

# Astrogliosis after ischemic stroke: Neuroprotection or neuroinflammation?

Amaia EliceGUI<sup>1,2</sup>, Thomas Schmitz<sup>1</sup>, Miren Revuelta<sup>1,3\*</sup>

<sup>1</sup>Department for Neonatology, Charité University Medical Center, Augustenburger Platz 1, 13353 Berlin, Germany

<sup>2</sup>Neuromuscular Diseases Research Group, Biodonostia Health Research Institute, Paseo Doctor Begiristain, 20014 San Sebastian, Spain

<sup>3</sup>Department of Physiology, Faculty of Medicine, University of the Basque Country UPV/EHU, Leioa, Spain

\*Author for correspondence:  
Email: miren.revuelta@ehu.eus

Received date: February 22, 2021  
Accepted date: March 05, 2021

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Citation: EliceGUI A, Schmitz T, Revuelta M. Astrogliosis after ischemic stroke: Neuroprotection or neuroinflammation? Am J Aging Sci Res 2021; 2(1):9-12.

## Abstract

Ischemic stroke is the main cause of disability worldwide affecting around 6 million deaths per year. A cascade of events following the ischemic insult induce energy failure, excitotoxicity and release of inflammatory mediators that provoke cell death and brain injury. In this process, astrocytes undergo a change on gene expression that leads to reactive astrocytes. In this review, different reactive astrocyte populations have been described, in which A1 neurotoxic astrocytes promote a pro-inflammatory environment and reduce neuronal survival, while A2 neuroprotective astrocytes secrete neurotrophic factors and cytokines that promote tissue repair and axonal regeneration. Therapies based on key targets promoting A2 neuroprotective and reducing A1 phenotypes should be further investigated, as they appear to be promising strategies for a clinically successful stroke therapy.

**Keywords:** Stroke, Reactive astrocyte, Neuroprotection, Neuroinflammation

## Abbreviations

BBB: Blood-Brain Barrier; GFAP: Glial Fibrillary Acidic Protein; ROS: Reactive Oxygen Species; TGF- $\beta$ : Transforming Growth Factor Beta; TNF $\alpha$ : Tumor Necrosis Factor Alpha; VEGF: Vascular Endothelial Growth Factor; BDNF: Brain-Derived Neurotrophic Factor; NF $\kappa$ B: Nuclear factor kappa B; IL-1 $\alpha$ : Interleukin 1 Alpha

## Introduction

Around 16 million people are affected by stroke annually worldwide, which contributes to nearly 5.7 million deaths per year. Besides, it is considered the largest cause of disability as half of the patients that survive require long-term health care and fail to live in an independent manner [1]. Ischemic stroke is the most common type of stroke and happens when blood flow to the brain is disrupted, leading to cell death and brain injury [2]. Age, low physical activity and individual risk factors such as smoking, diabetes, and high blood pressure increase stroke susceptibility. Ischemic stroke is more frequent in adult age and neurological outcomes are severe, where patients often suffer motor impairment, aphasia and inability to read after ischemic injury [3].

A wide cascade of events starts once blood flow is interrupted in the brain. Oxygen and glucose are decreased due to reduced blood supply, which provokes a rapid depletion of ATP. Energy failure is followed by cell membrane depolarization, calcium entry and extracellular glutamate accumulation. This leads to high excitotoxicity and release of pro-inflammatory cytokines. In the next hours following the injury, reactive oxygen species are increased causing oxidative stress, whereas together with a higher concentration of free cytosolic calcium and impaired mitochondrial function, cell death is triggered [4]. This pro-inflammatory environment which lasts from days to months is responsible for brain injury.

## Astrocyte Reactivity after Stroke

Astrocytes are the most abundant glial cells in the Central Nervous System (CNS). Under physiologic conditions, astrocytes have a wide variety of functions: apart from providing nutrients and trophic factors to neurons, they also take part in ion and water homeostasis, pH maintenance, and glymphatic system function [5]. Additionally, as they form tripartite synapses, they can modulate

neuronal activity and regulate synapse homeostasis by the regulation of excitatory and inhibitory synapses. Furthermore, astrocytes are part of the structure of the Blood-brain barrier (BBB) together with endothelial cells and pericytes, where they limit the traffic of different molecules to the brain parenchyma [6]. Maintaining this selective permeability and integrity is crucial for normal brain function.

After an ischemic insult, astrocytes undergo a rapid change of gene expression that leads to reactive gliosis. Reactive astrocytes present classical hallmarks such as overexpression of glial fibrillary acidic protein (GFAP) and other extracellular matrix proteins, cellular process increase, and cell body hypertrophy. Recently additional markers including lipocalin-2 (Lcn-2) and serpin3n, a serine protease inhibitor, have been described [7]. Indeed, astrocyte reactivity is coordinated by complex molecular mechanisms initiated through an inflammatory response that leads to the formation of a glial scar surrounding the injury site, which is considered a source of pro-inflammatory molecules, reactive oxygen species (ROS), and neurotoxicity, as well as axonal regeneration inhibitors [5]. This leads to an environment in which regeneration is inhibited and cerebral edema is triggered. However, the glial scar has also been described to have a neuroprotective role, as it demarcates the damaged tissue and maintains the inflammation in a localized area protecting cells against harmful substances released from the infarct core.

### Neuroprotective or Neuroinflammatory Population of Astrocytes after Stroke

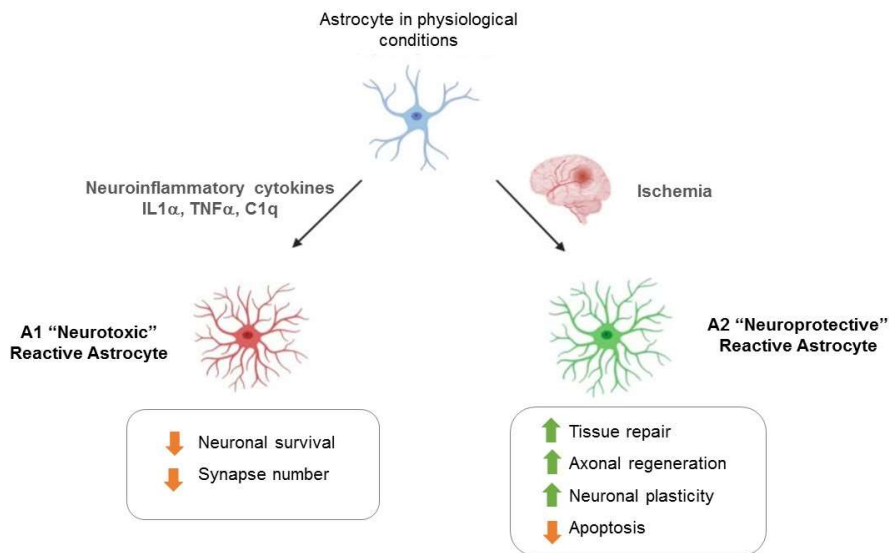
Recently, experts have described different reactive astrocyte phenotypes depending on the insult. Neuroinflammation and ischemia induce A1 and A2 reactive astrocyte phenotype respectively (Figure 1). A1 astrocytes upregulate many classical complement cascade genes shown to be harmful to synapses, and trigger neuronal and mature oligodendrocyte death [8]. They release neurotoxic and inflammatory factors including interleukin 6 (IL-6), IL-1 $\alpha$ ,

tumor necrosis factor alpha (TNF- $\alpha$ ), and nitric oxide, and activate the nuclear factor kappa B (NF $\kappa$ B) inflammatory pathway, which contribute to cell death and induce less synapses and neurite growth than healthy astrocytes. Besides, A1 reactive astrocytes lose synapse and myelin debris phagocytosis function. Specific markers complement 3, complement factor B and MX1S are expressed in A1 astrocytes. Activated microglia induce A1 phenotype by the release of IL-1 $\alpha$ , TNF- $\alpha$  and C1q cytokines [9]. A1 astrocytes appear in several neurodegenerative diseases such as Alzheimer's disease.

On the other hand, the neuroprotective A2 phenotype release anti-inflammatory cytokines and trophic factors that promote tissue repair. They express S100A10, which it is essential for cell proliferation, cell membrane repair and inhibition of cell apoptosis [10]. Besides, they express the anti-inflammatory cytokine transforming growth factor beta (TGF- $\beta$ ) that participates in neuronal survival and axonal regeneration, and synaptogenesis. TGF- $\beta$  together with glycoprotein gp130, signal transducer and activator of transcription 3 (STAT3) and interferon gamma (IFN- $\gamma$ ) pathways support the neuroprotective phenotype of astrocytes [11]. IL-4 and IL-10 are also anti-inflammatory mediators that secrete this astrocyte population. Additionally, A2 astrocytes are able to release other neurotrophic factors including nerve growth factor (NGF), basic fibroblast growth factor (bFGF), brain-derived neurotrophic factor (BDNF), glia-derived neurotrophic factor (GDNF), and vascular endothelial growth factor (VEGF) that protect neurons, enhance neurite growth and neuronal plasticity, and improve functional outcome after stroke [5,12].

### The Effect of Aging on Astrocyte Reactivity

Morphological and molecular changes are observed in the brain during aging and aging itself promotes astrocyte reactivity. This increase in astrocyte reactivity, however, is region-specific, and age-dependent changes in astrocyte gene expression contribute to age-



**Figure 1:** Depending on the stimulus, astrocytes can transform into different reactive astrocyte phenotypes. Pro-inflammatory cytokines IL1- $\alpha$ , TNF $\alpha$  and C1q that are released by activated microglia induce the A1 Neurotoxic phenotype, where neuron survival and synaptogenesis is decreased. Meantime, ischemia induces the transformation into the A2 phenotype, which triggers tissue repair, axonal regeneration and neuronal plasticity, contributing to a neuroprotective role.

related synapse loss, neuroinflammation, and cognitive decline [13]. However, homeostatic and neurotransmission function of astrocytes show minimal alteration during aging [14].

Clarke et al. demonstrated the upregulation of a group of potentially detrimental A1 reactive genes in the aging brain [13]. Aged astrocytes show upregulation of genes involved in inflammation and synapse elimination. The increased A1 neuroinflammatory phenotype induces the release of toxic factors and worsens neuron survival and excitatory neuronal function. Thus, A1 reactive astrocytes are a remarkable source of complement cascade components including C4b, C3, and C1q that trigger synapse loss, mainly in the aging hippocampus. Other genes regulating synaptic function are shown to be altered, such as Sparc, which is known to inhibit synapse function by blocking synapse formation and decreasing AMPA receptors [7,15]. Boisvert and colleagues observed that increased expression of Sparc observed in cortical astrocytes may inhibit synaptic function [14]. Besides, cholesterol synthesis was decreased in the aging brain, affecting directly neuronal dendritic elaboration and presynaptic function. Therefore, the activation of these genes aggravates synapse function and highlights the active role of astrocytes in synapse homeostasis.

As mentioned before, astrocytes play an important role in neuroinflammation and neuroprotection by the release of neurotrophic factors and both pro- and anti-inflammatory cytokines. However, during aging astrocytes adopt a pro-inflammatory phenotype, leading to a chronic and low-grade state of inflammation, named Inflammaging [16]. Aged astrocytes show less secretion of Wnt3, Insulin-like growth factor 1 (IGF-1), fibroblast growth factor receptor 2 (FGFR-2), and VEGF which have been associated with reduced ability for neurogenesis in the adult brain [12]. Aged astrocytes show impairment in their ability to promote neuronal differentiation and this is exacerbated after stroke, which worsens the recovery of the neurological injury and the restoration of dead cell populations [17]. In the aged ischemic brain, astrocyte reactivity increases exponentially and the forming glial scar is more exaggerated.

Besides, heterogeneity was found in different brain regions in mice. Regions that are more vulnerable to neurodegeneration, including the hippocampus and the striatum, showed an increase in astrocyte reactivity-related genes. These regions are more sensitive to oxidative stress, protein aggregation, and metabolic alterations [13]. In the human brain, increased GFAP expression has been observed in the hippocampus, frontal cortex, and temporal cortex [18].

Apparently, in the aging brain the increase of A1 reactive genes is mediated by the increase in microglial activation. In mice lacking A1 phenotype inducers IL1- $\alpha$ , TNF- $\alpha$ , and C1q, a significant reduction of aging-induced upregulation of reactive astrocytes was observed [13]. Thereby, Clark et al. demonstrated that microglia are the main responsible for the increase of A1 reactive astrocytes in the aging brain. However, it has been remarked that changes in aging-induced astrocyte reactivity are region-specific, while microglial reactivity is increased in a global way [19].

In age-related neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic Lateral Sclerosis (ALS), and Huntington's disease (HS), the increase of A1 reactive astrocytes has been noticeable, providing conditions that allow disease progression. The alteration of inflammatory- and

synapse-related genes will trigger a neurodegenerative environment. Complement component C3 was upregulated in regions associated with each disease in patient samples [8]. In AD, C3 has been described to be implicated in beta amyloid synthesis and clearance. In human AD samples, 60% of astrocytes in the prefrontal cortex expressed C3, showing that a big part of astrocytes may trigger neuroinflammatory pathways [8]. Besides, A1 astrocytes in ALS patients may gain toxicity by the secretion of extracellular vesicles containing SOD1, phospho-TDP-43, and FUS, which are crucial proteins that are altered in ALS and trigger motor neuron death [20]. In models of PD, blockage of A1 astrocyte conversion by a Glucagon-like peptide-1 receptor (GLP1R) agonist reduces the loss of dopaminergic neurons and behavioral deficits found in PD mouse models [21]. Therefore, as astrocytes have been demonstrated to be major contributors for the development of the mentioned neurodegenerative diseases, targeting their function would be an interesting way to improve synapse protection.

### **Astrocytes as Therapeutic Targets**

Astrocytes have been considered an important target for stroke therapy as apart from interacting and supporting neurons, they also modulate synaptogenesis, and trophic factor and cytokine release. Thereby, different strategies based on astrocyte modulation have been proposed in order to promote neuroprotection after ischemic injury. Downregulating the neurotoxic astrocyte population by cottonseed oil seems a promising strategy, as it has been demonstrated that reduces astrocyte activation and inflammation by the inhibition of TLR4/NF $\kappa$ B pathway [22]. Other TLR4 and NF $\kappa$ B inhibitors including polyphenols have also shown beneficial results by the reduction of inflammatory mediators and neuronal death [23,24]. On the other side, other strategies that increase the neuroprotective A2 astrocyte population and induce neurotrophic factor release have been proposed to improve neurological outcome after stroke. For example, neurotrophic factor administration including BDNF, IGF-1, and TGF- $\beta$  has shown positive functional recovery with decreased infarct volume and less neuroinflammation [25-27]. Unfortunately, the delivery of these factors is still challenging as they are rapidly degraded and subsequently have a poor half-life. Thereby, promoting neuroprotection in ischemic tissue is a promising strategy that should be further investigated.

### **Discussion**

In the last decade, there have been incredible advances in understanding the molecular mechanisms that occur after stroke and the role of astrocyte reactivity underlying this process. Indeed, the global understanding of the pathogenic and neuroprotective networks in the astrocyte has become a primary need for the development of new therapeutic approaches. We have observed that astrocyte heterogeneity separates reactive astrocyte populations in neurotoxic and neuroprotective astrocytes, in which different pathways are activated and cytokines are released. However, both phenotypes should not be considered as separated populations, but a spectrum of populations that will depend on the stage and severity of the disease. However, we have seen that astrocyte reactivity is essential to both delimit the injury site and release anti-inflammatory mediators. Besides, it must be taken into consideration that astrocyte responses are the result of complex activation cascades and other cell activation states including microglia. Thereby, strategies focusing on specific key targets for neuroprotection will be fundamental to

stroke and neurodegenerative disease therapy. The balance between the pro-inflammatory and neuroprotective states will be critical in the progression of the disease. Thus, further studies are needed to determine the potential of astrocyte modulation and develop clinically successful neuroprotective strategies.

## Acknowledgements

This work has been supported by Deutsche Forschungsgemeinschaft (SCHE 2078/2-1). Förderverein für frühgeborene Kinder an der Charité e.V.

## Author Contributions Statement

AE has been the responsible for writing the manuscript. MR has been involved in drafting the manuscript and revising it critically for important intellectual content. TS contributed to the final approval of the version to be published and acquisition of funding.

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