischemic lesions, vimentin+ cells were seen only in areas suffering permanent injury (28). It has also been shown recently that in the stab wound model all reactive astrocytes contain MAP-2, a protein that in normal brain is largely restricted to neuronal cell bodies and dendrites (33).

Some general conclusions can be derived from the results of the many studies summarized above. The stab wound model involves a hemorrhagic, necrotic lesion with large numbers of inflammatory cells infiltrating the edges of the wound. Hypertrophic astrocytes appear within a few hours in the 1-2 mm adjacent zone, too early for any significant increase of GFAP synthesis to occur. The next event is a rapid elevation of GFAP mRNA, followed by an increase in GFA protein that parallels the spread and increase in numbers of large, GFAP+ cells. That the gliotic reaction spreads throughout the ipsilateral cortex, and occasionally into the contralateral cortex, suggests induction by diffusible factors. There is also a significant hyperplasia of reactive astrocytes during the 3-6 d period post-lesion, but this is mainly restricted to the vicinity of the wound and affects only a minority of cells. Thus it appears that the majority of the reactive astrocytes are hypertrophied endogenous astrocytes and that their antecedents are both protoplasmic and fibrous astrocytes. All of these indicators of gliosis are mostly resolved by 3 weeks in areas that are not permanently damaged. A small zone of gliosis persists in the area of the lesion.

Toxic Lesions

The stab wound lesion involves a very complicated series of events - disruption of the vasculature, infiltration of hematogenous cells and serum, ischemia, anoxia, cell death - which makes analysis of the mechanism of gliosis very difficult. Gliosis following neuronal death induced by the systemic administration of a neurotoxin may afford a potentially simpler system for analysis, especially if inflammation is minimal or absent. Two such toxins have been thoroughly examined: trimethyl tin (TMT), which damages neurons in limbic structures (34), and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which targets the nigrostriatal dopaminergic pathway (35). Single intravenous doses of TMT in rats cause large dose- and time-dependent increases in GFAP in both hippocampus and frontal cortex (34). At a dose of 8 mg/kg, GFAP reaches a maximum of 4.5-fold in the hippocampus and 3-fold in the cortex at 35 d. The CA3 region of a hippocampus shows a 67-fold increase! These levels return to normal by 84 d. The GFAP concentration is paralleled by a dramatic increase in GFAP immunostaining, which also decreases by 84 days. [^{3}H-] thymidine labeling is maximal at 4-5 d after dosing, but less than 1% of the GFAP+ cells are labeled. A similar study has been performed on mice injected with a single subcutaneous dose of MPTP (12.5 mg/kg) (35). In these animals striatal GFAP increases 3-fold in 48 h followed by a decline to normal in 3 weeks (35). The authors verified that the blood-brain barrier was intact in these animals and that there was no increase in interleukin-1, indicating the absence of an inflammatory response. The gliosis induced by MPTP has a much more rapid onset and a quicker resolution than that induced by TMT, and the increase in GFAP is even faster than that observed in the stab wound model.

Gliosis has been more cursorily studied following the administration of the neurotoxins ibotenic acid, 6-hydroxydopamine and kainic acid, but these experiments required intracerebral injections that add the further complications of a needle wound (25,26,36). GFAP mRNA rises 1.4 fold in the ipsilateral striatum at 10 d following 6-hydroxydopamine lesions of the substantia nigra (36). It is probable the peak increase occurs before 10 d, since GFAP mRNA levels increase 4-fold in the striatum 2 d after an ibotenic acid lesion, and are already lower at 5 d (36). Other studies examined cell proliferation following similar excitotoxic lesions. In a striatal lesion induced with kainic acid many cells incorporated thymidine 3 and 6 d later, but none are astrocytes (25). Presumably they were primarily hematogenous cells and microglia. In another study ibotenic acid was injected into the caudate, which was then removed and dissociated for cell culture (26). The proliferating cells were identified as mostly of hematogenous origin, monocytes, macrophages, and T-helper cells (26). Thus there seems to be little proliferation of reactive glia in these lesions.

Genetic Diseases

Essentially all genetic disorders of the CNS that involve tissue destruction exhibit reactive gliosis. Studies in both humans and animals have been extensive, but mainly morphological. Only a few of the more pertinent and novel findings will be mentioned.

One of the more dramatic examples of gliosis is present in brains from cases of adrenoleukodystrophy, a

hereditary disorder of lipid metabolism. The pathological process, which usually starts in the occipital pole and progresses rostrally, initially involves demyelination followed by loss of axons and neurons. In extreme cases the entire occipital pole becomes a network of filament-rich astrocytic processes (37,38). This is an extreme case of gliosis progressing to a glial scar.

Astrocyte pathology in the cerebellar and demyelinating mouse mutants has been reviewed (39,40). While gliosis is present in most of these mutants, there are also astrocyte abnormalities that appear specific to the disorder. One of the interesting findings was the confirmation that ganglioside GD_3, which is elevated in several mutants characterized by neuronal degeneration (40), was localized on the surface of reactive astrocytes (41). This ganglioside is a major ganglioside in the immature CNS and is present on immature neuroectodermal cells, including glial precursors (42,43), but only low levels are found in the mature CNS. It is, however, highly enriched in multiple sclerosis plaques; a finding that first indicated it may be a major ganglioside of reactive glia (44). It is possible that GD_3 is a general marker for reactive astrocytes but is has not been looked for in most cases.

Gliosis in three hereditary animal diseases has been examined quantitatively: the Gunn rat (45), the twitcher mouse (46,47) and the brindled mouse (48). The former two mutants could be considered examples of the effects of neurotoxins: excess unconjugated bilirubin in the case of the Gunn rat, and psychosine production in the case of the twitcher mouse.

The elevated serum bilirubin in the Gunn rat, caused by lack of UDP-glucuronyl transferase, induces a marked hypoplasia of the cerebellum. The severity of damage varies among animals and it has been shown that the amount of GFAP in the cerebellum is inversely related to cerebellar weight (45). The increase in GFAP concentration averages about 4-fold that of heterozygous controls.

The twitcher mouse, an authentic model of human globoid cell leukodystrophy, has a deficiency of galactosylceramide-β-galactosidase, an enzyme that degrades cerebroside and psychosine (galactosylsphingosine), a known cytotoxin (49). This leads to severe demyelination in both CNS and PNS, infiltration of globoid cells (macrophages) and gliosis. Increased immunostaining for GFAP is seen in reactive astrocytes, in non-myelinating Schwann cells of the sciatic nerve, and in satellite cells in the trigeminal ganglion. These changes in GFAP expression coincide chronologically and topographically with the infiltration of macrophages, and precede the start of demyelination (46). This suggests either that the factors which trigger macrophage infiltration and activate reactive astrocytes are common or related, or that the macrophages themselves release astrocyte activating factors (see section below on mechanisms). GFAP increases 2-5-fold in the brain stem and spinal cord, and vimentin 4-5-fold (47). Double immunofluorescence staining shows that nearly every GFAP+ cell in the brain stem is also vimentin+ (47). Thus there is much more vimentin expression in this disease than in the injury models (discussed above). The immunostaining for GFAP in the PNS has been confirmed quantitatively. In normal nerves GFAP is undetectable on western blots (although some immunostaining is present), but twitcher sciatic nerves contain about 0.2 μg GFAP/50 μg of cytoskeletal protein, roughly 50-fold the normal level but still only 2% of the level in spinal cord (47). These results demonstrate that at least two types of peripheral glia, as well as astrocytes, can increase GFAP expression in response to pathological stimuli. Bergmann glia and radial glia, however, show no changes in immunostaining.

The brindled mouse, which has an X-linked deficit in copper absorption, shows an onset of gliosis as abrupt as that seen after stab wounds (48). At 10 days of age (P10) affected animals are not different from littermate controls in GFAP immunostaining, GFAP content, or GFAP mRNA levels. Only 2 d later affected animals have a 10-fold increase in GFAP mRNA levels in cortex and thalamus. GFAP immunostaining, GFAP content and GFAP mRNA levels are still elevated at P15, however, cell counts in the cortex show no differences in total cells, glia or neurons between brindled mice and normal controls.

Twitcher and brindled mice have a limited life span (40 d and 15-16 d, respectively) thus extended studies are not possible, but the gliosis shows no signs of resolution in either case. The Gunn rat does have a long life span and in these animals gliosis also does not diminish, and may progress as tissue destruction continues. This is in contrast to the models of neurotoxicity discussed above (TMT and MPTP) in which gliosis resolves with time.

Inflammatory Demyelination

Multiple sclerosis (MS) is the most common human inflammatory demyelinating disease. Severe fibrous gliosis is a prominent pathological feature of this disease and reactive astrocytes can constitute the major cell type in old, completely demyelinated plaques (50). Such glial scars stain intensely for GFAP and were the tissue chosen as the source for the first isolation of this protein (4).

The most common animal model of the pathological processes in MS is experimental autoimmune encephalomyelitis (EAE), which occurs in both acute and chronic-relapsing forms. Glial scars are not seen in acute EAE but are present in chronic-relapsing models in the guinea pig and mouse, which show persistent elevations of GFAP (50-52).

Gliosis in acute EAE in the Lewis rat has been most thoroughly studied and presents some unusual features. Smith et al. (53) were the first to show that, in animals sensitized to develop EAE, markedly enhanced immunohistochemical staining for GFAP appears in the spinal cord at about 10 days post-inoculation (dpi), coincident with the appearance of inflammation and the onset of clinical signs. This astrocyte reaction is widespread in both gray and white matter at all levels of the cord, is morphologically unrelated to the perivascular inflammatory lesions, and continues to persist after the resolution of clinical signs (about 20 dpi). This reaction appears to reflect hypertrophy of existing astrocytes since little hyperplasia is detected (54). The most surprising finding is that there is no apparent increase of GFAP content during the acute stages of the disease (11-14 dpi), although there is increased protein synthesis, including GFAP synthesis (53).

Most of these findings on gliosis in EAE have been confirmed and extended (55-57). In studies of the mechanism of inflammatory demyelination we discovered that the α_1-adrenoceptor antagonist, prazosin, can suppress clinical signs of EAE, block the extravasation of serum proteins into the cord parenchyma (edema) and delay the onset of the inflammatory response (58,59). This drug also delays the expression of the astrocyte reaction in EAE rats by a few days and suppresses the increased synthesis of vimentin (55). The prazosin treatment showed that the enhanced GFAP staining correlates with the onset of infiltration of inflammatory cells, but does not correlate with clinical signs or the amount of edema.

The discrepancy between increased immunocytochemical staining for GFAP and lack of change in GFAP content has been further explored (56). In the spinal cord of Lewis rats sensitized to develop EAE, GFAP increases gradually and significantly over time, and by 35 dpi reaches a level 1.5-2 times greater than controls. The GFAP content remained elevated through 65 dpi - the latest time examined. In this study increased GFAP immunostaining appears at 10 dpi, yet even at 14 dpi there is no significant increase in GFAP content. Other possible explanations for this early increase in staining in the absence of an increase in protein, such as an increase in soluble GFAP or modifications in the immunochemical properties of GFAP (e.g. an increase in epitope) have been ruled out. The most probable explanation, for which there is some evidence (60), is a physical change in glial filament bundles that permits them to bind more antibody (56).

This increase in GFAP is accompanied by increases in vimentin, which follows a similar time course (56). While some of this increase is undoubtedly due to inflammatory cells there is pronounced vimentin staining in reactive astrocytes (61) and in radially oriented astrocytes of the white matter late in the disease (56). Reactive astrocytes in spinal cord also show intense carbonic anhydrase staining, particularly in white matter, that is not present in controls (61). Although most attention has been paid to spinal cord, where the disease is more severe, gliosis is also present in the brain, primarily in white matter, and not related to inflammation. The reactive astrocytes in brain contain the astrocyte-specific Yb isoform of glutathione-S-transferase and, less frequently, vimentin (62). In contrast to spinal cord, astrocytes of the optic tract, a region of severe gliosis, do not stain for carbonic anhydrase (62).

In the same EAE model, GFAP mRNA follows a completely different time course and reaches higher relative levels than does GFA protein (57). EAE spinal cords have up to 3-fold more RNA than controls (57), similar to the increase in DNA (63,64), reflecting the infiltration of inflammatory cells. As early as 10 dpi elevated levels of GFAP mRNA can be detected relative to controls. During 11-14 dpi GFAP mRNA levels reach 6-8--fold control values then slowly decline throughout the time course, with a 4-fold increase still present at 65 dpi. However, coinciding with the height of inflammation and clinical signs at 12 dpi, GFAP mRNA drops to approximately 50% of the level at 11 dpi, then rises again at 13 dpi. This unusual event is accompanied by a similar transient dip in the level of mRNA for the 70 Kd neurofilament subunit. The levels of GFAP message contrast sharply with those of GFAP, which, as noted, increase only slowly and do not reach maximum values until after 4-5 weeks. Thus all indicators of gliosis: GFAP, GFAP mRNA and GFAP immunostaining, remain elevated at least through 65 dpi, more than 40 d after clinical signs have resolved (56,57).

The temporary decrease in message levels for GFAP and NF-70 may represent a temporary stress-induced reprogramming of transcription. There is a rapid increase in the mRNA for the 70 Kd heat shock protein (HSP70) relative to controls in spinal cord during the acute stages EAE. The mRNA rises from control levels at 7 dpi to 6-fold controls at 12 dpi, and drops again to control levels by 21 dpi (65). Thus HSP70 mRNA is a mirror image of GFAP mRNA, peaking when the latter dips.

There are also transient increases of c-fos mRNA and protein at the peak of disease (66).

CONCLUSIONS

The studies summarized here permit some generalizations.

Persistent or Resolvable Gliosis. In trauma models it appears that gliosis in areas of permanent tissue injury is permanent, whereas that induced in areas away from the lesion in transitory (28). This rule also seems to hold for damage caused by genetic lesions or by chronic inflammatory demyelination. On the other hand gliosis induced by toxins, which also cause permanent neuronal destruction, does resolve. In acute EAE, in which there is little tissue damage, gliosis persists for a long time, but may eventually resolve.

Hyperplasia and Hypertrophy. This issue seems finally to be settled in trauma models. There is hyperplasia but only in the immediate vicinity of the lesion. Most reactive astrocytes, and especially those at some distance from the lesion, are transformed from the endogenous astrocyte population. The origin of the dividing cells is not known. There appears to be little hyperplasia in the other models when it has been examined, but fewer studies have been done.

GFAP Immunostaining vs GFAP Content. In most cases the intensity of GFAP immunohistochemistry probably reflects GFAP content fairly well. However the results with sham-operated rats and in animals with acute EAE indicate that immunostaining must be interpreted cautiously.

Biochemistry. GFAP immunostaining, GFAP content and GFAP mRNA have been the common indicators of an astrocyte response. Vimentin expression has been proposed as a more specific indicator of permanent injury in ischemia (28) and may be in stab wound lesions as well. It is, however, also expressed in acute EAE where damage seems minimal, although scattered small lesions may persist. Ganglioside GD_3 has only been examined in persistent cases of gliosis and MAP-2 only following stab wounds. The time course, distribution and ubiquity of the vimentin, GD_3 and MAP-2 responses deserve more study.

Other early responses to brain injury are the induction of the transcription of heat shock genes (67) and of c-fos (68). In the stab wound model both heat shock (67) and c-fos (68) messages are significantly elevated 1-3 h after injury - several hours before the increase in GFAP mRNA (19). In acute EAE, however, all three messages are elevated at 10 dpi, but the time of maximal expression is different for each: 11 dpi for GFAP mRNA, 12 dpi for HSP70 mRNA and 11-12 dpi for c-fos mRNA (57,65,66). Thus neither the heat shock nor the c-fos responses appear to be directly linked to GFAP expression in either model.

Mechanisms. Brain injury models (69) and inflammatory demyelination both involve disruption of the blood-brain barrier, infiltration of serum and inflammatory cells, and activation of endogenous microglia. In brain injury models gliosis spreads far from the lesion; in EAE widespread gliosis occurs coincident with the onset of inflammation. These facts suggest that diffusible substances, such as serum factors, cytokines and other inflammatory mediators play an important role in triggering the gliotic response.

Speculations on mechanisms have focussed on two expressions of gliosis, astrocyte proliferation and GFAP expression. These may involve different mechanisms, since increased GFAP expression can occur in the absence of hyperplasia, although the converse has not been observed in vivo (but does occur in vitro, see below). Most of the relevant data have by necessity been obtained from in vitro studies.

There are many known mitogens for astrocytes. These include both well-characterized and uncharacterized growth factors, a discussion of which would be beyond the scope of this review. Of particular interest here are the inflammatory mediators interleukin-1 (IL-1) (70,71), tumor necrosis factor (TNF) (72,73) and interleukin-6 (72). IL-1, a product of activated macrophages, stimulates proliferation of rat astrocytes in vivo (71). Curiously it has no effect on cultured bovine astrocytes in the absence of serum (72). Astrocyte proliferation induced by TNF (72) is unusual in that it is associated with a marked down-regulation of GFAP mRNA expression and a reduction in GFAP levels, although vimentin mRNA is unaffected (74). This behavior has not been documented with other astrocyte mitogens. The significance of these in vitro studies for reactive gliosis remains speculative since the proliferative response is minimal in most gliotic conditions. It should also be noted that in the MPTP model the blood-brain barrier is intact and IL-1 can not be detected in the brain (35).

GFAP expression in cultured cells is influenced by dibutyryl cAMP (75,76), phorbol esters (76), serum factors (75), growth factors (77), and glucocorticoids (77). It is interesting that although hydrocortisone causes elevation of GFAP levels in vitro (77), corticosterone reduces GFAP content and mRNA levels in animals (78,79). Thus it may not be possible to extrapolate from the direct actions of an agent in vitro to the in vivo situation where indirect effects may predominate.

We are obviously far from a complete understanding of the complex phenomenon of reactive gliosis. The previously limited classification of astrocytes into protoplasmic, fibrous, radial, and Bergmann glia, is rapidly giving way to the idea that astrocytes also express region-specific properties, which may, in part, be a function of regional neuron-glial or glial-glial interactions (see ref. 3 for a discussion). The diversity in the manifestation of the gliotic response, which may be related to the diversity of astrocyte type and specific interactions, argues against a unified mechanism of gliosis.

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