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Review

Astrocytes: Key Regulators of Neuroinflammation

Emanuela Colombo[1] and Cinthia Farina[1,*]

Astrocytes are crucial regulators of innate and adaptive immune responses in the injured central nervous system. Depending on timing and context, astrocyte activity may exacerbate inflammatory reactions and tissue damage, or promote immunosuppression and tissue repair. Recent literature has unveiled key factors and intracellular signaling pathways that govern astrocyte behavior during neuroinflammation. Here we have re-visited *in vivo* studies on astrocyte signaling in neuroinflammatory models focusing on evidences obtained from the analysis of transgenic mice where distinct genes involved in ligand binding, transcriptional regulation and cell communication have been manipulated in astrocytes. The integration of *in vivo* observations with *in vitro* data clarifies precise signaling steps, highlights the crosstalk among pathways and identifies shared effector mechanisms in neuroinflammation.

A Controversial Role for Astrocytes in Neuroinflammation

Astrocytes are the most abundant glia cell type of the central nervous system (CNS) and are essential for brain homeostasis, as they provide metabolites and growth factors to neurons, support synapse formation and plasticity, and regulate the extracellular balance of ions, fluid and neurotransmitters [1]. Thanks to their strategic location in close contact with CNS-resident cells (neurons, microglia, oligodendrocytes and other astrocytes) and blood vessels, astrocytes participate in blood brain barrier (BBB) maintenance and permeability. They also play a role in the control of immune cell trafficking and activation. Astrocytes are immune-competent cells able to detect danger signals, respond via secretion of cytokines and chemokines, and activate adaptive immune defense [2,3]. CNS injury triggers a process leading to scar formation, whose impact on tissue homeostasis is ambivalent, as inflammatory and neurotoxic mediators are produced at injury site but remain confined to that area thanks to the glial scar [4]. Expression of the cytoskeletal glial fibrillary acidic protein (GFAP) is widely used for the identification of astroglia *in vivo* and *in vitro*, and upregulation of this marker in astrocytes is a typical hallmark for CNS pathologies [1]. GFAP is also widely expressed by progenitor cells of neurons, oligodendrocytes and astrocytes [5], and therefore its loss during development in constitutive GFAP knockout (KO) mice may have a long-term impact on cell types other than astrocytes. Pioneering studies in such mice have shown no gross alterations in brain architecture and BBB tasks in young animals, but white matter pathology in old mice, indicating a physiological relevance for those intermediate filaments only during aging [6,7]. Yet, GFAP plays a role during CNS infection and autoimmunity, as young GFAP KO mice show more severe clinical expression of ***Toxoplasma encephalitis*** (**TE**, see Glossary), *Staphylococcus aureus*-induced **brain abscess** and **experimental autoimmune encephalomyelitis (EAE)** than wild type (wt) animals [8,9] (Table 1). To better evaluate the role of astrogliosis during CNS injury in adult animals, Sofroniew and colleagues have generated inducible transgenic mice where selective ablation of proliferating astrocytes is achieved in adult mice by ganciclovir administration (GFAP-TK mice) [10] (Table 1). *In vivo* experiments in distinct disease models (**brain injury (BI)**, **spinal cord injury (SCI)** or EAE) consistently show that the loss of reactive astrocytes during the early phases of injury results in exacerbation of clinical signs and motor

Trends

Astrocytes are active players in neuroinflammation, and their response may be beneficial or detrimental for tissue repair, depending on the kind of stimuli offered by the inflamed milieu.

The regulatory function of the astrocyte *in vivo* is regulated by specific signaling pathways. Transgenic animals carrying mutations in astrocyte proteins involved in ligand binding, transcriptional regulation, and cell communication have been instrumental to dissect astrocyte signaling in distinct neuroinflammatory models.

TGFβ, IFNγ, gp130, estrogen, STAT3, BDNF, and FasL support the protective phenotype of the astrocyte, whereas IL17, sphingolipids, TrkB, SOCS3, NFκB, chemokines and VEGF trigger damaging pathways.

During neuroinflammation, astrocytes are exposed simultaneously to a plethora of stimuli leading to a complex network of intracellular events. However, distinct activation modes may share signaling steps and effector mechanisms.

[1]Institute of Experimental Neurology (INSpe), Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy

*Correspondence: farina.cinthia@hsr.it (C. Farina).

 http://dx.doi.org/10.1016/j.it.2016.06.006

Table 1. Impact of Astrocyte Depletion in Neuroinflammatory Models[d].

Transgenic model	Disease model	Effects of deletion				Refs
		Disease severity	Inflammation	Demyelination	Neuronal damage	
GFAP$^{-/-}$	EAE	↑	=	=	N.A.	[8]
	TE	↑	↑	N.A.	N.A.	[9]
	Brain abscess	↑	↑	N.A.	N.A.	[9]
GFAP-TK	Forebrain Stab injury[a]	↑	↑	N.A.	↑	[10]
	SCI[a]	↑	↑	↑	↑	[11,16]
	Cortical contusion Injury[a]	↑	↑	N.A.	↑	[12]
	EAE[a]	↑	↑	N.A.	N.A.	[15]
	EAE[a]	↑	↑	↑	↑	[13]
	EAE[b]	↑	↑	N.A.	N.A.	[14]
	EAE[c]	↓	↓	N.A.	N.A.	[15]

[a]Ganciclovir (GCV) administration pre- or immediately post-injury.
[b]GCV administration after disease onset.
[c]GCV administration 30 days post immunization.
[d]Key. ↑, worsening in specific disease outcomes; ↓, improvement in specific disease outcomes; = no difference; N.A., not assessed.

deficits, scar disorganization, spreading and persistence of inflammatory cells, BBB alterations, and, when analyzed, demyelination and neuronal death [10–16] (Table 1). By contrast, astrocyte depletion during the chronic phase of EAE ameliorates disease expression and reduces leukocyte infiltration into the CNS [15] (Table 1). These data indicate that the outcome of astrogliosis is regulated in a time- and context-specific manner, and that stimuli from the microenvironment during neuroinflammation may shift astrocyte action from beneficial to detrimental for the neural tissue. The direct impact of the loss of a structural protein on glial inflammatory signaling remains, however, to be elucidated. Thus, the development of novel therapies for neuroinflammatory disorders relies on the identification and selective targeting of specific astrocytic functions. To this end, the GFAP promoter has been instrumental to drive or abolish gene expression in astroglia *in vivo* and study astrocyte function in distinct neuroinflammatory models. Table 2 details the impact of single molecular perturbations in the astrocyte on disease outcomes. In the next paragraphs we discuss how the protective versus detrimental behavior of glia cells is regulated by surface or cytoplasmic proteins, transcription factors and released mediators, and integrate the information derived from *in vivo* studies with *in vitro* findings to highlight the crosstalk among distinct pathways and identify shared effector mechanisms in neuroinflammation.

Protective Astrocyte Signaling Pathways Regulated by Cytokines, Growth Factors and Hormones

In vivo studies have provided compelling evidence that astrocyte responses to certain cytokines, growth factors and hormones are protective, as their absence worsens CNS injury (Table 2 and Figure 1).

The first protective pathway is mediated by the glycoprotein gp130, an essential signal transducer for members of the IL6 cytokine family. The investigation of TE and EAE in mice lacking gp130 in GFAP-positive cells (GFAPcre-gp130$^{fl/fl}$ or **floxed mice**) has demonstrated that astrocytic gp130 signaling is crucial for glia cell survival and control of disease expression (Table 2 and Figure 1) [17,18]. In fact GFAPcre-gp130$^{fl/fl}$ mice display astrocyte apoptosis, larger areas of parasite-induced tissue necrosis, higher mortality after TE infection [17] and

Glossary

Brain abscess: focal brain infection induced by stereotactic injection of *Staphylococcus aureus*.
Brain injury (BI): contusion or stab injury performed with a steel-tipped piston or a blade respectively.
Experimental autoimmune encephalomyelistis (EAE): T cell-mediated autoimmune disease of the central nervous system with clinical and neuropathological similarities to multiple sclerosis. It is induced by active immunization with myelin extracts, purified myelin proteins, or immunogenic myelin peptides; or by adoptive transfer of myelin-reactive T lymphocytes.
Floxed mice: transgenic mice where two LoxP sites are positioned in intronic regions flanking one or several essential exons of the target gene. Recognition of LoxP sites by Cre recombinase leads to excision of LoxP-flanked DNA. Thus, tissue-specific gene deletion is achieved by breeding floxed mice with transgenic mice expressing Cre recombinase under the control of a tissue-specific promoter.
Spinal cord injury (SCI): contusion, compression, distraction, dislocation, transection or chemical damage of the spinal cord.
Stroke: induced by moderate to severe middle cerebral artery occlusion.
Toxic demyelination: acute demyelination induced by diet enriched with Cuprizone (a copper-chelating agent toxic to oligodendrocytes).
***Toxoplasma* encephalitis (TE):** systemic parasitic infection followed by a chronic stage with cyst formation in the brain. It is induced by oral or intraperitoneal administration of *Toxoplasma gondii.*