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Reactive Astrogliosis in the Injured and Postischemic Brain

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1. GENERAL COMMENTS

Reactive astrocytes appear within days of a disturbance or injury to the brain. They form part of a gliotic or scar tissue, which can take on a very complex form, depending on the type and intensity of the disturbance. Reactive astrocytes are usually characterized by morphological changes and increases in intermediate filaments. However, other more functional characteristics are not as well-characterized. In particular, glial interactions with surviving neurones are not very well-defined. Some attention has been paid to glial upregulation of protective factors and changes in expression of extracellular matrix, but changes in membrane properties and homeostatic mechanisms have received very little regard. This is true for injury in general, but is especially true following ischemic injury.

2. GLIAL SCAR VERSUS GLIOTIC TISSUE: TERMINOLOGY AND CELLULAR COMPOSITION

The terms gliosis, reactive astrocytosis, and glial scar are not very well defined, and most authors use them without a clear definition. Quite often it is assumed that gliosis is a stereotypic reaction of brain tissue to injury. Although it is true that gliosis is one of the most sensitive markers for any type of brain disturbance, the resulting reorganization of the tissue is complex and highly dependent upon type and intensity of the disturbance.

"Gliosis" or "reactive gliosis" are terms used to indicate long-term changes (a few weeks to permanent) in the structure of astrocytes and microglia in response to disturbances in the neuronal microenvironment. These disturbances can be so subtle and small that neuronal appearance remains unchanged. In fact, two studies (Nedergaard and Hansen, 1988; Kraig et al., 1991) have shown that artificially induced successive waves of spreading depression in otherwise healthy brain tissue, can cause gliosis with no lasting change in neurones. It is

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also known that gliosis can be associated with the aging brain in areas close to the pial surface (Penfield, 1932). In any case, there is no disruption of brain structure or blood–brain barrier (BBB) and glial limitans. Therefore, the terms "gliosis" or "reactive gliosis" only imply that some lasting change has occurred in astrocytes and/or microglia with no necessary change in neurones or brain architecture.

The terms "glial scar" or "scar tissue", on the other hand, inherently suggest that some type of structural damage has occurred. Since brain damage is not restricted to neurones and glial cells, formation of scar tissue often involves cells of the mesodermal lineage. These mesodermal elements (fibroblasts, meningeal cells, and epithelial cells) interact with astrocytes to form a new barrier to the outside; the glial limitans. This new barrier functions to reisolate the brain parenchyma from the outside environment, replacing the destroyed BBB (at injuries involving capillaries) or pia mater (when injuries involve the dura mater and leptomeninges). Scar formation is also highly associated with cellular proliferation since cells must replace and fill in the path of destruction. The majority of proliferating cells following a disruptive injury consist of astrocytes and microglia (glial cells: "glia" being greek for "glue"). Therefore, unlike "gliosis" or "reactive gliosis," a "glial scar" formation is concordant with glial proliferation and disruption of the glial limitans or blood–brain barrier.

Two other commonly encountered terms used when discussing glial cells following injury are "isomorphic gliosis" and "anisomorphic gliosis". Isomorphic injury implies that gliosis is associated with progressive loss of myelination and a parallel arrangement of astrocytic fibers, but the basic organization of the tissue is left intact. On the other hand, anisomorphic gliosis is composed of glial elements arranged in a netlike mesh, resulting in a complete reorganization of tissue (Courville, 1964). In essence, the terms "isomorphic" and "anisomorphic" bear some resemblance to the terms "gliosis" and "glial scar" (as defined above) respectively.

Although both astrocytes and microglia are generally involved in all types of injury, this chapter will be focusing on properties of astrocytes following injury. The microglial reaction (reactive microgliosis) to ischemia is dealt with in the following chapter. Reactive astrocytes following almost any type of injury are characterized morphologically by an increased size (hypertrophy) with enlarged and extended processes. These two criteria (hypertrophy and changes in processes) seem to be the extent one can stereotype the astrocytic reaction in gliosis (Malhotra et al., 1990). An increase in the astrocyte-specific intermediate filament GFAP is a reliable sign of underlying astrogliosis, however, it is not seen in all cases (da Cunha et al., 1993; Hatten et al., 1991) There is increasing evidence of GFAP– glutamine synthetase+ subtypes of astrocytes also respond to a loss of neurones with functional changes, without becoming GFAP+; at least in the rat hippocampus (Jabs et al., 1997).

3. CELL PROLIFERATION DURING REACTIVE ASTROGLIOSIS

Proliferation studies using autoradiography with tritiated thymidine suggest a low proliferation rate of astrocytes in the normal adult brain: a 1 h pulse labeling will result in identifying 40–50 astrocytes in S-phase throughout the brain (reviewed in Korr, 1986). Some older work with varied exposure times to radioactive thymidine suggests that both a proliferating and nonproliferating pool of astrocytes exists. This work suggests a constant transfer rate from the proliferating to the nonproliferating pool, accompanied by a smaller rate of loss of cells from the nonproliferating to the proliferating pool (Korr, 1980; Korr et al., 1983). An increase of the total number of astrocytes is prevented by a constant attrition rate of astrocytes from the nonproliferating pool: some of these cells become pyknotic and die by phagocytosis mediated by microglia (Korr, 1980). These studies have yet to be verified with the appropriate cell type specific markers, but they do suggest a dynamic equilibrium of proliferating astrocytes in the adult brain, that can be modified by neuronal events and that has to be under close control by a variety of factors.

The equilibrium between the two pools of astrocytes appears to be dynamic: rats kept in an enriched environment show an increase in astrocyte proliferation rate (Altman and Das, 1964) and number (Diamond et al., 1966) compared to impoverished littermates. Consistent with two pools of astrocytes, another study showed that the supraoptic nucleus contains a population of GFAP+ as well as a population of GFAP–/S100+ cells, both of which are regarded as astrocytic in nature. After adrenalectomy or lactation, cells from the S-100+/GFAP– pool shifted to the S-100+/GFAP+ pool, whereas salt loading had the opposite effect (Gary and Chronwall, 1995). There are conflicting reports on whether these shifts are accompanied by proliferation (Gary and Chronwall, 1995; Paterson and Leblond, 1977). However, it does appear that there are two pools of astrocytes differing with respect to proliferation and expression of cytochemical markers.

In practice, the idea of two pools of astrocytes increases the complexity of the picture following injury, but it may help explain some of the seemingly conflicting results regarding astrocyte proliferation. The majority of studies suggest that proliferation of astrocytes after injury is only a minor occurrence in gliosis compared with reactive hypertrophy (Miyake et al., 1988, 1989). The increase in GFAP+ cells observed is not a result of proliferation but rather of the transfer of cells from the GFAP– pool (Miyake et al., 1992) with the bulk of proliferating cells appearing to be microglial in nature. However, proliferation of astrocytes is observed at sites of injury and neuronal loss (Janeczko et al., 1993). It therefore appears that during injury involving neuronal loss, astrocyte proliferation occurs at the site of loss, whereas areas more remote from the injury experience GFAP upregulation (possible transfer from GFAP– pool) and astrocyte hypertrophy (David and Ness, 1993).

Questions remaining to be addressed involve the nature of the two pools of astrocytes in the mature brain. Are the proliferating cells truly astrocytes or are they glial precursor cells? Since many investigations used radioactive thymidine where the label will be transferred to all daughter cells, the daughter cells might subsequently upregulate their GFAP, giving the impression that GFAP positive cells are in the S-phase. Thus, it is still open if all subpopulations are equally capable of entering the cell cycle or if this capability is exclusively the property of an immature precursor subpopulation. A further complication is that many investigators consider GFAP– cells labeled with radioactive thymidine as microglia, contributing to underestimation of astrocytic proliferation after injury.

4. FUNCTIONAL PROPERTIES OF REACTIVE ASTROCYTES

The qualitative change in functional properties of astrocytes seems to be independent of the type and extent of the injury or disturbance. This section will review the injury-induced long-term changes of astrocytes that are based on functional rather than structural parameters, so the reader can get an impression of the associated change in the interaction between astrocytes and surviving neurones.

Whenever possible this review will consider *in situ* measurements rather than results collected from cell cultures, since there are doubts that cell cultures represent a good model for reactive gliosis. GFAP is overexpressed in cultures, as are several membrane receptors, and it seems that only a few selected features reflect *in situ* measures. For example, there are no endfeet in cultured astrocytes and it seems this model is only of interest to study trigger mechanisms of gliosis rather than functional properties of reactive astrocytes.

4.1. Cell Membrane Properties

In situ astrocytes express different membrane properties than those known in cultured astrocytes. There are two subtypes of astrocytes as first described by Steinhauser and coworkers in the hippocampus (Steinhauser, 1993) and confirmed for the spinal cord (Chvatal et al., 1995) and brain stem (Akopain et al., 1997). The cells of the first subtype are termed passive glial cells and are positive for both GFAP and glutamine synthetase, in addition to being well coupled to each other. They express time and voltage independent channels (Fig. 1). Their properties therefore conform to the classical glial pattern. The second subtype is given the name "complex" glial cell because of its complex current pattern. They express voltage dependent potassium channels, especially the A-type, delayed, and inward rectifying (Fig. 1), and TTX-sensitive sodium channels. Cells belonging to the complex subtype are generally GFAP–, but glutamine synthetase+ and only weakly coupled to other glial cells.

If reactive gliosis is elicited in the hippocampus by kainic acid induced epileptic seizures, the amount and intensity of GFAP+ cells increases in the CA1 layer, accompanied by a neuronal loss of 60% (Jabs et al., 1997). Passive

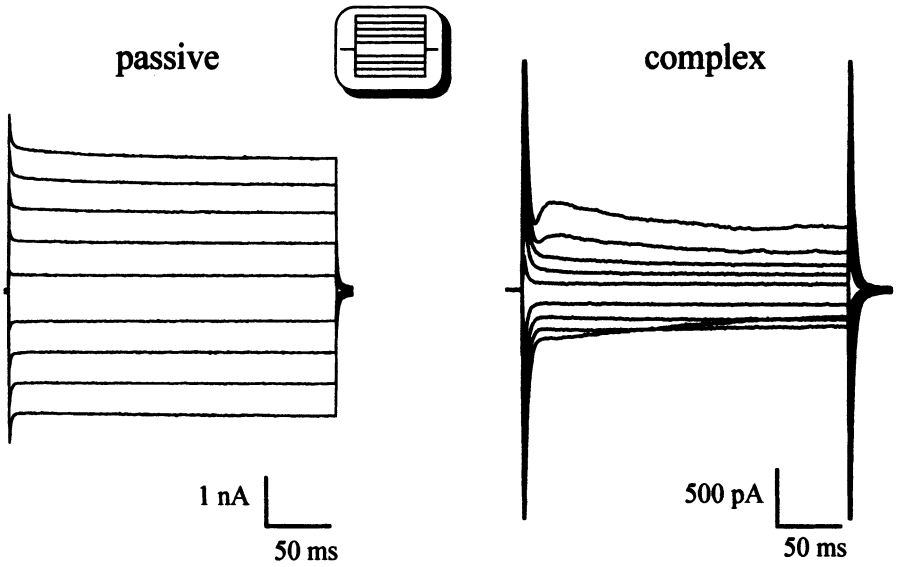

Fig. 1. Current pattern of a passive and complex hippocampal glial cell *in situ.* Shown are the superimposed current traces as a response to the applied voltage pattern shown in the inset. Holding potential was –80 mV. Successive depolarizing and hyperpolarizing voltage command steps were given for 200 ms in 20 mV increments and the responding current pattern was recorded.

(GFAP+) cells do not exhibit any changes in their membrane properties; however, there are dramatic changes in complex cells. The most striking changes in complex cells after seizures is that they are now never coupled and the TTX-sensitive sodium current is lost. Moreover, passive currents of these complex cells are reduced and the input resistance is increased. The conductance of the delayed potassium current is reduced by 40% and the rate of inactivation of the voltage-dependent, inactivating inward potassium current is increased five times (Fig. 2). The complex cells are probably in a position to react to neuronal activity with changes in their ion currents due to depolarization by neuronally released potassium and transmitter substances (Jabs et al., 1997). Remarkable is the complete lack of coupling by these complex cells in the gliotic tissue. It is possible that these cells are the progenitor cells responsible for proliferation after neuronal loss. Newly formed progenitor cells might then lose their voltage-gated channels, begin expressing GFAP and mature into passive cells to join the glial syncytium at a later stage.

There are also changes reported to transmitter receptors on astrocytes after gliosis. The 5-HT_{5A} receptor couples negatively to adenylyl cyclase in astrocytes. Following a needle wound, the 5-HT_{5A} receptor mRNA is upregulated dramatically in GFAP+ astrocytes (Carson et al., 1996). The authors speculate

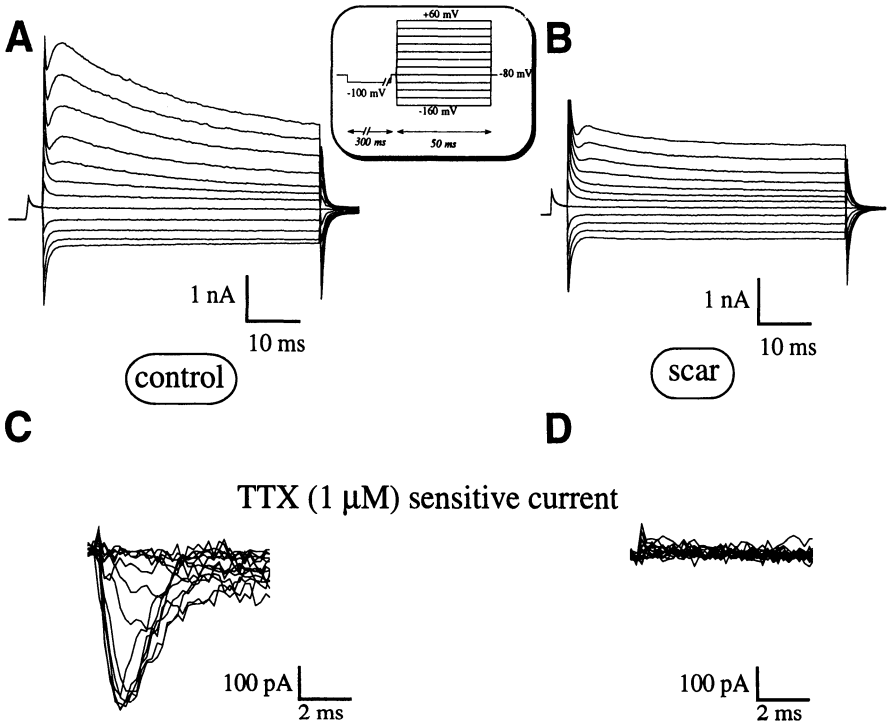

Fig. 2. Patch clamp recording of two different astrocytes. **(A, C).** Complex astrocyte from normal tissue (rmp = –87 mV). **(B, D).** Complex astrocyte from gliotic tissue (rmp = –87 mV). **(A, B).** Current traces recorded during voltage steps from –160 to +60 mV (50 ms, 10 mV, increments, R_s compensated), after 300 ms prehyperpolarization to –100 mV (not shown). **(C, D).** TTX-sensitive current. Voltage step protocol described in (A) was applied with and without TTX (1 μ*M*) and the corresponding current traces were subtracted. In (C) the fast (<1 ms) activating, inactivating properties of the TTX-sensitive current reveal a typical voltage-gated Na^+ channel. In contrast, in (D) in the cell of gliotic tissue, this Na^+ current component was not observed.

that exposure of serotonin released by surviving neurones would lead to reduced intracellular concentrations of cAMP. Such a mechanism may allow neurones to control the extent of gliosis in vivo, since it was shown for cell cultures that gliosis-like changes are mediated by an increase in the cAMP concentration. In addition, it was found that complex glial cells in gliotic hippocampi have an inward current evoked by kainate that is 50% less than those from normal hippocampi, albeit the difference is not statistically significant (Jabs et al., 1997). Since this kainate receptor is permeable for calcium, it would make these reactive astrocytes less sensitive to neuronal input.

4.2. Homeostatic Properties

Clearing the extracellular space of excess potassium released by active neurones was one of the first functional properties assigned to astrocytes. However, there is a long-standing controversy whether reactive astrocytes from gliotic tissue are capable of regulating external K^+ concentrations. Walz et al. (1996) found that most GFAP+ coupled astrocytes from gliotic hippocampus increased their internal K^+ concentration if the external K^+ concentration was raised. If the external concentration increased from 3.5–10 m*M*, internal concentration responded with a 25 m*M* increase. There appears to be two independent mechanisms contributing to this ability, both being able to compensate for the failure of the other one. The first mechanism is energy-dependent (Na^+, K^+-ATPase) and the second is independent of the internal ATP concentration (Donnan-mediated KCI fluxes). Therefore, it would appear that reactive astrocytes have very effective potassium clearance properties, despite the loss of neighboring neurones. Moreover, there seems to be a safety mechanism that allows potassium uptake, despite energy-depletion.

Astrocytes in the normal brain form a syncytium that is involved in spatial buffering of potassium and in calcium/spreading depression waves. Because of detrimental aspects of gliotic tissue following injury, it is questioned whether glial cells maintain this spatial buffering ability. Hossain et al. (1994a,b) investigated reactive astrocytes in vivo with antibodies against Connexin 43, a major component of astrocytic gap junctions. They found a substantial loss of immunocytochemically detectable Connexin 43 at sites depleted of neurones, whereas sites in the immediate vicinity increased punctate connexin 43 staining. Consistent with this, Jabs et al. (1997) and Walz et al. (1996) found extensive dye coupling in GFAP+ astrocytes (passive glial cells) in the hippocampal slice following kainic acid-induced injury. Thus, physiological and immunocytochemical data both indicate that reactive astrocytes possess and use gap junctions surrounding sites of neuronal loss. This, therefore, implies that reactive glial cells do not lose there spatial buffering capacity, but rather augment it (increased coupling).

Termination of transmitter action by uptake and subsequent metabolism is another important function of astrocytes. Unfortunately, there are no comparative studies available on transmitter uptake systems and their efficiency in normal and gliotic tissues. However, Petito et al. (1992) have shown that an increase in astrocytic glutamine synthetase occurs rapidly after ischemia. This suggests that metabolism and possibly uptake of glutamate into astrocytes increases after ischemia and might be an indication that astrocytic systems that terminate transmitter action are upregulated after injury.

4.3. Extracellular Matrix

It is becoming increasingly clear that extracellular matrix and cell surface molecules play a large role in the complex cell–cell and cell–matrix interactions

in development and following injury. Because of the role glial cells play in maintenance and support of neuronal function, it is not surprising that these cells are responsible for synthesizing and secreting the majority of extracellular matrix molecules. Glial-derived matrix provides the tract for leading axons. This glial matrix can direct growth of extending axons in two ways, either by providing a favorable substrate (promoting growth) or by producing an inhibitory substrate, forming a barrier to growth cones. Glial cells do this by secreting or expressing cell adhesion molecules and proteoglycans, respectively. However, adding to the complexity is the fact that these extracellular matrix molecules may bind growth factors and other ligands serving as coreceptors or local reservoirs to effect temporal patterns of secreted trophic–tropic factors (Sudhalter et al., 1996).

Favorable substrates for growth and development can be expressed on the surface of cells as well as be secreted into the extracellular environment. Substrate adhesion molecules which are expressed by glia during development comprise neural cell adhesion molecules (NCAM) and N-cadherin (McKeon and Silver, 1995; Gilmore and Sims, 1996). Both N-cadherin and NCAMs are associated with active growth cones. Two substances secreted by glia that aid neurite extension are glia-derived nexin (GDN) and an extracellular matrix adhesion molecule laminin. Glia-derived nexin is a 43 kDa protein which can serve as a neurite-promoting factor and a specific serine protease inhibitor (McKeon and Silver, 1995). The specific protease inhibitory activity is associated with reduced degradation of extracellular matrix thereby prolonging neurite growth promoting action. Laminin is also secreted by glia into the extracellular matrix and serves a similar function to cell surface adhesion molecules. Expression of laminin is present only in developing axon tracts very similar to expression of NCAM. It is suggested that interaction between GDN and laminin is necessary for prolonged neurite outgrowth (McKeon and Silver, 1995).

The ability to inhibit axonal growth is a necessary component in the repertoire of mechanisms the nervous system uses during development and following injury. Two extracellular matrix glycoproteins, janusin and tenascin, are very interesting because they are able to direct advancing growth cones as well as promote neuronal differentiation (Faissner and Schachner, 1995). Tenascin is predominantly synthesized and expressed by astrocytes in the developing brain. However, following a stab wound injury, astrocytes in and immediately surrounding the stab wound re-express tenascin, perhaps providing the reason axons are unable to permeate glial scar tissue. Tenascin, when distributed in discreet concentrations, functions to inhibit advance of growth cones and thereby redirect neurite extension. It is also postulated that this inhibitory/repulsive activity is involved in setting up boundaries, segregating nuclei and neural pathways (Faissner and Schachner, 1995). Janusin is another glycoprotein from the same family as tenascin, but is expressed by oligodendrocytes at the onset of myelination. Astrocytes appear to be able to induce janusin expression in differentiated oligodendrocytes, perhaps through secretion of platelet derived growth

factor (PDGF) and/or bFGF (Faissner and Schachner, 1995). Therefore, these two glycoproteins can serve as axon guidance mechanisms during development, with janusin being expressed in central nervous system (CNS) myelin and tenascin being expressed during development and following stab wounds in the adult CNS (Faissner and Schachner, 1995).

Another family of extracellular matrix signaling molecules are the proteoglycans. Proteoglycans are made up of a protein core with various glycosaminoglycan (GAG) side chains. It is the composition of the GAG side chains that enable the different proteoglycans to be classified. The four major GAG side chains found in the nervous system are chondroitin sulfate, dermatan sulfate, heparin sulfate, and keratan sulfate. Heparin sulfate proteoglycan (HSPG) is secreted by astrocytes and interacts with laminin to promote neurite outgrowth, whereas keratan and chondroitin sulfate proteoglycans (CSPG) can interact with laminin resulting in inhibition of neurite extension (McKeon and Silver, 1995). Interestingly, astrocytes in the adult CNS re-express tenascin and CSPG following a stab wound to the CNS (Faissner and Schachner, 1995) or following kainic acid-induced gliosis in the hippocampus (Bovolenta et al., 1992). This is in contrast to astrocytes in immature CNS, perhaps illustrating some terminal differentiation of glial cells from development to maturity (McKeon and Silver, 1995).

Therefore, it appears that reactive glial cells in the mature CNS are no longer able to express the necessary extracellular matrix molecules for promoting axon extension. Instead, they express inhibitory matrix molecules like tenascin and chondroitin sulfate proteoglycans, likely playing a role in the inability of axons to penetrate the glial scar.

4.4. Upregulation of Neurotrophic Factors

Cultured astrocytes produce several neurotrophic factors of which nerve growth factor (NGF), basic fibroblast growth factor (bFGF), neurotrophin 3 (NT3), and ciliary neurotrophic factor (CNTF) are the most prominent (for review *see* Rudge, 1993). These factors support the neurite outgrowth and survival of neurones from various preparations. After brain injury, the synthesis of all these neurotrophic factors is upregulated in an obvious effort of the tissue to promote neuronal survival in this period of additional stress. The source of these molecules is not clear; neurones and glia that survive injury have an increased production rate of these factors. Neurotrophic factors are considerably upregulated in tissue where a majority of the neuronal cell bodies are lost as a result of necrosis and delayed neuronal death (Crutcher et al., 1979). Immunocytochemistry and *in situ* hybridization also demonstrated an upregulation of these neurotrophic factors in reactive astrocytes (Rudge, 1993). Interestingly, it was shown that this mRNA expression is much higher in reactive astrocytes at or near borderlines of an injury than further away in the parenchyma, despite similar morphological appearance of the astrocytes. The targets for the increased astrocytic secretion of neurotrophic factors in gliotic tissue could be any cell type involved. Neurones are an obvious target considering the beneficial effects

of these factors in the period immediately after an injury. Nonetheless, these neurotrophic factors can also recruit and activate more astrocytes and microglia/macrophages, as well as affect cells of the mesodermal lineage, thereby complicating the picture.

4.5. Other Biochemical Changes

Most enzymes and signal molecules are upregulated in reactive astrocytes. Care has to be taken when measuring the activity of substances in gliotic tissue, since activated microglia are contributing to many of the observed changes. Of course, many changes will be a direct reflection of astrocytic size increase and proliferation. An exhaustive list of affected molecules in reactive astrocytes was recently presented by Eddleston and Mucke (1993). Some important changes involve the upregulation of the immune response; reactive astrocytes express antigens in an MHC-dependent manner and are involved in cytokine production (Suzumura et al., 1986; Traugott and Lebon, 1988). Other functionally important secretions involve protease–protease inhibitor complexes and apolipoprotein E.

5. TRIGGER MECHANISMS FOR REACTIVE ASTROCYTES IN ISCHEMIC TISSUE

So far the discussion has been confined to to an overview of general properties of gliotic/scar tissue and reactive astrocytes, irrespective of the nature of injury. There are many modifications that are dependent on the type of injury (trauma, ischemia, toxins, infection, and degeneration). The following two sections will focus on events during and after ischemia. This section will investigate the possible mechanisms that could trigger reactive astrocytosis during and after an ischemic insult.

There are a number of possible mediators of reactive gliosis. Given the complexity of the nervous system, it most certainly involves an integration of multiple stimuli. Possible mechanisms for activation of astrocytes following an ischemic episode are illustrated in Fig. 3. Astrocytes could be responding to energy depletion caused by the ischemic insult directly or spreading depression waves radiating from the ischemic core into the penumbra and healthy tissue (*see* Chapter 2). Neuronal signals, such as glutamate, ATP, or potassium may also be involved in activating astrocytes directly in the vicinity of the damaged neurones. In addition, cytokines or growth factors released from microglia or astrocytes themselves (as autocrine signals; *see* Fig. 3) could cause reactive astrocytosis. Of course, it is likely that a combination of several or even all of these factors act in conjunction and cause the process of gliosis. In general though, factors responsible for the transformation of astrocytes into reactive species during ischemia and/or the postischemic period remain uncertain. However, a number of observations make certain mechanisms more likely than others.

For instance, astrocytes are much better equipped to withstand an ischemic episode than neurones. Astrocytes withstand energy depletion with ion gradients

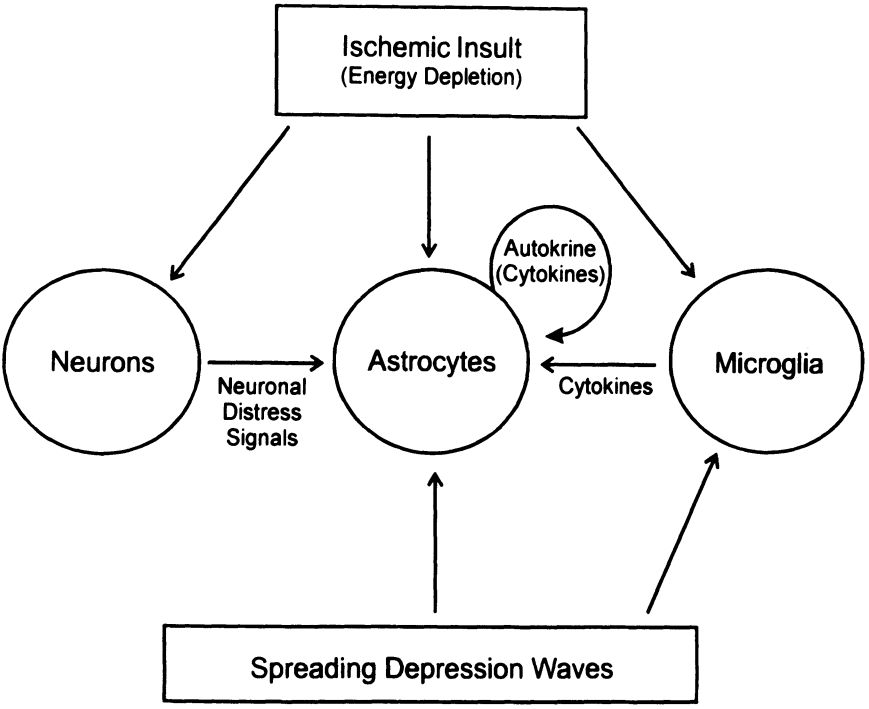

Fig. 3. Schematic drawing of possible factors involved in triggering the transformation from normal to reactive astrocytes.

intact longer than neurones (Harold and Walz, 1992). This ability results from three primary reasons. First, glycogen reserves enable astrocytes to produce ATP in the absence of external glucose and oxygen for longer than neurones. Second, there is no driving force for potassium. The astrocytic potassium equilibrium potential and membrane potential are almost identical with no significant sodium or chloride conductance. Therefore leakage currents are minimal and so is compensatory Na^+, K^+-ATPase pumping. After blockade of the Na^+, K^+-ATPase because of lack of ATP, the ion gradients therefore disperse slower in astrocytes than in neurones. Third, astrocytes can withstand a long term complete depolarization, as it happens in the core of a focal ischemic insult, much longer than neurones with complete recovery of their ion gradients. These differences indicate that astrocytes can withstand an ischemic episode longer than neurones (Silver et al., 1997), but this does not yet prove that an ischemic insult *per se* is responsible for reactive astrocytosis. At this point, there is no evidence for such a trigger mechanism.

A phenomenon that accompanies focal ischemia and thought to be a possible mediator of reactive gliosis is spreading depression (*see* Chapter 2). Spreading depression waves radiate from the core of a focal injury into the penumbra and

then into healthy tissue. In healthy tissue, spreading depression waves seem to be able to activate microglia (approx 1 h) prior to their ability to activate astrocytes (approx 3 h). Thus, activation of microglia precedes that of astrocytes following spreading depression waves (Gehrmann et al., 1993; Kraig et al., 1991). Although no additional studies have been conducted, the possibility remains that spreading depression waves activate microglia, which in turn activate astrocytes. Alternatively spreading depression waves (which involve astrocytic gap junctions and cell membrane ion channels) may activate astrocytes directly, albeit with a somewhat slower time course than microglia.

Still other possible mediators of the gliotic response include the plethora of cytokines and growth factors known to increase their expression following injury. Glial cell line-derived neurotrophic factor (GDNF) (Humpel et al., 1994), NGF (Stauss et al., 1994), and bFGF (Riva et al., 1994) following kainate-induced excitation, TGF-β1 following an ischemic episode (Lehrmann et al., 1995) or penetrating brain injury (Lindholm et al., 1992), and IL-1β following fluid percussion traumatic brain injury (Fan et al., 1995). Still other studies have shown that administration of CNTF (Kahn et al., 1995), CSFs (Balasingam et al., 1994), γ-IFN (Balasingam et al., 1994; Yong et al., 1991), and TNF-α in vivo, will result in glial responses similar to those seen following injury. The large number of changes seen in cytokine concentrations, after an insult to the CNS, is strong evidence that they play a crucial role in modulating and possibly initiating the responses seen following injury. Activated microglia produce and secrete several cytokines and toxic intermediates. The three cytokines released (IL-1, TNF-α, and TGF-β1) have several effects on the surrounding environment. IL-1 and TNF-α can aid in the recuritment of blood-borne macrophages and T-lymphocytes across the BBB as well as cause reactive astrogliosis. TGF-β1 inhibits astroglial and microglial proliferation and activation while at the same time, in conjunction with IL-1 and TNF-α, increasing production of NGF in astrocytes (Saad et al., 1991). TNF-α has also been shown to mediate oligodendrocyte damage/degradation (Schwartz et al., 1994). Obviously, the role of cytokines in the generation of the injury response is complex, making elucidation of trigger mechanisms far from easy. However, with knowledge of the many factors released and the many functions of the different cell types following injury, researchers may be able to resolve factors and functions important for optimal recovery.

6. PATTERN OF REACTIVE GLIOSIS IN ISCHEMIA

6.1. Transient Global Ischemia

Various models of transient global ischemia reveal the same trend of astroglial activation (Petito et al., 1990; Petito and Halaby, 1993, Schmidt-Kastner et al., 1990). There were two different patterns of the astrocytic reaction, depending on if the astrocytes were associated with an area that showed neuronal necrosis and subsequent neuronal loss or if the astrocytes were located in

an area that proved to be resistant to neuronal injury. In both areas there was, within the first 2 d, an increase in GFAP content. This increase was permanent in the area with necrosis and returned to control levels in areas with no permanent injury. However, in both areas there was a transition from GFAP– to GFAP+ cells (Petito, 1986) with the transition, again, being transient in the noninjured area. In the necrotic area, the astrocytes showed persistent hypertrophy and an increase in processes, the hallmarks of reactive gliosis. In the noninjured area, the hypertrophy was small and transient. The expression of vimentin in astrocytes was the most dramatic. It showed a clear correlation with necrosis of neurones, whereas the astrocytes in the non-necrotic areas did not show any detectable vimentin. Proliferation of astrocytes was also only apparent in the necrotic area.

6.2. *Focal Ischemia*

Occlusion of the middle cerebral artery (MCA) or cortical photothrombosis causes focal ischemia. The astrocytic reaction to this model was investigated (Inuzuka et al., 1996; Yamashita et al., 1996; Witte and Stoll, 1997). The increases in astrocytic GFAP were investigated and found, not only in the ischemic areas, but also in remote, nonischemic areas of the same hemisphere and often in the contralateral hemisphere. It is assumed that reactive gliosis occurs at sites of neuronal loss, where the number and intensity of reactive astrocytes increases at the lesion site steadily over approx 1 wk. In the ipsilateral hemisphere, there is a weaker and more diffuse GFAP increase that is transient, not associated with neuronal damage and in all likelihood caused by spreading depression waves (*see* Chapter 2). In many cases, as mentioned above, there is also an astrocyte activation in the contralateral hemisphere, which seems to be caused by changes in the neuronal connectivity pattern.

The heterogeneous expression of astrocytic activation in both global and transient ischemia is similar to expression of matrix molecules (David and Ness, 1993) and intermediate filament-associated proteins (Yang et al., 1997) in response to a penetrating wound. In both the traumatic and ischemic injury, the astrocytes at the site of loss of neurones show quantitatively and qualitatively a more pronounced reaction. The functional purpose, other than to possibly seal off the lesioned site, is unknown.

7. CONCLUSIONS

It is apparent, from the pattern of gliosis, that the creation of a border around a lesioned area or that the sealing of a breach in the BBB has absolute priority in the remodeling of tissue in the gliotic reaction. The facilitation of neuronal regeneration seems to take a lower priority. Interestingly, the presence of reactive astrocytes in otherwise healthy tissue seems to cause partial ischemic tolerance of the affected tissue for a certain time period (Witte and Stoll, 1997). This may be a result of more efficient homeostatic mechanisms

and/or secretion of neuroprotective factors in gliotic tissue. Whether or not the neuroprotection afforded by gliotic tissue can extend to repair processes like regeneration after injury is a more complicated issue and may depend on the circumstances and location.

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