Neuroglia in the Aging Brain

Edited by

Jean de Vellis

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Astrocytic Reaction After Traumatic Brain Injury

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1. INTRODUCTION

Astrocytes and microglia respond to a great variety of lesions in the nervous tissue of the central nervous system (CNS). In this sense, although astroglial cells react in a relatively constant manner, the intensity and time course of the changes are largely dependent upon the type of lesion involved.

The present review fundamentally addresses traumatic lesions affecting the CNS. This type of lesion has been extensively studied from the experimental perspective and constitutes one of the most reliable and easily reproducible models for analyzing the reactive changes taking place in the glial component of nervous tissue.

One of the earliest morphological studies of glial reaction to traumatic injury of the CNS was carried out by Wilson in 1926 *(1)*. This author employed conventional histological techniques (cresyl violet and trichromic stains) to study the trajectory of therapeutic punctions made in two human brains. In this context, a glial reaction was observed, with connective tissue participation in the repair processes. However, the application of metallic impregnation techniques allowed more detailed research of the glial reaction in these lesions, and afforded a clear distinction between cell types such as astrocytes and microglia. In this sense, the classical studies of Penfield et al. were of great interest *(2–4)*. These authors, by producing punction lesions in the brain, detected a series of cellular changes in the astrocytes that comprised early edema, astrocyte proliferation, fibrous morphological transformation of the astrocytes, astrocyte hypertrophy, and participation of the connective tissue in the glial scar. In addition, the intensity of the glial reaction was related to the amount of necrotic tissue produced.

2. INTERMEDIATE FILAMENT PROTEIN EXPRESSION IN ASTROCYTES

Both glial fibrillary acidic protein (GFAP) and the protein vimentin (VIM) have been described in astrocytes. GFAP is considered to be characteristic of mature astrocytes *(5)*, whereas VIM—initially described in mesenchymal cells—has been detected in radial glia and in mature astrocytes *(6,7)*. Changes in the expression of both glial antigens are thought to occur in the course of normal astroglial development. Thus, in several mammal

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species, VIM-GFAP transition takes place in the first weeks of life after birth, as a result of which GFAP and VIM may be temporarily coexpressed by most astrocytes during such transition periods *(7–11)*. Moreover, biochemical studies have revealed changes in the presence of messenger RNA (mRNA) encoding for both GFAP and VIM during this period *(12)*.

Once development has been completed, astrocytes express variable amounts of GFAP. Whereas the concentration of the latter is high in fibrous astrocytes of the white matter *(13)*, it may be very low in certain regions of the brain cortex—where protoplasmic astrocytes (at least in the rat) cannot be detected by the habitual immunohistochemical techniques *(14)*—with the exception of layer I astrocytes that form the *glia limitans*, which are clearly GFAP-positive. However, astrocytes in special locations such as the cerebellum, retina, optic nerve, and major tracts of white matter continue to coexpress GFAP and VIM even in the adult animal *(7,15–17)*. In vitro immunoelectron microscopic studies of GFAP and VIM location by Abd-El-Basset et al. *(18)* have shown that in both astrocytes and their precursors, VIM and GFAP copolymerize in the same individual intermediate filament; as a result, the GFAP/VIM ratio present in these intermediate filaments reflects the degree of differentiation and functional status of these cells.

Gliosis is the typical astroglial response to CNS lesions. The term encompasses two phenomena: Astroglial proliferation and hypertrophy *(14)*. Astroglial hypertrophy (proliferation will be addressed in the corresponding section below) is the most important and easily detectable characteristic of reactive astrocytes. It may be demonstrated by argentic impregnation techniques (Cajal's gold sublimate, for example), though at present the immunohistochemical demonstration of increased GFAP (and occasionally VIM) expression is clearly the most reliable parameter.

2.1. *Time Course*

The early character of astrocyte reaction seems to be a constant finding in traumatic damage of the CNS—regardless of the lesion model employed. Thus, in experimental cerebral wounds, increased GFAP immunopositivity becomes detectable a few hours to two days after producing the lesion—with a maximum intensity peak that rarely exceeds 7 d *(18a–27)*. In relation to this phenomenon, the findings have been similar to those reported for other experimental models of traumatic injury of the CNS *(28–34)*.

It is interesting to point out that the early onset of astrocyte reaction (expressed by an increase in GFAP positivity) is very similar to that observed in other types of nervous tissue lesion *(35–59)*. In different types of lesion, the data obtained from biochemical studies—both as regards GFAP quantification *(20,36,55,59–62)* and GFAP mRNA assay *(46,55,60,62,63)*—clearly confirm the immunohistochemical findings. Likewise, GFAP mRNA *in situ* hybridization studies *(55,64)* have shown the astrocytes to be responsible for GFAP synthesis.

Based on electron microscopic studies of the first evolutive stages of experimentally induced cerebral lesions, we have found (nonpublished personal observations) reactive astrocytes to exhibit a manifest edematous appearance, with a very electron-transparent hyaloplasm and very scarce glial filaments (though the latter subsequently increase greatly in number). In this sense, the ultrastructural image is scantly compatible with the intense GFAP immunopositivity detected in light microscopic immunohistochemical studies. Considering that the intermediate filaments are highly dynamic structures *(65)*, a pool of kinetically active disassembled subfilamentous units would be expected to be in

dynamic equilibrium with assembled filaments *(66)*. The surprising increase in astrocyte GFAP immunoreactivity so shortly after injury, and the apparently paradoxical ultrastructural absence of glial filaments, could be accounted for by a rapid increase in GFAP synthesis, which would expand the pool of subfilamentous units and thus increase the immunopositivity of the reactive astrocytes that nevertheless still require a period of time to complete filament assembly.

As seen above, the constant pattern of findings has led to widespread consensus on the rapid establishment of reactive astrocyte changes in highly diverse CNS lesions. However, variable results have been obtained as regards the permanent or transient nature of such changes, depending on the experimental model involved. An added difficulty is the tendency to carry out studies that conclude too early—thus frequently preventing the recording of reliable data on the true duration of the glial reaction.

Cerebral lesions exhibit a gradual decrease in astrocyte reaction over time, reflected by a drop in GFAP immunoreactivity. In this sense, the reaction is seen to have clearly decreased after approximately three weeks *(20)*. After this period of time, only a narrow band of immunopositive astrocytes remains in the cortex surrounding the lesion *(22)*. Biochemical studies have shown the amount of both GFAP *(67)* and GFAP mRNA *(68)* to return to values very close to normal after these three weeks. Likewise, in mild contusive brain lesions, immunopositivity practically returns to normal 30 d after injury *(28)*.

The duration of the glial reaction is much more controversial in other types of CNS lesion involving astrocyte response. Thus, in ischemic lesions the reaction is seen to remain stable after three months *(49)*, though it may descend to negligible levels after six months *(44)*. In turn, deafferentiation lesions appear to induce a transient astroglial reaction. As an example, deafferentiation caused by the injection of a neurotoxic agent (ibotenic acid) induces a glial reaction in the projection areas of the injected zone that disappears 5–6 mo later *(45)*. The astrocytes of a given region undoubtedly respond to lesions in a subcortical nucleus projecting fibers to that region. In this sense, astrocytes have been shown to respond to cholinergic but not to dopaminergic deafferentiation *(36)*. Additional examples of transient reactions are the glial response produced in the olfactory tract by sectioning the olfactory nerve—with regression occurring after one month *(35)*—and the glial reaction in the facial nucleus after compression of the facial nerve (with regression after 40 d) *(57)*.

However, other lesion models appear to induce a permanent glial response. Thus, in Wallerian degeneration of the optic nerve, the astrocyte reaction seems to persist for at least one year and apparently indefinitely *(69,70)*. Likewise, in unilateral sections of the subcommissural fornix, glial response persists for at least one year *(56)*. Toxic lesions *(40,57,71)* in turn lead to a practically permanent presence of reactive astrocytes.

In other situations, the greater or lesser persistence of the glial reaction is related to the severity of the damage produced. This has been observed following the induction of variable intensity brain ischemia *(53)*, and in toxic lesions *(44)*, where the astrocyte reaction persists after four months in certain areas of the hippocampus and fades in others—in close correlation to the degree of neuronal degeneration produced.

2.1.1. Other Intermediate Filament Proteins in Astrocytes

Although much less frequently than GFAP, VIM has also been used as a reactive astrocyte marker in traumatic lesions of the CNS. In cerebral injuries after 5 d of evolution, our group *(19)* has detected a reactive astrocyte band measuring 300–350 μm in thickness sur-

rounding the punction trajectory. By applying techniques for the detection of VIM and GFAP, we were able to show that 60% of the reactive astrocytes coexpressed VIM and GFAP—the remaining astrocytes only being positive for GFAP. In no case did we observe simultaneously VIM-positive and GFAP-negative astrocytes. Other authors have also reported the coexpression of VIM and GFAP in reactive astrocytes *(50,53)*. By using this same lesion model, Takamiya et al. *(25)* have detected reactive astrocytes that transiently express VIM from 2–10 d post-lesion, with a maximum expression peak after 3–5 d. This latter observation has also been reported in other types of acute necrotic lesions *(53)*.

The expression of VIM has also been described in reactive astrocytes in noninvasive traumatic injuries, such as brain contusions *(28,31)* or spinal cord compression *(29)*. VIM expression has likewise been recorded in ischemic lesions *(44,49,52,54)*, toxic lesions *(44)*, in lesions involving retrograde degeneration *(42,59)*, or in Wallerian degeneration processes of the CNS *(50,56)*.

As pointed out at the start of this study, VIM is regarded as an immature astrocyte marker in the course of astroglial development; consequently, VIM expression by reactive astrocytes could reflect a temporal regression of these cells to immature states. In this sense, Abd-El-Basset et al. *(18)* have observed in vitro that highly mobile astrocyte precursors (proastroblasts and young astroblasts) possess intermediate filaments exclusively composed of VIM (proastroblasts) or composed of a heteropolymer in which the GFAP/VIM ratio is low (young astroblasts). In contrast, in more mature (and thus less mobile) astroblasts, and in immobile astrocytes, the intermediate filaments are composed of heteropolymers with a high GFAP/VIM ratio. These authors suggest that heteropolymer formation allows the astroglia to regulate the "stiffness" of its intermediate filament network by increasing or decreasing the GFAP/VIM ratio. The reactive astroglia could behave in the same manner, thus responding to different functional situations that may require increased cell mobility.

The idea that reactive astroglia undergoes a certain regression toward more immature states has been supported by new evidence in recent years. Thus, nestin (a type of intermediate filament expressed by neuroepithelial stem cells of the embryonic CNS) *(72)* is known to be expressed by reactive astrocytes (along with GFAP) in lesions of the CNS *(73–75)*.

In punction-induced cerebral lesions, reactive astrocytes *(27)* have been found to coexpress GFAP and a certain intermediate filament associated protein (IFAP) found in radial glia and derived elements, but not in the adult CNS.

2.2. *Spatial Spread*

In experimental cerebral wounds, astrocyte reaction is frequently not limited only to the proximity of the lesion, but extends through the damaged hemisphere and even to the opposite hemisphere (i.e., theoretically unaffected by the traumatism). The degree of homolateral spread and the presence or absence of contralateral involvement seems to depend upon the size of the lesion. Thus, in small cerebral wounds *(18,22,23,25)*, the astroglial reaction is circumscribed to 1 mm of tissue surrounding the lesion, or alternatively extends throughout the ipsilateral cerebral cortex *(18,22,25)* but without reaching the contralateral hemisphere. A similar situation is observed in percussion-induced contusive brain lesions *(33)*. It is interesting to note that the astroglial reaction in the damaged hemisphere is later in developing with a slower and more persistent spread in subcortical regions than in the cortex *(18a,22)*. In contrast, when the amount of damaged tissue is

greater *(24),* the reaction likewise extends to the cortex opposite to the side of the lesion, albeit with less intensity. Unpublished observations by our group corroborate this finding. In addition, we have established that VIM is only expressed by the reactive astrocytes immediately adjacent to the punction trajectory, as a result of which this protein clearly cannot be employed for studying more distant glial responses. In nontraumatic brain lesions that nevertheless involve abundant damaged nervous tissue, as in experimental laser-induced injuries *(53),* astroglial reaction in the contralateral hemisphere can also be detected. According to these authors, the expression of VIM by the reactive astrocytes is restricted to the areas in proximity to the lesion.

A phenomenon requiring more detailed analysis is the presence of astrocyte reaction in the undamaged hemisphere. According to Moumdjian et al. *(24),* a number of causes may be postulated:

1. Distant tissue diffusion of soluble factors that induce astrocyte activation and which are initially released at the site of the lesion;
2. Wallerian degeneration necrosis of nerve fibers with cortico-cortical projections that traverse the *corpus callosum,* thereby triggering an astroglial reaction contralateral to the lesion that is in turn incremented by the astrocyte reaction produced by deafferentiation itself;
3. Migration of reactive astrocytes away from the wound site, through the *corpus callosum,* with secondary colonization of the contralateral hemisphere.

In this sense, it has been shown that transplanted astrocytes are able to migrate *(77–82)*—an important migration route being the parallel tracts of myelinated nerve fibers. However, native astrocytes do not appear to possess such mobility *(83).*

In order to evaluate the contribution of each of the mechanisms proposed above in accounting for the spread of astrocyte reaction to the contralateral hemisphere *(24),* a number of surgical procedures have yielded the following results:

1. Callosotomy alone induces mild gliosis in both hemispheres;
2. Unilateral brain lesion only produces severe ipsilateral and moderate contralateral gliosis;
3. Callosotomy with unilateral brain lesion induces effects similar to those of the second procedure above.

According to these authors, if astrocyte migration through the *corpus callosum* were a factor to be taken into account, then the contralateral gliosis seen in rats subjected to lesion plus callosotomy (which thus prevents migration) would have to be less than that recorded in rats with lesion only. However, no differences are observed between the two experimental groups. On the other hand, if Wallerian degeneration of axons projecting to the contralateral hemisphere—with the resulting deafferentiation—were an important consideration in the genesis of contralateral gliosis, then animals belonging to the lesion plus callosotomy group would be expected to develop more contralateral reactive gliosis than the group subjected to lesion only. Not only is this not the case, but callosotomy alone moreover induces less gliosis than the contralateral reaction of the brain lesion alone. As a result, Moumdjian et al. *(24)* clearly favor soluble factors diffusing from the lesion site as the basic mechanism underlying the observed contralateral glial reaction.

An additional factor must be considered in the origin of distant (and even contralateral) astrocyte reactions. In effect, it is well established that astrocytes are interlinked by gap junctions *(84).* These cells therefore form a type of "astroglial syncytium" throughout the nervous parenchyma. In experimental astroglial reactions, the immuno-

histochemical expression of connexin-43 (a predominant protein in interastrocyte gap junctions) is found to be increased *(85,86)*. This suggests that transformation to reactive astrocytes implies an increase in gap junctions and thus in the efficacy of the "astroglial syncytium." In this way, certain intracellular signals that trigger gliosis could pass from one cell to another—covering considerable distances in a relatively brief period of time. However, the fact that VIM expression by reactive astrocytes never extends beyond the injury zone could be in conflict with the idea of possible intercellular signals that diffuse across gap junctions to play an important role in the genesis of distant astroglial reaction.

3. ASTROCYTE PROLIFERATION

The use of double labeling techniques (GFAP and thymidine or similar compounds such as bromodeoxyuridine, widely employed in recent years) is essential for studying astrocyte division in CNS lesions. By applying these methods to nervous tissue lesions with scant production of necrotic material and an intact blood-brain barrier, most studies have shown that the astrocytes do not divide *(51,87,88)*. In contrast, deafferentiation lesions do seem to show astrocyte proliferation *(89)*.

In experimental traumatic lesions of the CNS *(23,25,90–99)*, astrocyte proliferation is generally an early and very brief phenomenon that only takes place between 24 h and 8 d after lesion induction. There is no evidence of astrocyte proliferation in later stages of the evolutive course, and the maximum mitotic peak is reached 3–4 d after injury. The labeling index is low and rarely exceeds 10%. Similar results have been obtained in other traumatic lesion models *(28,100)*.

Regional differences in astrocyte proliferation have been detected. Thus, Topp et al. *(26)* and Garcia-Estrada et al. *(91)* have found the labeling index to be higher in the cortex than in the hippocampus, whereas Janeczko *(93)* has reported greater proliferation in the white matter and deep-lying regions of the lesion. Although the proliferative phenomena appear to be limited to the damaged hemisphere *(23,25,93)*, other researchers have found a slight spread of astrocyte division towards the contralateral hemisphere *(101,102)*.

Thus, it seems clear that although many nervous tissue lesions exhibit an important number of GFAP-positive reactive astrocytes, the low cell proliferation figures recorded indicate that most such cells are preexisting GFAP-negative astrocytes that have become GFAP-positive.

4. PHAGOCYTIC ROLE OF THE ASTROGLIA

Although the microglia/macrophages are the principal cells in charge of eliminating foreign, nocive, or degenerated elements in nervous tissue injuries, evidence suggests that astrocytes also possess a certain phagocytic capacity.

In vitro research has shown that astrocytes in neonatal or early postnatal rats uptake polystyrene spheres *(79)* within lysosomal structures, where they are retained for several weeks. These cells are also able to engulf yeast cells *(103)* and latex particles *(104)*. Ronnevi *(105,106)* has in turn described astrocyte phagocytosis of synaptic buttons in the neonatal cat.

On the other hand, the phagocytosis of degenerative tissue debris by astrocytes has been described under pathological conditions such as nerve fiber injury and degeneration *(63,107–116)*, toxic lesions *(76,117,118)*, experimental allergic encephalitis or traumatic brain injury *(64)*.

However, ultrastructural studies carried out by our group involving experimental cerebral wounds and Wallerian degeneration of the optic nerve have failed to provide evidence of phagocytosis by astrocytes. In the case of Wallerian degeneration of the optic nerve, we have seen *(69)* an increase in lipid droplets and dense bodies within astrocytes. Although the dense bodies often adopt peculiar morphologies, we have never observed unequivocal images of astrocyte phagocytic activity. We consider that no clear phagocytic role can be attributed to these cells—at least in the experimental setting involved. In contrast, astrocytes may be implicated in the metabolic processing of certain products originating from intense tissue degeneration—a phenomenon that could explain the presence of lipid droplets.

An interesting experimental approach to the problem of a possible phagocytic role for the astroglia involves the *in situ* administration of particulate material—most of which will obviously be engulfed by the microglia/macrophages. A number of authors have applied this experimental design, using different materials. Thus, it has been found that astrocytes in the neonatal rat phagocytose exogenously injected carbon particles *(119),* and that astrocytes in adult rats are able to capture latex particles *(83).*

In a recent study in adult rats subjected to the local injection of colloidal carbon in cerebral wounds, Al-Ali and Al-Hussain *(120)* have observed that astrocytes are able to phagocytose carbon particles. However, this phenomenon was only recorded in astrocytes in brains subjected to two successive injections of colloidal carbon spaced one week apart; no uptake was seen in brains subjected to a single injection. The evident conclusion of this study is that astrocytes only act as phagocytes in the event of "professional" macrophage saturation, i.e., astrocytes would appear to function as a second line of defense.

5. NEWLY-FORMED *GLIA LIMITANS*

In extensive traumatic lesions of the CNS, meningeal cells of fibroblastic appearance (meningocytes) penetrate deeply within the cavity of the lesion and establish close relations with the astroglial cells. The repair process leads to the appearance of a newly-formed *glia limitans,* which closely resembles the normal *glia limitans.* This normal component of the surface of the nervous organs is known to be composed of a series of astrocytic prolongations externally lined by a continuous basal membrane, beyond which the connective elements of the leptomeningeal territory are found *(84).*

Our group has conducted structural studies of the meningeal regeneration process and the peculiar meningo-astroglial relations that are established in the course of the repair of cerebral lesions in rats *(121).* These experiments show that the appearance of a basal lamina limiting the lesion cavity is a crucial event, for it establishes a clear separation between the mesodermal or neuroectodermal elements. According to our results, patches of electron-dense material similar to the basal lamina are already apparent 10 d after inducing the lesion. After 14 d, practically the entire wound cavity is delimited by a typical basal lamina intimately attached to the underlying nervous parenchyma. This basal lamina always rests upon glial filament-rich cell prolongations identifiable as corresponding to astrocytes. Collagen microfibrils—either isolatedly or forming small bundles—as well as macrophages or regenerative meningocytic elements habitually appear in proximity to the astrocytic prolongations—though always covered by the basal lamina. The *glia limitans* thus regenerated is clearly identifiable 14 d after lesion induction. A feature of this newly-formed *glia limitans* is the marked irregularity of its surface *(121);* as a result, the meningeal territory frequently presents long solid glial cords, always totally enveloped

by basal lamina, and incompletely covered by thin and delicate meningocytic prolongations (and even somata). Whole astrocyte somata are occasionally seen to form part of the glial cords, and always associated to the corresponding basal lamina cover. This irregularity of the surface of the newly-formed *glia limitans* has been confirmed in different types of lesion *(69,122–125)*. A more recent study by our group *(121a)* has shown the glial cords to coexpress GFAP and VIM in the meningeal territory.

As regards the appearance of the basal lamina during the lesion repair process, our findings basically coincide with the ultrastructural observations of other authors, though with minor chronological differences *(180,127,142,143)*.

Thus, the glial reaction leading to the appearance of a newly-formed *glia limitans* seems to be similar in response to different types of lesion. As a result of their plasticity and repair capacity, the subpial reactive astrocytes are able to extend their prolongations (and even somata) to the subarachnoid space.

Although ultrastructural observations suggest meningeal elements to induce basal lamina formation by astrocytes and the development of newly-formed *glia limitans*, evidence from experimental models also strongly supports this hypothesis. In astrocyte and meningocyte cultures *(144,145)*, structures similar to *glia limitans* and even irregular deposits of electron-dense material analogous to the basal lamina are seen to form in the contact zones between both types of cell. Experiments have shown that meningocytes may even be essential for *glia limitans* formation: The destruction of meningocytes with 6-hydroxydopamine (6-OHDA) causes disorganization of the *glia limitans* and covering basal lamina *(146–149)*. Meningocyte destruction with 6-OHDA implies a decrease in the concentration of fibrilar collagen types I, III and VI, together with laminin, fibronectin and type IV collagen in relation to the *glia limitans (135)*. Moreover, steroids topically applied to cerebral wounds, which would cause a decrease in the proliferation of meningeal elements, has been found to induce a lesser organization of the astrocyte prolongations forming the *glia limitans*, with the appearance of laminin-negative zones at this level *(126)*.

Biochemical studies support the idea that the basal lamina is synthesized by astrocytes. These cells have been shown to produce molecules that form part of the basal lamina, including laminin *(127–131)*, fibronectin *(131,132)* and proteoglycans *(133,134)*. On the other hand, the meningeal cells are able to produce *(135)* fibrilar collagen types I, III and VI, fibronectin, laminin, type IV collagen, and heparan-sulfate type proteoglycans. Thus, it seems possible that meningeal cells not only regulate astrocyte synthesis of the basal lamina but also contribute in part to production of the latter. In addition, astrocytes would be able to interact with the extracellular matrix by means of adhesion molecules such as the neural cellular adhesion molecule (NCAM) *(136)*, or tenascin *(137–139)*—which are likewise expressed by these cells.

6. GROWTH FACTORS AND CYTOKINES

Nonreactive astrocytes possess few growth factors. However, when activated they are known to express multiple factors, cytokines and other substances *(140,141)*. Astrocyte expression of these diffusible compounds varies according to the type of lesion and region involved. Likewise, astrocyte reaction differs between zones close to the lesion and areas located at a distance from the injury site *(141)*.

This section deals with the cytokines and growth factors predominantly implicated in reactive gliosis—fundamentally in traumatic lesions of the CNS.

Three steps may be contemplated in the expression of diffusible factors in post-lesion gliosis, with reference to astrocytes: (a) The origin of the factors that activate astrocytes; (b) The factors expressed by astrocytes in response to injury; and (c) Actions of the diffusible factors secreted by reactive astrocytes upon neighboring structures.

(a) Astrocyte reaction to injury is a response to different soluble activating factors that in turn induce astrocytes to produce growth factors and cytokines. The source of these triggering substances is the nervous tissue affected by the lesion. Injury itself causes the destruction of many cells, including neurons which release factors that are normally found dissolved in the cytoplasm but are not habitually secreted. An example of such behavior is afforded by the basic fibroblast growth factor (bFGF) *(150)* and the ciliary neurotrophic factor (CNTF) *(151)*. These two factors, and particularly bFGF, seem to play a special role in astrocyte activation. In addition to destruction of nervous tissue, injury causes vascular rupture, with the subsequent loss of blood-brain-barrier integrity. Additional diffusible factors and cytokines from the extravasated blood plasma (thrombin, platelet-derived growth factor [PDGF], epidermal growth factor [EGF] and insulin, and so on) are thus added and contribute to activate the astrocytes. The extravasated mononuclear cells, together with the microglia, participate in the inflammatory reaction secondary to injury not only by removing the altered tissue debris but also by releasing cytokines such as interleukins (IL) IL-1 and IL-6, interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α) and growth factors such as transforming growth factor-beta (TGF-β) *(152–156)* and bFGF *(157)*. All these agents induce the expression of specific reactive astrocyte receptors such as bFGFr *(158)*, CNTFr *(151)*, and EGFr *(159)*. The binding of these factors to their corresponding receptors located on the astrocyte membrane, induces the synthesis of further diffusible factors, and thus stimulates and modulates astrocyte response.

(b) The first sign of astrocyte activation corresponds to the biochemical or *in situ* hybridization detection within the cytoplasm of the mRNA encoding for the different soluble factors. However, astrocyte response does not become effective until the corresponding proteins are synthesized and secreted to the exterior, where they in turn act upon the specific target cells. Once the astrocytes have been activated, a factor synthesis cascade is triggered in sequence over time. In this sense, Cook et al. *(157)* inform of the existence of both early genes (i.e., which express their mRNA in the first 24 h following injury) and late genes (those with mRNA expression after 72 h).

The early activation of certain genes encoding for soluble factors points to the existence of a rapid astrocyte response, though the first soluble factors to be demonstrated by immunohistochemical techniques appear around three days after the lesion is induced. These factors are bFGF *(102)*, CNTF *(151)*, vascular endothelial growth factor (VEGF) *(104)*, and TGF-β *(160–162)*. Their expression is maintained for prolonged periods of time. Posteriorly, about 5–6 d after injury, the astrocytes express other factors such as insulin-like growth factor-2 (IGF-2) *(161)*, IL-1, IL-6 and TNF-β *(154)*, and nerve growth factor (NGF) *(163,164)*. This latter factor has been detected by Goss et al. *(165)* after 24 h.

(c) The factors synthesized by the reactive astrocytes are secreted to the exterior, and act in paracrine fashion upon the neighboring cells (neurons, oligodendroglia or its precursors, endothelium, meningeal cells in the event that destruction affects the surface of the nervous organ, and macrophages located within the lesion site). They also interact with the secreting astrocytes themselves, in an autocrine manner. The action of the diffusible factors always takes place from the exterior; to this effect, it is essential for the factors to be secreted to the exterior, where they in turn bind to specific receptors located on the target cell surface membrane. The fact that astrocytes secrete numerous factors suggests that they play a special role in controlling the nerve tissue repair process, though response

varies according to the region where the lesion is produced. The factors secreted by activated astrocytes act upon many different cells. In this sense, astrocytes may be regarded as the neuralgic center of the repair process, for their activation triggers "explosive" action upon many structures that intervene in the nerve tissue repair process.

These factors exert a protective effect upon the neurons. In this sense, bFGF *(157,166),* NGF *(167),* IGF-1 *(168)* and the leukemia inhibitor factor (LIF) *(169)* diminish neuronal death in the zones close to the lesion site. However, reactive astrocytes stimulated by IL-1 may exert a neurotoxic effect by producing nitric oxide *(170).*

Astrocytes are important target cells of factors secreted by their own activated astrocytic counterparts. These factors exert the following effects:

Astrocyte proliferation is stimulated by bFGF *(171,172),* EGF *(173,174)* and PDGF *(67).* Fibronectin, expressed by reactive astrocytes, is a potent stimulator of astrocyte division *(175,176).* Many factors also inhibit astrocyte proliferation, including IFN-γ *(177–179),* and TGF-α 1, which inhibits the mitotic effects of FGF and EGF *(180).* This stimulating and inhibiting behavior of factors produced by the astrocytes themselves supports the idea of their regulatory influence upon gliosis.

Astrocyte hypertrophy, with increases in GFAP and VIM synthesis. These actions are performed by bFGF *(171,181–183).* However, Reilly et al. *(184)* observed that bFGF produces a decrease in GFAP mRNA in astrocytes in vitro, whereas TGF-α 1 increases it. Logan et al. *(155)* described a TGF-α 1 stimulating effect upon GFAP synthesis. CNTF is also a potent inducer of astrocyte hypertrophy *(67),* stimulating the synthesis of GFAP and VIM—actions that are in turn enhanced by TNF-γ *(64,101).* According to Kahn et al. *(185),* CNTF appears to specifically increase astrocyte GFAP synthesis, though without affecting VIM levels.

According to Ridet et al. *(141),* these soluble factors induce astrocyte synthesis of surface molecules and extracellular matrix. Astrocyte expression of intercellular adhesion molecules (ICAM-1) is stimulated by different growth factors and cytokines such as IL-1 *(186).* NGF *(163)* stimulates the synthesis of neural cellular adhesion molecules (NCAM), although interleukin-beta 1 *(186)* and IFN-γ *(140)* stimulate ICAM production. Furthermore, NCAM and components of the extracellular matrix such as fibronectin, tenascin or heparan sulfate favor axon growth of the neurons damaged by the wound. However, when astrocytes synthesize other proteoglycans such as keratan sulfate or chondroitin sulfate, such action is effectively inhibited *(187,188).* Other proteoglycan actions have been reported, including the ability of heparan sulfate to bind to FGF type 4 receptors and stimulate the cell in the absence of growth factor.

Activated astrocyte factors induce astrocyte production of other growth factors and of an increased number of receptors for their own secretory products thereby incrementing reactive astrocyte response to certain trophic factors and cytokines. bFGF induces NGF expression *(150,157)* and increases the number of bFGF receptors present both in astrocytes and in other cells *(157,158,172).* Although EGF is not synthesized by reactive astrocytes, the latter do exhibit receptors for this factor *(159);* as a result, these cells become susceptible to EGF action. In vitro studies have shown EGF to stimulate astrocyte production of bFGF, TGF-β 1 and NGF *(140).* In turn, TGF-β 1 also induces NGF synthesis in astrocytes *(163,189).* Interleukin-1 is synthesized by reactive astrocytes *(154),* and it has been shown to stimulate astrocyte synthesis of NGF *(190,191).* IGF-1 also stimulates the production of its own receptors on hypertrophic astrocytes *(102).*

Many of the growth factors synthesized by reactive astrocytes induce these same cells to produce NGF—one of the main functions of which is the induction of a trophic effect upon the damaged neurons, thereby contributing to their survival. In addition, NGF stimulates the synthesis of its own receptors by the same reactive astrocytes *(192)*.

Not all growth factors and cytokines exert a stimulating effect upon astrocytes. Certain diffusible substances synthesized by inflammatory cells and by the reactive astrocytes themselves tend to buffer glial reaction. In this sense, IFN-γ inhibits astrocyte proliferation and the synthesis of extracellular matrix molecules *(191)*. In turn, interleukin-10 (produced by microglia/macrophages) buffers astrocyte reaction *(193)*, with a decrease in bFGF mRNA levels within the astrocytes *(194)*.

Certain growth factors secreted by reactive astrocytes stimulate the proliferation of oligodendroglia precursors in adults *(102,157,162)*. In contrast, Amur-Umarjee et al. *(195)*. have observed an inhibitory effect of astrocytes upon oligodendrocyte remyelinization capacity in vitro.

Other growth factors in turn induce vascular proliferation and restoration of the blood-brain-barrier. In this context, bFGF appears to intervene in vessel neoformation *(182)*. VEGF (synthesized by astrocytes and inflammatory cells) seems to stimulate vascular formation following injury *(196)*, while TGF-α 1 would favor reconstitution of the blood-brain barrier *(155)*.

When the lesion affects the surface of the nervous organ, these factors induce neoformation of the *glia limitans*. Logan et al. *(155)* detected TGF-α 1 in astrocytes, and TGF-α 1 mRNA in meningeal cells.

Astrocytes release factors that stimulate microglial proliferation; these cells concentrate in the lesion site, and in turn increment astrocyte reactivity. This is the case of TGF-α 1 *(197)* and bFGF *(198)*. In this way a mutual stimulation circuit is established based on the positive feedback principle, between astrocytes and microglia/blood mononuclear cells, thus favoring and reinforcing astrocyte activity in gliosis.

Other elements have also been implicated in the regulation of glial response, including norepinephrine via beta-adrenergic receptor action *(91,199)*, and corticoids *(192)*.

To summarize, the reactive astrocyte plays a prominent role in the regulation and modulation of the scar tissue resulting from damage of the nervous system. This action is largely, though not entirely, mediated by the cascade activation of different growth factors and cytokines, which in turn interact with each other upon the surrounding cells and structures.

7. GLIAL REACTION IN RELATION TO AGE

Most authors consider that astrocyte reaction to lesions of the CNS is less important during the fetal and neonatal period than in adults. In general, immature astrocyte response to injury is similar though less intense than mature astrocyte reaction in the adult *(70,152,193,200–202)*. An important difference in astrocyte response to lesion between the fetal and adult stages is the absence of glial scar formation following CNS injury in the neonatal period *(18,21,203,204)*. However, according to some authors, the response is more dependent upon the type and/or location of the lesion than on astrocyte immaturity. In this sense, glial scars have been described following neonatal injury *(70,152,193,200–203)*.

According to most researchers, the absence of glial reaction in neonatal lesions is a consequence of astrocyte immaturity *(205,206)*. However, Smith et al. *(207,208)*, are of

the opinion that the absence of a glial scar is due to some specific property of the immature astrocytes, and is not a consequence of any cellular incapacity. According to Berry et al. *(18a),* the absence of a glial reaction could be attributed to the microglia/macrophages rather than to the astrocytes as such. In neonatal animal models, debris elimination is faster and more effective thereby avoiding persistent macrophage presence within the lesion site *(152,193,202,206).*

A critical period seems to exist in the early neonatal stages, after which the capacity to develop an "adult"-like glial response to CNS injury is acquired. A number of authors have pointed out the coincidence in time between the critical period in which the "adult" response to lesions is acquired and the myelinization phase *(168,207,208).* This coincidence has led to the idea that the presence of myelin and/or oligodendrocyte degradation products determines the formation of a glial scar. However, as has been demonstrated by Berry et al. *(18a),* lesions are followed by normal healing in myelin-deficient mutants.

The existence of changes in the astrocyte population (particularly hypertrophy, with increased GFAP expression) associated to advanced age is generally accepted. However, unlike in the neonatal period, very few studies have been carried out in individuals of advanced age, and the results obtained are moreover contradictory. According to some authors, astrocyte response in old animals is more intense, extensive, and prolonged in time *(209),* and proliferation is both greater and earlier *(26).* In contrast, Kane et al. *(46)* have observed no increase in astrocyte hypertrophic response in such individuals. In this sense, the conflicting results obtained may be partly attributed to the experimental methodology employed.

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