B. Castellano López and M. Nieto-Sampedro (Eds.)
Progress in Brain Research, Vol. 132

CHAPTER 30

Glial activation in the immature rat brain: implication of inflammatory transcription factors and cytokine expression

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Introduction

The postnatal brain holds a unique plasticity which is lost during maturity. This is determined by ongoing events, such as neuronal differentiation, growth of neuronal dendritic trees, presence of exuberant synaptic contacts and the establishment of mature neuronal circuits (Uylings et al., 1990). These special neuronal and neuroanatomical characteristics correlate with an important stage in glial differentiation and in the acquisition of the adult glial phenotypes. During the first 2 weeks of postnatal life in the rat, two different types of microglial cells coexist: in the white matter tracts there are *amoeboid microglial cells*, which appear during prenatal life deriving from monocytes or primitive/fetal macrophages (Boya et al., 1987; Ling and Wong, 1993; Dalmau et al., 1997), and maintain several macrophage characteristics such the expression of major histocompatibility complexes (MHC) (Ling et al., 1991); in the gray matter, there are *primitive ramified microglial cells* (Dalmau et al., 1998), a transition form towards the formation of *adult ramified microglial cells* found in the mature central nervous system (CNS) and derived from the progressive ramification of amoeboid cells (Boya et al., 1991; Wu et al., 1993; Dalmau et al., 1998).

It is also during the second week of postnatal life in the rat when there is first evidence of the typical mature astroglial cells found in the adult brain, stellated and containing glial fibrillary acidic protein (GFAP) as their main cytoskeletal component. Characteristically, astroglial cells of white matter tracts show hypertrophy and an increase in GFAP content, which is accompanied by the expression of the antioxidant proteins metallothioneins (Acarin et al., 1999a; Acarin et al., 1999d). These intrinsic 'activation' of glial cells in white matter tracts during the first 2 weeks of postnatal life is probably a consequence of important ongoing processes, such as myelination, remodeling and the establishment of adult connexions (Innocenti et al., 1983a,b; Uylings et al., 1990). Moreover, in vitro studies have demonstrated the neuronotrophic properties of immature astrocytes, which show an important expression of growth factors and production of extracellular matrix proteins (Hatten et al., 1991; Kimelberg and Norenberg, 1994). Therefore, this stage of neural development maintains a unique plasticity that provides an optimal experimental model for the study of neuronal affectation and the glial response associated to degenerative and regenerative processes.

Particularly, the period between days 6 and 10 of postnatal life in the rat has been defined as the *plasticity window period*. The brain response to traumatic injury during this period has been extensively studied by Kolb and coworkers (Kolb, 1990; Kolb

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et al., 1994, 1996a,b, 1998), who have provided evidence that the immature rat cortex responds to injury by triggering an important phenomenon of dendritic sprouting and cortical reorganization which determines a lack of abnormalities in cortico-cortical and subcortico-cortical connections and normal performance in behavioral testing.

In this chapter, we will review the present knowledge on the mechanisms of glial cell activation in the immature brain, particularly focusing on the participation of cytokines and transcription factors in the development of the glial response. This review is based on up to date bibliography, including the studies we have performed using a model of cortical excitotoxic injury in the postnatal day 9 rat brain.

Cytokines and inflammatory transcription factors in the immature brain

Cytokines are a group of low molecular weight proteins which are classically known to play a crucial role in the initiation, propagation and suppression of the inflammatory response in peripheral tissues. Their effects are mediated by modulating immune cell activation, proliferation, migration and the production of other molecules (Di Santo, 1997; Grell and Scheurich, 1997). However, in the last decades, much attention has been focused on the possible functions of cytokines in the CNS, where they have been implicated in development (Mehler and Kessler, 1997), in normal adult brain function (Schobitz et al., 1994) and in several pathophysiological processes (Giulian and Lachman, 1985; Woodroofe et al., 1991; McGeer and McGeer, 1997). Although the precise functions of cytokines have not yet been elucidated, cytokines, such as interleukin-1β (IL-1β), tumor necrosis factor (TNFα), interleukin-6 (IL-6) and transforming growth factor β1 (TGF-β1) are thought to play a key role in the modulation of glial cell function, by acting as important intercellular messengers involved in neuronal–glial interrelationship and the crosstalk between astrocytes and microglial cells. The presence and cellular expression of cytokines in the brain varies according to the changing CNS conditions: during development, in adulthood or after brain damage.

In the last decade, it has been shown that cytokines play a significant role in several important processes during brain development, including neuronal survival, cellular differentiation, synaptic regulation and neuritic outgrowth (Mehler and Kessler, 1997). Specifically, expression of the pro-inflammatory cytokine IL-1β is developmentally regulated, and IL-1β mRNA, protein and its bioactivity are barely undetectable in postnatal rat brains (Chang et al., 1994; Szaflarski et al., 1995; Hagberg et al., 1996; Silverstein et al., 1997), being only found in scattered endothelial cells throughout the brain (Acarin et al., 2000a). In contrast, detectable mRNA levels of the pro-inflammatory cytokine TNFα have been reported in several studies (Mizuno et al., 1994; Szaflarski et al., 1995; Silverstein et al., 1997), and this cytokine is mainly found in certain neuronal populations throughout the immature brain and in astrocytes of the corpus callosum (Acarin et al., 2000a). Developmental IL-6 mRNA and protein levels are also lower than in the adult (Gadient and Otten, 1994; Pousset, 1994; Hagberg et al., 1996; Prechel et al., 1996), although IL-6 mRNA is present in the late embryonic rat cortex (Pousset, 1994) and the protein is observed in neurons and white matter astroglial cells of the early postnatal brain (Acarin et al., 2000a). Finally, constitutive expression of the anti-inflammatory cytokine TGF-β in neurons and glia has also been reported during development. Basal expression is usually related to the TGF-β2 and TGF-β3 isoforms (Morganti-Kossmann and Kossmann, 1995), and constitutive expression of TGF-β1 is only observed in different neuronal populations until the second postnatal week, diminishing in adulthood (Acarin et al., 2000a). Interestingly, a recent study showed that, although microglial cells are the main cell type producing cytokines in vitro, cytokines are not expressed in vivo by the amoeboid microglial cells found in the white matter tracts during development (Hurley et al., 1999).

In immune cells of non-neural tissues, the actions of cytokines are mainly mediated by the transcription factors signal transducers and activators of transcription (STAT) and the nuclear factor κB (NF-κB), which regulate a variety of genes involved in inflammation, cellular adaptation to stress and cell death (Schindler and Darnell, 1995; Baldwin, 1996; Perkins, 1997; Baeuerle, 1998; Liu et al., 1998). In the last decade, activation of these transcription factors has also been implicated in the brain response to

stress and damage, as their target genes include several proteins involved in glial cell development and the neuronal and glial response to neural damage, like the cytoskeletal protein GFAP, the antioxidant proteins metallothioneins, cell adhesion molecules and MHC complexes (Baldwin, 1996; Cattaneo et al., 1999; Lee et al., 1999). In this regard, STATs and NF-κB transcription factors are likely to play a key role in the glial changes of gene expression observed both during development and after injury.

The transcription factor NF-κB is formed of two subunits (p65 and p50) and it is found in an inactive form in the cytoplasm. Upon several extracellular signals including specific receptor stimulation, a complex cascade of kinases becomes activated, ending up in the phosphorylation of IκB, the inhibitory molecule bound to NF-κB. After phosphorylation, IκB becomes ubiquitinated and is degraded by a proteasome, releasing activated NF-κB which is then free to translocate to the nucleus, bind the promoter region of several genes or cooperate with other transcription factors (Baldwin, 1996; Woronicz et al., 1997). In this way, NF-κB participates in brain development and plasticity (Bakalkin et al., 1993) and it is found in higher amounts in immature animals than in adulthood (Bakalkin et al., 1993), mainly in specific neuronal populations, in some blood vessels throughout the brain and in glial cells of white matter tracts (Acarin et al., 2000b).

The STAT family of proteins are also found in an inactive monomeric form in the cytoplasm. Activation of specific cytokine receptors induces activation of one or several kinases of the family of janus kinases (JAKs), that phosphorylate the tyrosine residues of STATs, activating them. In addition, growth factor receptors with tyrosine kinase activity can also activate STATs independently of JAK activation. To date, seven members of the STAT family have been described in mammals STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6. Upon activation STAT proteins form homodimers or heterodimers and translocate to the nucleus, interacting with specific DNA regions located in the promoters of cytokine-regulated genes (see Schindler and Darnell, 1995; Pellegrini and Dusanter-Fourt, 1997 for review). The JAK-STAT signaling pathway is known to play a key role during neuronal and glial differentiation in the developing CNS (Bonni et al., 1997; Cattaneo et al., 1999). Among the different members of the STAT family, STAT3 and STAT6 are the more strongly expressed isoforms during development (Planas et al., 1997a; De Fraja et al., 1998), although STAT1 is also observed (Planas et al., 1997b; De Fraja et al., 1998). Specifically, STAT1 and STAT3, which can homodimerize or heterodimerize upon phosphorylation, seem to be the most important members involved in glial differentiation and the glial response to injury, as they mediate the signal transduction of the ciliary neurotrophic factor (CNTF), a key factor in the differentiation of CNS progenitor cells into GFAP producing astrocytes (Bonni et al., 1997; Kahn et al., 1997; Rajan and McKay, 1998; Nakashima et al., 1999), and STAT3 mediates the signaling pathway of IL-6, a cytokine which is achieving a strong importance in developmental and postinjury processes (Hirano, 1998).

Glial response to injury in the immature rat brain

The glial response in the immature brain can be divided into three distinct sequential phases. The first stage will be called the *early phase*, which develops within hours after the injury and is characterized by the first changes in endogenous glial cells, metabolic changes in the damaged neurons and leaking of the blood–brain barrier (BBB). This is followed by an *acute phase* determined by the time of neuronal cell degeneration, leukocyte infiltration, a massive presence of microglia/macrophages and important structural and metabolic changes in astrocytes. Finally, scar tissue is formed during the period of *glial scar formation* which appears within the following 7–10 days depending on the type of injury.

Early phase

Astroglial activation

The first documented signs of astroglial activation following immature brain injury is the activation of the transcription factors STAT3 and NF-κB, which are localized in the astroglial nucleus within a few hours following an excitotoxic lesion (Abbracchio et al., 1995; Acarin et al., 1998a, 2000b). This early

activation of astroglial cells is likely to be mediated by signals originating from damaged neurons or induced by subtle changes in the extracellular milieu. The particularities of astroglial cells as extracellular sensors, cells expressing ion receptors and the presence of their blood vessel endfeet, enable them to carry out a very rigorous control of the extracellular media, detecting slight alterations (Norenberg, 1994; Aschner, 1998). Moreover, several of the reported STAT3 or NF-κB inducers are potentially present in the injured immature brain. Neurotrophic and growth factors such as CNTF, epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), all of them activators of STAT3 (Schindler and Darnell, 1995), are present in the developing brain (Lee et al., 1997) and in the adult brain after injury (Iihara et al., 1994; Hu et al., 1997; Planas et al., 1998), and could contribute to this transcription factor activation. In regards to NF-κB, glutamate itself is a potent activator; either directly through *N*-methyl-D-aspartate (NMDA) receptor binding, or indirectly by causing neuronal depolarization and oxidative damage (O'Neill and Kaltschmidt, 1997), common pathophysiological pathways in several types of injury. Additionally, nerve growth factor (NGF) and the cytokines IL-1 and TNFα, other important NF-κB triggering factors, are also upregulated after postnatal lesions. In particular, NGF mRNA is observed after intraventricular NMDA injections (Springer et al., 1994), and both IL-1 and TNF mRNAs were detected after hypoxia–ischemia and excitotoxicity (Szaflarski et al., 1995).

It is interesting to note that activation of these transcription factors in astrocytes precedes upregulation of GFAP, cell hypertrophy and metabolic changes. Actually, several genes implicated in reactive astrogliosis, like those encoding GFAP or metallothionein I–II (Acarin et al., 1999d) have been shown to be modulated by STAT proteins (Kahn et al., 1997; Cattaneo et al., 1999; Lee et al., 1999) or NF-κB (Krohn et al., 1999) binding to their promoters. Noteworthy, at the end of this early phase of activation, astroglial cells responding to excitotoxic and ischemic damage express IL-6 (Hagberg et al., 1996; Acarin et al., 2000a), which is maintained until the formation of the glial scar. STAT3 is in fact the main signal transducer of IL-6 actions and a STAT3-target gene itself, which leads us to think that this cytokine may be a key factor in the mechanism of astroglial activation.

Microglial activation

Microglial cells become activated within the first hours following several types of injury to the developing CNS, including aspiration lesions (Milligan et al., 1991), stab wounds (Kaur et al., 1987), hypoxia/ischemia (McRae et al., 1995; Towfighi et al., 1995; Ivacko et al., 1996; Li et al., 1998; Benjelloun et al., 1999; Bona et al., 1999) and excitotoxicity (Acarin et al., 1996, 1999c). During this early phase, gray matter microglial cells undergo morphological and metabolic changes towards activated forms, they retract their processes and become pseudopodic, increase lectin binding, upregulate receptor complement 3 expression and mildly express MHC class I (McRae et al., 1995; Towfighi et al., 1995; Ivacko et al., 1996; Li et al., 1998; Acarin et al., 1999c; Bona et al., 1999). In addition, after hypoxia, amoeboid microglial cells of white matter tracts express the inflammatory related enzyme inducible nitric oxide synthase (You and Kaur, 2000). However, no signs of microglial STAT3 or NF-κB activation are detected during this early period of time, which implies that these early changes in microglial cells do not involve gene activation mediated by these inflammatory transcription factors.

Acute phase

Astroglial response

This stage is achieved within the first days postinjury, and is determined by events, such as neuronal degeneration, disruption of the BBB and leukocyte extravasation, which trigger important changes in glial cell morphology towards fully reactive forms and metabolically activated cells. It is well know that different types of postnatal brain damage, such as excitotoxicity (Burtrum and Silverstein, 1993), hypoxia/ischemia (Burtrum and Silverstein, 1994; Benjelloun et al., 1999), traumatic injury (Balasingam et al., 1996) or axonal damage (Oblinger and Singh, 1993) trigger an increase in the expression of cytoskeletal proteins like GFAP and vimentin, that contribute to cell hypertrophy and

motility. These cytoskeletal changes are also accompanied by a phagocytic activity of astroglial cells and changes in gene expression and cell metabolism, like the upregulation of antioxidant proteins, such as metallothioneins (Penkowa and Moos, 1995; Acarin et al., 1999a,d). Moreover, during this stage, astrocytes proliferate and migrate (Janeczko, 1988; Hatton et al., 1993; Janeczko, 1994), contributing to restore the structural integrity of the tissue. All these features of reactive astrogliosis are also accompanied by changes in gene expression. Following excitotoxic damage, reactive astrocytes maintain the activation of STAT3 and NF-κB, and express the cytokine target genes of these transcription factors: TNFα, IL-6, and IL-1β to a lesser extent (Acarin et al., 2000a). Similarly, levels of IL-1β, TNFα and IL-6 are upregulated following postnatal ischemia (Szaflarski et al., 1995; Hagan et al., 1996; Hagberg et al., 1996; Silverstein et al., 1997) and IL-1β and TNFα are increased in response to lipopolysaccharide (LPS) maternal treatment (Cai et al., 2000). In the last few years, in vivo studies have shown that astrocytes are important sources of these pro-inflammatory cytokines, especially following excitotoxic/ischemic damage or traumatic injury, both during development and in adulthood (Botchkina et al., 1997; Holmin et al., 1997; Gong et al., 1998; Orzylowska et al., 1999; Pearson et al., 1999).

Microglia/macrophage response

During the acute phase, the presence of neuronal and/or axonal debris, massive tissue disruption and BBB breakdown induces postnatal brain microglial cells to become further reactive, to show MHC class II expression, to upregulate other macrophage markers and even become phagocytic cells (Yee et al., 1990; Morioka and Streit, 1991; Greensmith and Navarrete, 1994; Acarin et al., 1999c; Bona et al., 1999). In addition, this acute phase is characterized in some circumstances by an important infiltration of leukocytes from the bloodstream (Benjelloun et al., 1999). These are probably recruited by different types of chemokines including the monocyte chemoattractant protein-1, which have been demonstrated following postnatal excitotoxic/ischemic injury (Ivacko et al., 1997; Szaflarski et al., 1998; Bona et al., 1999). Moreover, reactive microglia/macrophages express the anti-apoptotic gene bcl-2 (Benjelloun et al., 1999) and some of them produce the pro-inflammatory cytokine IL-1β (Acarin et al., 2000a), which may also play an important role in the development of the inflammatory response by inducing leukocyte extravasation, increasing MHC expression in microglial cells, and triggering the astroglial production of other cytokines (Morganti-Kossmann and Kossmann, 1995; Rothwell and Hopkins, 1995).

Actually, microglial cells are assumed to be the cell type expressing IL-1β after brain damage; the most extensively studied cytokine in neurodegeneration (for review see Morganti-Kossmann and Kossmann, 1995; Rothwell and Strijbos, 1995; Touzani et al., 1999). However, massive microglial production of IL-1β has been shown in strong inflammatory responses like those occurring after postnatal LPS challenge (Lee et al., 1993b; Sebire et al., 1993; Van Dam et al., 1995), but most studies identifying IL-1β expressing cell types after excitotoxic/ischemic damage both in the adult and developing brain point to an early IL-1β induction in microglial cells followed by a late astroglial expression (Zhang et al., 1998; Orzylowska et al., 1999; Pearson et al., 1999; Acarin et al., 2000a).

Interestingly, following an excitotoxic lesion in the immature brain, only a small subpopulation of reactive microglia located at the periphery of the degenerating area showed a very transient pulse of NF-κB or STAT3 activation within 24 h, early in the acute phase of the response (Acarin et al., 2000b). As changes in microglial morphology have already taken place, these pulses of STAT3 and NF-κB activation seem to occur once microglial cells are already activated, and may represent a response to very specific stimuli. In contrast, in the adult brain, reactive microglia/macrophages strongly activate STAT1 and STAT3 after ischemia (Planas et al., 1996, 1997b; Justicia et al., 2000), suggesting that both the developmental stage and the specific type of injury may modulate glial STAT activation. Likewise, microglial NF-κB activation is only induced in specific situations. Although no studies are available in the developing brain, we learn from the adult brain that microglial cells activate NF-κB in pathological situations which involve strong inflammatory processes and massive peripheral blood cell recruitment

like LPS treatment, multiple sclerosis and spinal cord injury (Kaltschmidt et al., 1994; Bauer et al., 1997; Bethea et al., 1998; Gveric et al., 1998; Heese et al., 1998).

Glial scar formation

Astroglial response

The formation of gliotic tissue, usually referred as glial scar, is the long-term result of the glial response. The glial scar is mainly composed of hypertrophied astrocytic processes, but it also contains macrophages and extracellular matrix. Glial scars do not develop after embryonic or newborn brain injury (Moore et al., 1987; Berry et al., 1999), but they form around 1 week after traumatic injury to rats of 6 days of age and older (Firkins et al., 1993; Janeczko, 1994; Acarin et al., 1998b). Glial scar formation implies achievement of maximal astroglial hypertrophy, accompanied by the peak expression of cytoskeletal proteins and the antioxidant proteins metallothioneins. Astrocytes forming postnatal brain scars show a strong mRNA and protein production of metallothionein isoform III (Acarin et al., 1999a), in addition to the isoforms I–II expressed in previous phases. Metallothionein III is an antioxidant protein, but also a potent inhibitor of neuritic growth and has been named growth inhibitory factor (GIF) (Uchida et al., 1991; Palmiter et al., 1992). This inhibitory molecule could modulate the activity of growth factors and participate in the well known inhibitory properties of glial scar.

Interestingly, some glial scar reactive astroglial cells still contain STAT3 and NF-κB in their nuclei and express IL-1β, TNFα and IL-6 (Acarin et al., 2000a,b), supporting the idea that these transcription factors autopotentiate astroglial hypertrophy and metabolic activity. In addition, glial scar reactive astrocytes modulate their own activation by expressing the growth factor and anti-inflammatory cytokine TGF-β1 (Suzumura et al., 1993; Hu et al., 1995; Jones et al., 1998). Furthermore, TGF-β1 upregulates the expression of different extracellular matrix components like several collagen types, fibronectin, tenascin and thrombospondin, and promotes the synthesis of protease inhibitors, providing a net effect of extracellular matrix accumulation (for review see Massagué, 1990) and suggesting its important role in glial scar formation both in the adult and immature brain.

Microglia/macrophage response

As mentioned before, macrophages are also an important component of the glial scars in the immature brain. Scar macrophages derived from endogenous microglial cells and originating from blood monocytes express the cytokine IL-1β and produce the anti-inflammatory cytokine TGF-β1 (Acarin et al., 2000a). Interestingly, the accumulation of microglia/macrophages observed during the acute phase of the microglial response decreases with time, showing reduced numbers in the glial scar. Actually, the fate of brain macrophages once their phagocytic activity is over remains controversial. After an excitotoxic lesion in the immature brain, we have observed an accumulation of macrophages in the expanded perivascular spaces of blood vessels located within the glial scar together with an increase in the number of meningeal macrophages (Acarin et al., 2000b), suggesting a possible elimination of macrophages through the bloodstream following excitotoxic brain injury. However, the possible apoptotic death of macrophages has been reported in a model of postnatal ischemia (Benjelloun et al., 1999).

Diversity of the glial response

An important point to consider is that glial cells react to brain injury in different ways, by undergoing changes in their cytoskeleton, migrating, proliferating and increasing expression of distinct families of genes (Ridet et al., 1997). Whether none, some or all of these events occur will highly depend on the grade of neuronal death, axonal degeneration, tissue damage and BBB disruption, that is to say, the milieu in which the glial response takes place.

In this sense, in the last years we have been using an experimental model based on an NMDA-induced excitotoxic injury in the neocortex, which causes sublethal affectation of distal connected thalamic nuclei, and allows us the study of the glial response in different conditions. Our studies show that in the excitotoxically damaged cortex versus the distal tha-

lamus, astroglial and microglial protein upregulation and de novo gene expression display two different patterns (Acarin et al., 1998b, 1999c,d), which could be determined by different mechanisms of glial cell activation. In this sense, activation of STAT3 and NF-κB is only observed in cortical areas affected by severe tissue damage, neuronal degeneration and BBB disruption (Acarin et al., 1999c). In contrast, the milder glial response occurring in the distal thalamus is not preceded by immunocytochemically detectable STAT3 and NF-κB activation, although microglial and astroglial response do occur. As no vasculature damage and no leukocyte recruitment takes place in the thalamus (Acarin et al., 1999c), no inflammation occurs and neither expression of IL-1β or TNFα (potent inducers of NF-κB) or IL-6 (activator of STAT3) are observed (unpublished observations), suggesting that appropriate stimuli for STAT3 or NF-κB activation are not provided in sufficient amounts in these sublethal conditions.

Neuronal–glial signaling following an excitotoxic process in the immature rat cortex

The chronological study of the activation of previously mentioned inflammatory transcription factors and cytokine expression in the excitotoxically lesioned immature cortex, together with our previous works on the evolution of the glial response in an excitotoxic model, has provided us some insights into the putative mechanisms of neuronal–glial and glial–glial crosstalk following an excitotoxic immature brain damage.

Within the first hours after the excitotoxic insult, affected neuronal cells undergo metabolic changes and alterations in gene expression, which are accompanied by BBB disruption and the first evidence of glial cell activation. These very early neuronal changes occur within 2–4 h postinjury and include activation of the transcription factor NF-κB (Acarin et al., 2000b), induction of the neuroprotective cytokine IL-6 (Acarin et al., 2000a) and de novo expression of the immediate early genes c-fos and c-jun (unpublished observations), important sensors of neuronal stress (Sharp, 1993). As it is summarized in Fig. 1, activation of the NMDA receptor itself by exogenous application of NMDA can trigger activation of NF-κB (Ko et al., 1998), which can increase the expression of one of their target genes, the multifunctional cytokine IL-6 (Sparacio et al., 1992), which at the same time modulates c-jun and c-fos expression (Schindler and Darnell, 1995).

Interestingly, the transcription factor STAT3, responsible for the signal transduction mechanisms of IL-6 (Gruol and Nelson, 1997), is not activated in these excitotoxically damaged neurons, but it is observed in surrounding astroglial cells (Fig. 1) (Acarin et al., 1998a, 2000b), suggesting that IL-6 could be induced in damaged neurons to promote interactions with neighboring glial cells. Another piece of evidence of the importance of this signaling mechanism in glial cell activation is the fact that several of the features of reactive astrocytosis which occur at later stages (Fig. 2) appear to be regulated by this IL-6-induced transcription factor: GFAP upregulation (Kahn et al., 1997), metallothionein expression (Lee et al., 1999), proliferation (Fukada et al., 1996; Grandis et al., 1998) and IL-6 expression (Van Wagoner and Benveniste, 1999), supporting an autocrine action of this cytokine in astroglial response.

In addition, astroglial cells also show NF-κB activation within hours after the excitotoxic lesion (Fig. 1). NF-κB can be induced by oxidative stress and other changes in the extracellular milieu which occur in an excitotoxic process. Noteworthy, this transcription factor also modulates the expression of the GFAP gene (Krohn et al., 1999) as well as the cytokines IL-1β, TNFα and IL-6 (Sparacio et al., 1992; Friedman et al., 1996; O'Neill and Kaltschmidt, 1997), all of them produced by astrocytes during the acute phase of the response (Fig. 2) (Acarin et al., 2000a).

This pattern of cytokine production and activation of inflammatory transcription factors in astrocytes leads us to postulate that during the acute phase of the response, IL-6 and TNFα produced by the astrocytes themselves may modulate their own response and the reaction of neighboring microglial cells and blood vessels. Likewise the above commented actions of IL-6, it is well documented that TNFα plays an important role in triggering inflammatory processes, microglial and astroglial cell proliferation and scar formation (Merrill, 1991; Ganter et al., 1992; Thery and Mallat, 1993; Muñoz-Fernandez and Fresno, 1998).

Moreover, IL-1β is also an important participant

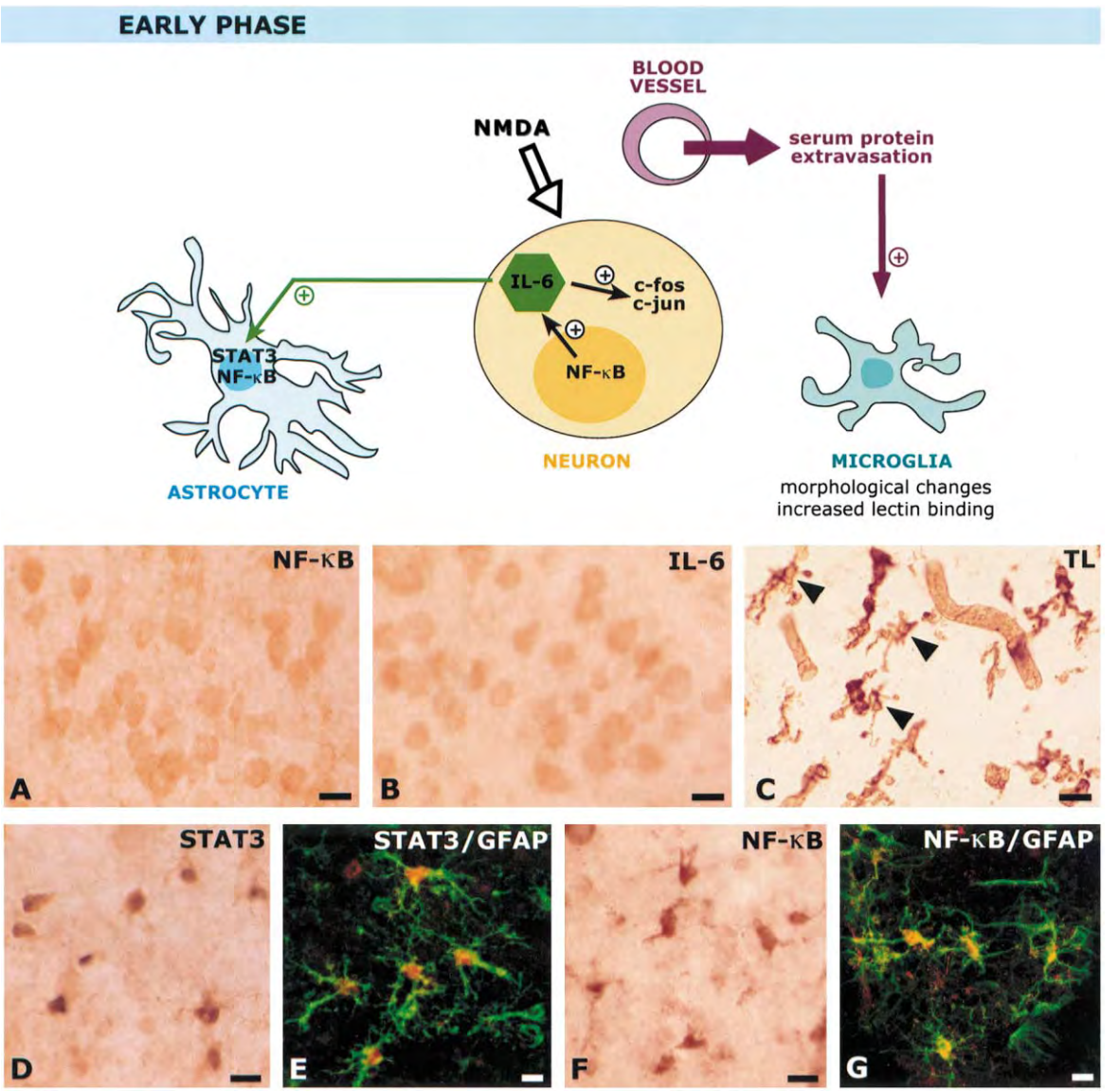
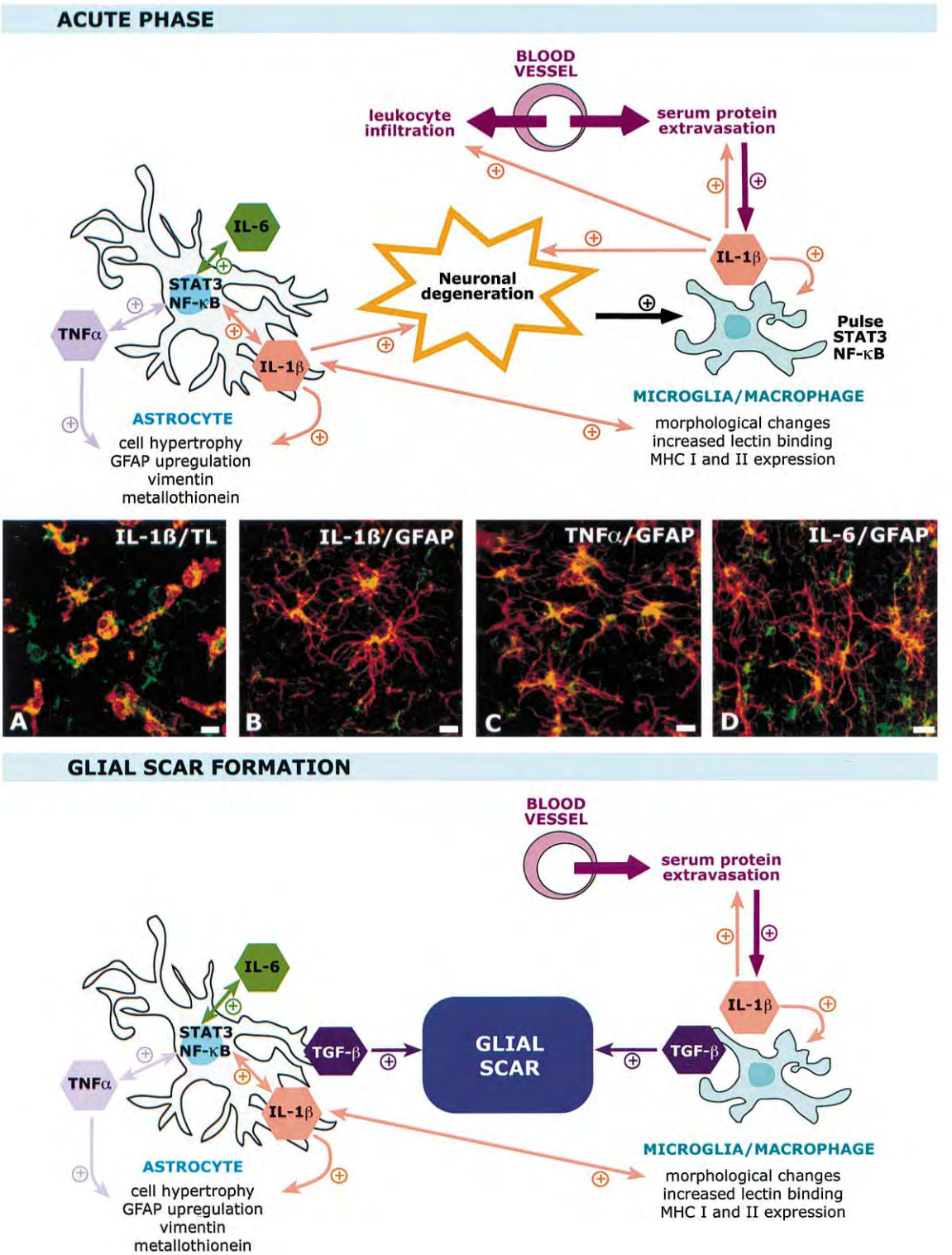


Fig. 1. Schematic representation of the events occurring during the early phase following an excitotoxic injury to the immature rat brain. Significant features include activation of NF-κB (A) and expression of IL-6 (B) in NMDA-damaged neurons, morphological changes and increased tomato lectin (TL) binding in microglial cells (arrowheads in C), and activation of STAT3 (D,E) and NF-κB (F,G) in astrocytes. E and G are confocal micrographs showing STAT3/NF-κB labeling in red and GFAP staining in green, yellow indicates colocalization. Scale bars: 10 μm.

Fig. 2. Schematic representation of the events occurring during the acute phase (top) and the stage of glial scar formation (bottom) following an excitotoxic injury to the immature rat brain. Both in the acute phase and in the glial scar, reactive astrocytes express the cytokines IL-1β, TNFα and IL-6 (B–D) and reactive microglia/macrophages express IL-1β (A). Moreover, scar glial cells also produce TGF-β1. A–D are confocal micrographs showing cytokine labeling in green and GFAP/TL staining in red, yellow indicates colocalization. Scale bars: 10 μm.


ACUTE PHASE
BLOOD VESSEL
leukocyte infiltration
serum protein extravasation
IL-6
STAT3
NF-κB
TNFα
IL-1β
Neuronal degeneration
IL-1β
Pulse STAT3 NF-κB
ASTROCYTE
cell hypertrophy
GFAP upregulation
vimentin
metallothionein
MICROGLIA/MACROPHAGE
morphological changes
increased lectin binding
MHC I and II expression
IL-1ß/TL
IL-1ß/GFAP
TNFα/GFAP
IL-6/GFAP
A
B
C
D
GLIAL SCAR FORMATION
BLOOD VESSEL
serum protein extravasation
IL-6
STAT3
NF-κB
TNFα
TGF-β
GLIAL SCAR
TGF-β
IL-1β
IL-1β
ASTROCYTE
cell hypertrophy
GFAP upregulation
vimentin
metallothionein
MICROGLIA/MACROPHAGE
morphological changes
increased lectin binding
MHC I and II expression

in the modulation of astroglial–microglial crosstalk following postnatal excitotoxic injury. This cytokine is produced by microglial and astroglial cells and it can modulate both glial responses and affect BBB permeability and neuronal degeneration. However, in spite of the amount of studies reporting IL-1β induction after several kinds of brain damage, its role after injury remains to be clarified (for general reviews on IL-1β functions see Morganti-Kossmann and Kossmann, 1995; Rothwell and Strijbos, 1995; Zhao and Schwartz, 1998; Rothwell, 1999). Reported IL-1β functions after brain injury are mainly pro-inflammatory, as they include leukocyte extravasation (Shrikant et al., 1994), astroglial growth (Giulian and Lachman, 1985; Giulian et al., 1988; Merrill, 1991), and raising the production of inflammatory mediators like nitric oxide, free radicals and other cytokines (Lee et al., 1993a; Thery and Mallat, 1993; Benveniste, 1995; Chao et al., 1995; Merrill and Benveniste, 1996; Kitamura et al., 1998). In addition, several reports have recently pointed to an important role of IL-1β in mediating and exacerbating neuronal damage in the adult (see Rothwell et al., 1995 for review), as well as in the injured postnatal brain (Martin et al., 1994). Therefore, it seems that IL-1β is able to inhibit, exacerbate or induce neuronal damage, and its overall effects may depend on the cytokine concentration, the environment and the other factors present at the injury site. Accordingly, it should be taken into account that the specific actions of each cytokine are obscured by the fact that their activities overlap and regulate one another to form a complex cytokine network. It is well known that the hallmarks of cytokine biology are pleiotropism, functional redundancy and feedback. In general, following a postnatal excitotoxic injury, cytokines are present at very early times, and these may contribute to several of the ongoing processes, such as the regenerative attempts of neurons and the modulation of the astroglial and microglial responses, therefore contributing to the progression of immature brain injury.

New insights: blockade of NF-κB activation, a role in neuroprotection?

Given the importance of the glial response in the evolution of neural damage and the lesion outcome, the better characterization of glial activating factors as well as their secretion products could help in the understanding of the mechanisms underlying neuronal degeneration and will allow us, in the future, to interact with glial cell metabolism as a therapeutic target, in order to modulate the activity of these cells in several degenerating conditions, improving the neuropathological outcome.

In this sense, we have recently focused our studies in the possible neuroprotective effects of blocking the activation of these transcription factors. The treatment with a non-steroid anti-inflammatory drug, such as a fluorated salicylate, which blocks constitutive NF-κB activation in the postnatal brain in vivo (Acarin et al., 2000c), has shown that blocking glial NF-κB following a postnatal excitotoxic lesion causes an important downregulation of the glial response which is accompanied by a significant reduction of lesion volume (Acarin et al., 1999b; González et al., 2000), therefore providing neuroprotection. These studies are encouraging, although more studies are needed to further evaluate the effects of the blockade of these transcription factors after brain damage, in order to better characterize the beneficial inhibition of their target genes and to elucidate the mechanisms underlying neuroprotection.

Abbreviations

BBB	blood–brain barrier
CNS	central nervous system
CNTF	ciliary neurotrophic factor
EGF	epidermal growth factor
GFAP	glial fibrillary acidic protein
GIF	growth inhibitory factor
IL-1β	interleukin-1β
IL-6	interleukin-6
JAK	janus kinase
LPS	lipopolysaccharide
MHC	major histocompatibility complex
NF-κB	nuclear factor-κB
NGF	nerve growth factor
NMDA	*N*-methyl-D-aspartate
PDGF	platelet-derived growth factor
STAT	signal transduction and activator of transcription
TGF-β	transforming growth factor β
TNFα	tumor necrosis factor alpha

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QUERIES:

?#1: Please update Acarin et al., 2000a (page 395)
?#2: Please provide page nos. for González et al., 2000 (page 396)
?#3: Lai and Baumann, 1997 not mentioned in the text. Delete? (page 397)