Title
Astrocyte Reactivity: Subtypes, States, and Functions in CNS Innate Immunity

Journal
Trends in Immunology, 41(9)

ISSN
1471-4906

Author
Sofroniew, Michael V

Publication Date
2020-09-01

DOI
10.1016/j.it.2020.07.004

Peer reviewed

HHS Public Access

Author manuscript

Trends Immunol. Author manuscript; available in PMC 2021 September 01.

Published in final edited form as:

Trends Immunol. 2020 September ; 41(9): 758–770. doi:10.1016/j.it.2020.07.004.

Astrocyte reactivity: Subtypes, states and functions in CNS innate immunity

Michael V Sofroniew[1]

[1]Department of Neurobiology, David Geffen School of Medicine, University of California, Los Angeles, CA 90095, USA

Abstract

Astrocytes are neural parenchymal cells that ubiquitously tile the central nervous system (CNS). In addition to playing essential roles in healthy tissue, astrocytes exhibit an evolutionarily ancient response to all CNS insults referred to as astrocyte reactivity. Long regarded as passive and homogenous, astrocyte reactivity is being revealed as a heterogeneous and functionally powerful component of mammalian CNS innate immunity. Nevertheless, concepts about what astrocyte reactivity comprises and what it does are incomplete and sometimes controversial. Here, we discuss the goal of differentiating reactive astrocyte subtypes and states based on composite pictures of molecular expression, cell morphology, cellular interactions, proliferative state, normal functions and disease-induced dysfunctions. We present a working model and conceptual framework for characterizing astrocyte reactivity in its diversity.

Astrocyte diversity in health and disease

Astrocytes are **glial cells** (sees Glossary) of neural progenitor origin that contiguously tile the entire mammalian central nervous system (CNS) where they constitute one of the most abundant cell populations and provide multiple activities essential for CNS functions in health and disease [1]. Astrocytes interact with both neural and non-neural cells, including neurons and their synapses, **oligodendrocytes**, **oligodendrocyte progenitor cells (OPC)**, **microglia**, various peri-vascular cells, **meningeal fibroblasts** and circulating immune cells. In heathy CNS tissue, astrocytes maintain homeostasis of extracellular fluids, ions and transmitters [2], provide glucose metabolites as energy substrates to neurons [3], modulate local blood flow [4], help regulate drainage of interstitial fluid [5], play essential roles in synapse development and plasticity [6], and exhibit dynamic activities crucial for neural circuit function, neurological function and behavior [7].

In addition to their functions in healthy CNS, astrocytes exhibit an evolutionarily ancient response to CNS injury and disease commonly referred to as astrocyte reactivity that was long regarded as homogeneous and functionally passive [1, 8–10]. Instead, over the last

Correspondence to MVS: sofroniew@mednet.ucla.edu.

twenty-five years, experimental studies using causation-testing transgenic manipulations and other technologies have revealed that astrocyte reactivity can lead to a diverse set of potential changes in astrocyte morphology, molecular expression and functions that can powerfully influence outcomes in all types of CNS disorders including traumatic injury [11–13], autoimmune inflammation [14–18], microbial infections [19–21], tumor formation [22, 23], exposure to environmental toxins [24, 25], peripheral metabolic disorders [26] and neurodegenerative diseases [27–29]. An extensive body of literature shows that reactive astrocytes can respond to diverse molecular signals that derive from many cell types including neurons, other glia, local stromal cells, microbes, serum proteins, as well as blood borne immune cells and molecules, from metabolic disorders in other tissues (Figure 1A); in response to such non-cell autonomous stimuli, astrocytes can produce a multitude of molecular signals that can influence many different neural and non-neural cell types including cells involved in innate immune responses (Figure 1B) (reviewed in detail elsewhere [1, 8, 9, 20–23, 30–32]).

The identification of diverse astrocyte roles in both health and disease has fueled interest in the possibility that different astrocyte subtypes may exist that exert different functions. The concept of astrocyte heterogeneity is not new. Over a hundred years ago, classical neuroanatomists described various morphological subtypes in healthy CNS that are common across mammals, including bushy astrocytes in grey matter, fibrous astrocytes in white matter, border-forming **glia limitans astrocytes** along meninges and many others [2]. Recent and ongoing studies are expanding information about structural, genetic and functional diversity of astrocytes across the healthy CNS [33–41] and are beginning to identify its developmental origins [42, 43]. In addition, there is a steadily growing interest in characterizing the diversity of astrocyte reactivity and understanding how it is regulated. In this regard, information obtained with different methodologies is accumulating rapidly and there is a need for guidelines on assimilating this information. Here, we present a working model and conceptual framework for categorizing diverse forms of astrocyte reactivity, consistent with current information, as discussed below; the model recognizes and encompasses the need for flexibility for future expansion and modification as information accrues (Key Figure, Figure 2).

Concepts of cell subtypes and states

Advances in RNA sequencing and proteomics are enabling detailed assessment of molecular expression differences among cells. Nevertheless, it should be emphasized that meaningful definitions of differences in reactive astrocyte subtypes or states cannot be based solely on differences in molecular expression, but should be based on composite pictures that also include information about cell morphology, proliferation, molecular expression, functions, and cellular interactions. From a technical perspective it is also noteworthy that astrocytes are exquisitely sensitive to being isolated and rapidly undergo molecular changes associated with reactivity. If not controlled for, technically-induced artifactual changes can markedly skew or confound interpretation of molecular expression data. Here, with these points in mind, we refer to different reactive astrocyte **subtypes** as cells with permanent fundamental differences in the aforementioned composite ‘pictures’. In contrast, we refer to differences in astrocyte reactivity **states** as temporary changes in molecular expression and function that

do not alter basic cell features and can progressively change or be reversible over time. Comparable examples among neurons would be that of hippocampal pyramidal neurons and cerebellar Purkinje neurons, representing distinct neuronal subtypes, and each of these might exhibit state changes such as long term potentiation (LTP) or long term depression (LTD). Analogous concepts are needed, but do not yet exist for astrocytes or reactive astrocytes.

Reactive astrocyte subtypes

Concepts of what might constitute reactive astrocyte subtypes are not well developed and We posit that there is a need to establish criteria for discriminating amongst them. Considerable information is available from decades of structural and cell biological studies that should not be neglected. The first clues for distinguishing among different mammalian reactive astrocytes subtypes derived from examining their structure, proliferative state, the types of cells they interacted with, and the tissue architecture to which they contributed [1, 30, 44]. Based on these criteria, we propose that at least two fundamentally different subtypes can be readily discriminated at this time: (i) astrocytes that are newly proliferated and organized into a new and permanent tissue architecture that forms borders around areas of overt tissue damage or inflammation (Key Figure, Figure 2C), and (ii) astrocytes that do not proliferate and retain the basic cell structure, tissue architecture, and functional interactions they exhibited in healthy tissue (Key Figure, Figure 2D). Basic features of these two broad subtypes can be summarized as follows:

i. **Proliferative, border-forming reactive astrocytes** surround damaged tissue that contains leukocyte infiltration, stromal-cell proliferation and fibrosis after trauma, ischemia, infection, autoimmune inflammation, toxin accumulation, blood-brain barrier (BBB) leak or neurodegenerative disease (Key Figure, Figure 2C) [1, 8, 11, 29, 32]. These new astrocyte borders separate and isolate damaged, inflamed and fibrotic tissue from adjacent viable neural tissue in a manner analogous to astrocyte limitans borders that separate neural from non-neural tissue along the meninges in healthy CNS [32]. Loss-of-function studies from multiple laboratories show that border-forming reactive astrocytes interact with non-neural stromal and immune cells, which they attract, instruct and corral (reviewed elsewhere [13, 32]). Border-forming astrocytes are essential for reforming the BBB around lesions and for protecting and preserving adjacent functional neural tissue, such that loss or attenuation of these cells leads to increased spread of inflammation and serum proteins, increased loss of neural tissue, and decreased functional recovery in rodents [11, 12, 32, 45]. New astrocyte borders and reorganized tissue architecture are essentially permanent [32]. Newly proliferated astrocytes can derive from different progenitor sources, including proliferation of local astrocytes [13, 46–48] and periventricular neural progenitors [45]. At present, it appears that proliferation of reactive astrocytes is associated primarily with border formation and that most border forming astrocytes around CNS lesions are newly proliferated [13, 32]. Nevertheless, the degree to which reactive astrocyte proliferation may or may not occur in other contexts is not clear and is not well-studied. Because astrocytes divide rarely in healthy tissue [13], proliferation is an important means of discriminating

different subtypes of reactive astrocytes, those that proliferate, and those that do not.

ii. **Non-proliferative reactive astrocytes** maintain their locations and fundamental features of cell structure, cellular interactions and functions in neural tissue that is not overtly damaged and retains its tissue architecture, but nonetheless responds to injury or disease (Key Figure, Figure 2D) [1, 8, 49]. Depending on the nature and severity of the insult, non-proliferative reactive astrocytes exhibit variable changes in molecular expression [30, 50] and variable degrees of cellular hypertrophy [1]. Notably, the discrete, non-overlapping cellular domains exhibited by astrocytes in healthy grey matter are preserved such that hypertrophy occurs primarily within these individual cellular domains [51]. Thus, non-proliferative reactive astrocytes are likely to continue functionally interacting with the same cellular elements that they interact with in healthy tissue, including for example, neurons, synapses and blood vessels. In support of this notion, reactive astrocytes in rodents are reported to promote synapse recovery after axonal injury via Stat3-dependent mechanisms (*(GFAP)-Cre/Stat3-loxP* conditional knockout mice) [52]; moreover, loss of functions via atypical reactive astrocytes has been associated with seizure genesis following repetitive mild head trauma [53]; also, low glutathione concentrations -- which can occur in stroke -- can dysregulate astrocyte-mediated vasodilation [54]. Nevertheless, in spite of these few early studies, surprisingly little is currently known about how different forms of non-proliferative astrocyte reactivity alter essential astrocyte functions. As discussed above, in healthy CNS, astrocytes interact with many cell types and exert a multitude of activities crucial for normal CNS function. Dissecting the adaptative and maladaptive effects of non-proliferative astrocyte reactivity after different types of CNS insults represents a large and important challenge for future studies.

These two broad categories of proliferative border-forming astrocytes or non-proliferative hypertrophic reactive astrocytes should not be considered an exhaustive characterization of potential reactive astrocyte subtypes. As available information grows, additional subtypes, or subdivisions of these two are likely to emerge. It is recommended that classifying different subtypes be based on composite pictures that take into account multiple forms of information about differences in astrocyte morphology, cell lineage, proliferative state, molecular expression, cellular interactions and functions. Defining such differences is an important goal for future research. This working model and framework (Key Figure, Figure 2) is intended as a beginning categorization to be expanded upon.

A brief comment on nomenclature. There is a widespread practice of referring to reactive astrocytes as ‘scars’ (glial scar, astroglial scar and so forth). As our understanding of adaptive reactive astrocyte functions grows, we advocate that it is time to move on from this outmoded practice which is increasingly out of synchrony with definitions of scar tissue in other tissues (Box 1).

Astrocyte reactivity states

The potential changes in molecular expression that can be undergone by reactive astrocytes is now being revealed as remarkably broad and heterogeneous across disorders and tissue regions, and fluid over time during disorders [23, 34, 50, 55–61]. For example, reactive astrocytes associated with stroke [50], LPS injection [50], traumatic brain injury [57], autoimmune disease (e.g. the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS)/MS) [17, 61], or different neurodegenerative disease models from mice and/or humans [58, 60, 62, 63], all exhibit transcriptome changes that vary substantially both in the number and types of genes whose expression change. Significant differences in transcriptome changes can also be observed (i) at different stages during the same disorder, for example at different times after stroke [50] or autoimmune attack (EAE) [61], and (ii) among different cohorts of locally intermingled reactive astrocytes in the same tissue samples evaluated by single cell or single nuclei RNA profiling [61, 63]. Notably, in many of the above cases, transcriptome differences appear to occur among reactive astrocytes that do not exhibit obvious differences in other features such as cell morphology, proliferation or cellular interactions. Hence there is a need for conceptually evaluating changes in reactive astrocyte molecular expression and function; these would be plastic, context-dependent, and can change over time without changing other basic cell characteristics (Key Figure, Figure 2B–E). When such changes and differences are observed among reactive astrocytes, it seems more appropriate to refer to them as differences in reactive astrocyte states rather than to regard the myriad and highly nuanced molecular differences among reactive astrocytes as being indicative of different subtypes. Such changes in reactive astrocyte states may be finely graded and directly continuous with diverse functional states observed in healthy tissue (Key Figure, Figure 2A).; they may evolve over time after or during disorders; and they may in certain instances, resolve over time, if triggering cues are removed (Key Figure, Figure 2B–E). In this regard, it may be useful to regard differentially expressed genes and other molecular changes as interchangeable tools used by the same astrocyte subtypes as required in different contexts and induced by different triggers, rather than as markers of different astrocyte subtypes. Thus, reactive astrocytes that do not change their basic subtype may exhibit substantially different changes in gene expression and cell function over time during the same disorder, or in response to different disorders. Understanding the potential for diversity and subtilty of different astrocyte reactivity states is only beginning and is an important topic for future research.

Molecular signatures and nomenclature

Large scale molecular screening studies are revealing different molecular expression profiles of reactive astrocytes in different disorders based either on bulk analyses of whole tissue samples, or on single cell analyses [17, 23, 34, 50, 55–61, 63]. Such observations raise questions about whether there are consistent patterns of molecular expression that might serve as ‘signatures’ for particular astrocyte functional subtypes or states and what type of nomenclature might be appropriately applied. For example, in some single-cell molecular analyses, astrocyte clusters most prevalent in baseline healthy tissue are referred to as ‘homeostatic’, and astrocytes in diseased tissue that are most different from ‘homeostatic’ astrocytes, are referred to as ‘disease-associated’ [63]. Caution is urged with regards to this

practice. First, astrocytes in healthy CNS exhibit multiple different molecular expression patterns that are associated with homeostatic functions [36]. Second, assigning the term 'homeostatic' only to astrocytes in healthy tissue implies that reactive astrocytes are by definition 'nonhomeostatic', which can be misleading because disease-associated changes undergone by certain reactive astrocytes can exert homeostatic activities that preserve BBB function [64], tissue integrity [32], and neurological functions [65]. Third, there is already substantial evidence that there are multiple molecular expression patterns associated with reactive astrocytes in different disorder contexts rather than a single 'disease-associated' pattern [17, 23, 34, 50, 55–61, 63]. Caution is also urged with regards to assigning functional attributes to molecular 'signatures' purely on the basis of correlative association. For example, early transcriptome profiling studies identified an 'A1' molecular signature of 12 genes associated with a neurotoxic astrocyte subtype that emerged after exposure to specific cytokines secreted by microglia exposed to lipopolysaccharide (LPS), and an 'A2' molecular signature of 12 genes associated with a neuroprotective subtype after ischemic stroke [50, 66]. Notably, the assignment of these two proposed molecular signatures to two potential reactive astrocyte subtypes with different functions was based entirely correlative based on evidence, and the functions of all of these genes are not known; in addition, to date, no causation testing loss- or gain-of-function experiments have directly linked any of these proposed marker genes to either 'toxic' or 'protective' functions of reactive astrocytes. Thus, the predictive value of these proposed marker genes as 'signatures' to identify specific astrocyte functional subtypes or states is untested and any attempts to correlate expression of some or all of these markers with potential 'toxic' or 'protective' astrocyte functions in different contexts is speculative and potentially misleading. In line with this uncertainty, studies thus far have found few, and in some cases none, of the 'A1' marker genes detectably expressed in various CNS neurodegenerative disorders or models including Huntington's disease (HD) [58, 60], amyotrophic lateral sclerosis (ALS) [55], Alzheimer's disease (AD) [56, 67], prion disease [68] and glioblastoma [23]. At present, to avoid such issues, it seems most appropriate to label molecularly-related astrocyte clusters neutrally, followed by experimental causation-testing dissection of effects attributable to specific molecules [61].

Reactive astrocyte adaptive functions

Astrocyte reactivity was first recognized over 120 years ago. For decades it was regarded as a simple stereotypic response of little interest beyond being a marker of past or ongoing tissue pathology; and functional speculations were long centered around potential detrimental effects of 'glial scars' (but see Box 1) [1, 69]. The advent of transgenic technologies allowed causation-testing loss-of-function experiments to probe reactive astrocyte functions *in vivo* in specific injury or disease models. Early studies yielded surprises and unexpectedly revealed that genetically targeted ablation of proliferative border-forming reactive astrocytes in mice led to increased spread of inflammation, increased neuronal degeneration, failure of BBB repair and reduced recovery of certain neurological functions [11, 12]. Subsequent studies have confirmed and built on these observations. Although a detailed account is beyond the scope of this article, briefly, there are now numerous loss-of-function studies from multiple laboratories showing that transgenically attenuating astrocyte reactivity via selective deletion of a wide variety of different types of

molecules, including membrane receptors, transcriptional regulators, or effector molecules, can exacerbate tissue injury and worsen functional outcome in models of essentially all forms of CNS disorders, including infection [19], traumatic injury [65], autoimmune attack [14, 15, 70]; stroke [45, 71]; and multiple degenerative disease models [28, 72–75] as reviewed in detail elsewhere [31, 32]. Moreover, recent evidence suggests that reactive astrocytes can contribute to synapse remodeling and circuit reorganization [6, 33, 37], and even the longstanding view that reactive astrocytes are the primary cause for the failure CNS axon regeneration has been overturned [57, 69, 76, 77]. Together, these findings support the concept that in multiple disorder contexts, normal reactive astrocytes can contribute adaptive functions that help preserve neural tissue and maintain neurological functions. Nevertheless, how reactive astrocytes implement the broad adaptive effects that are revealed by loss-of-function experiments is poorly defined; therefore, dissecting the cellular and molecular mechanisms that mediate reactive astrocyte effects in specific disorder contexts represents a key goal for future study.

Reactive astrocyte maladaptive effects

Reactive astrocytes also have the potential to contribute to maladaptive effects in various ways through loss- or gain-of-functions that occur in response to non-cell-autonomous reactivity-driving signals. As described above, in healthy CNS tissue, astrocytes exert many essential functions that are crucial for neural circuit function, neurological function and behavior [7]. The degree to which essential astrocyte functions might be attenuated or lost by reactive astrocytes and thereby cause detrimental effects in different disorder contexts is poorly understood and represents yet another important goal for future study. In this regard, a reduced proliferative response of reactive astrocytes in the aged brain can contribute to age-related vulnerability to traumatic injury in mice [78], and this is interesting as it might also contribute to increased vulnerability to aging-related neurodegenerative conditions [29]. As a counterpoint to the loss of beneficial astrocyte functions, there is a potential for reactive astrocytes to exert detrimental or maladaptive effects through inappropriate gain-of-functions, which might be driven by chronic exposure to reactivity triggers (Key Figure, Figure 2F,G). For example, transgenically-targeted molecular deletion studies show that attenuation of pro-inflammatory signaling mediated by NFkB, VEGFA or Ccl2 in reactive astrocytes, can improve neurological outcome during experimental autoimmune inflammation in transgenic mice [79–81]. Furthermore, mounting evidence shows that chronic astrocyte exposure to reactivity triggers during autoimmune or neurodegenerative disorders, can help drive excessive inflammation [32, 61]. In these contexts, astrocyte reactivity may be regarded in the same light as inflammation, i.e. normal astrocyte reactivity, similarly to normal inflammation, is an adaptive response to CNS injury and disease that can be considered an essential component of CNS innate immunity. However, under certain circumstances, chronic astrocyte reactivity, like chronic inflammation, may benefit from attenuation. From another angle, the opposite may also be true, such that in some cases it may be important to augment pro-inflammatory signaling and attenuate anti-inflammatory signaling by reactive astrocytes. For example, it is well-documented that reactive astrocytes can control and limit the spread of inflammation after traumatic injury, stroke and other conditions, particularly through Stat3-mediated signaling [11, 32, 65]. However, in the

context of tumor formation, recent studies in mice show that reactive astrocytes may be co-opted by tumor cells to inappropriately suppress local inflammation via Stat3-mediated mechanisms and thereby, aid tumor growth and invasive spread [22, 23, 82, 83]. Stat3-mediated astrocyte suppression of local inflammatory responses and phagocytosis may also reduce amyloid clearance in mouse models of AD-like amyloid toxicity [84, 85]. Thus, reactive astrocyte dysfunctions and detrimental effects are regulated in very specific ways by definable molecular signaling events that are highly context specific. Beneficially modulating specific reactive astrocyte functions will thus require a detailed understanding of specific mechanisms that operate in different contexts, and which might potentially involve the induction of opposite effects for different scenarios.

Discriminating astrocyte disease states from normal reactivity states

As outlined above, astrocyte reactivity should in the first instance be regarded as an ancient, conserved and normal physiological response that evolved to protect neural tissue, maintain tissue homeostasis and preserve neurological functions after diverse insults. When trying to understand disease mechanisms, normal reactive astrocyte subtypes and states should not be conflated with disease states that are caused by cell-autonomous astrocyte dysfunctions, which can contribute to neuronal dysfunction and neurodegeneration either prior to astrocyte reactivity or by inducing abnormal reactivity states (Key Figure, Figure 2G,H). For example, gene mutations or polymorphisms in HD [58, 86], familial ALS [55], Alexander disease [87], AD with **APOE4** polymorphism [56], infections with prions [68] or viruses [88], or exposure to environmental toxins [25], can all directly lead to disease-induced cell-autonomous astrocyte dysfunctions that worsen the outcomes of disorders or disorder models. Such cell-autonomous astrocyte dysfunctions can begin before detectable evidence of astrocyte reactivity (Key Figure, Figure 2H), as seen for example in a mouse model of HD, where early dysregulation of extracellular K^+ homeostasis contributed to neuronal hyperexcitability and symptom onset [86]. Alternatively, disease-induced cell-autonomous astrocyte dysfunctions can, in a disorder specific manner, either blunt, amplify, or change normal astrocyte reactivity responses, e.g. resulting in loss of essential astrocyte functions and increased neurodegeneration [56], gain of detrimental astrocyte effects and excess inflammation [25], or seizure genesis [88] im mouse or zebrafish experimental models. It is important to distinguish normal astrocyte reactivity from such astrocyte disease states (Key Figure, Figure 2G). Historically there has been a widespread misbelief that normally occurring astrocyte reactivity is a maladaptive event that *per se*, causes tissue damage and should be globally attenuated. There is no evidence for this misbelief. Instead, there is a need to differentiate physiologically adaptive astrocyte reactivity from disease-induced astrocyte dysfunctions, and to target dysfunctions in a context specific manner.

Reactive astrocytes and CNS innate immunity

As functions of reactive astrocytes are elucidated, cumulative evidence points towards reactive astrocytes as integral and essential components of multicellular CNS innate immunity. Astrocytes have long been recognized to respond to activators of innate immune responses [89], including all forms of microbial pathogens [20, 21], environmental toxins [24, 25] and diverse forms of tissue damage as discussed above (Figure 1A). Astrocytes in

healthy tissue express many of the receptors to **PAMPs** and **DAMPs** known to trigger innate immune responses [90] (Figure 3), in particular **TLRs**, including TLR4 [90], which is sometimes erroneously attributed only to microglia. Astrocytes exchange molecular signals and interact directly with other innate immune cells such as microglia [91], various perivascular cells and blood borne leukocytes [8, 32]. Astrocytes n both elaborate and respond to a wide variety of cytokines, chemokines, growth factors and other molecules used for inter-cellular communication during innate immune responses, as discussed above (Figure 1). Reactive astrocytes can attract, instruct and corral inflammatory and immune cells (reviewed in detail elsewhere [32]). Multiple genetic deletion studies show that attenuating astrocyte reactivity disrupts CNS innate immune responses and leads to the spread of infection or inflammation [32], and CNS regional differences in astrocytes can contribute to regional differences in predisposition to West Nile virus infection in mice [92].

An interesting emerging role for astrocytes in innate immunity is their participation in the formation of **adventitial** (**perivascular**) **cuffs** that harbor foci of activated immune cells [14, 32, 93]. Adventitial cuffing is emerging as important aspect of immune responses within host tissues across multiple organs as platforms for tissue resident immune memory and tertiary lymphoid organs [94]. In CNS, adventitial cuffing is well recognized in certain microbial infections and autoimmune inflammation, and astrocytes have long been recognized in borders around such perivascular cuffs in humans and experimental rodent models [1]. Transgenically targeted experimental disruption of astrocyte border formation leads to spread of inflammatory cells away from perivascular cuffs into the neural parenchyma resulting in loss of neural tissue in mice [14, 32]. Mechanistically, astrocyte processes form tight junctions via proteins claudin 1 and 4, which restrict or corral immune cells within the perivascular space when blood-borne leukocytes cross the endothelial BBB to gain entry into perivascular spaces in human co-culture models [93]. Astrocytes can also produce multiple cytokines and chemokines capable of recruiting and instructing lymphocytes, suggesting that astrocyte borders around such cuffs may serve to attract and then restrict the spread of blood borne immune and inflammatory cells [32, 81]. Adventitial cuffs of antibody-producing B cells are formed during CNS viral infections, and local antibody production represents an important means of clearing viral infections that is regulated at least in part, by INFγ and IL10 signaling in mouse models of alphaviral encephalomyelitis infection [95–97]. Reactive astrocytes can produce both INFγ and IL10 as well as other B cell attracting chemokines (Figure 1B) [32] and it will be interesting to study potential astrocyte roles in this process. Another relevant aspect of astrocyte reactivity might involve priming to alter innate immune responses to future potential insults as part of what is becoming known as innate immune memory that can be orchestrated by chromatin modification and epigenetic reprogramming [98]. Thus, multiple new roles for astrocytes are emerging in multicellular CNS innate immune responses that merit further investigation.

Concluding remarks

Reactive astrocytes are essential components of multicellular CNS innate immunity that respond to all forms of CNS injury and disease. There is mounting interest in strategies to manipulate astrocyte reactivity in CNS disorders (Box 2). Doing so will require a detailed understanding of what astrocyte reactivity is, what it does and how it is regulated under

varying conditions (see outstanding questions). Accumulating evidence argues that astrocyte reactivity cannot be parsed into a few broad and stereotypic programs that are either beneficial or detrimental to the host. Instead, multiple findings point towards reactive astrocytes as being able to adopt a broad spectrum of nuanced changes that are disorder and context specific. There is a growing need to develop a detailed understanding of how specific astrocyte functions change during different disorders and how this may vary in different tissue regions. This knowledge is required to be able to develop evidence-based therapeutic approaches that might beneficially modulate astrocyte reactivity in a disorder- and context-specific manner.

Acknowledgements

Work in the author's laboratory is supported by US National Institutes of Health (NS084030); the Dr. Miriam and Sheldon G. Adelson Medical Foundation; Paralyzed Veterans Foundation of America; and Wings for Life. The author declares no conflicts of interest.

Glossary:

Adventitial cuffs
Perivascular spaces rich in extracellular matrix; harbor collections of tissue resident immune cells. In CNS, surrounded by corralling astrocyte cell processes

APOE4
Apolipoprotein E , member of a f mily of fat-binding proteins; involved in cholesterol metabolism. The human *APOE* allele 4 (*APOE4*) is a polymorphic variant of the *APOE* gene, associated with an increased risk of developing Alzheimer's disease

Astrocyte
Neural parenchymal cells derived from neural ectoderm and neural progenitor cells; execute multiple functions crucial for neural circuit activity and neurological functions in health and disease

Astrocyte reactivity
Diverse changes in molecular expression, cell structure, functions and intercellular interactions undergone by astrocytes in response to all forms of CNS insults

Astrocyte borders
Limitans borders formed by newly proliferated astrocytes around scar tissue formed by mesenchymal cells and fibrotic extracellular matrix that replaces CNS parenchymal cells lost to injury or disease. Astrocyte scar-borders delineate neural from non-neural tissue similar to astrocyte limitans borders along all surfaces to the meninges

States
Defined here as changes in molecular expression and cellular function that do not alter basic cell features or subtype, and may be temporary and/or progressively change over time

DAMP

Damage- or **D**anger-**A**ssociated **M**olecular **P**atterns, molecular motifs associated with cellular injury and tissue damage; recognized by TLRs and other receptors; trigger innate immune signaling and activation

Fibrosis
Deposition of extracellular matrix, including collagens, proteoglycans, fibronectins and other molecules secreted by diverse scar-forming cells

Glial cells
CNS parenchymal cells, resident throughout neural tissue; provide multiple essential functions that enable neural circuit activity and neurological functions. CNS macroglia, astrocytes and oligodendroglia, derive from neural ectoderm and neural progenitor cells. CNS microglia are of non-neural origin and derive from the yolk-sac

Glia limitans astrocytes
interface directly with fibroblast-lineage cells of the pia mater of the meninges around the entire CNS and thereby delimit neural from non-neural tissue

Mesenchymal or stromal scar
Scar tissue formed by non-neural stromal cells including pericytes, perivascular fibroblasts, meningeal fibroblasts, fibrocytes and inflammatory cells, and fibrotic extracellular matrix

Meningeal fibroblasts
innermost layer of the meningeal covering of the CNS, the pia matter; composed of fibroblast-lineage cells interfacing directly with astrocytes that form the glia limitans

Microglia
CNS resident non-neural glia that share many properties with macrophages but are not of bone marrow origin and instead derive from the yoke sack and populate the CNS during development

Oligodendrocyte
Glial cells that form myelin sheaths in the CNS

Oligodendrocyte progenitor cells (OPC)
Precursor cells that proliferate to replace oligodendrocytes during normal turnover in the healthy CNS, and also proliferate after CNS injury

PAMP
Pathogen-**A**ssociated **M**olecular **P**atterns , small molecular motifs associated with microbes, recognized by TLRs and other receptors ; trigger innate immune signaling and activation

Subtypes
defined here as cells with permanent fundamental differences in a composite picture of cell structure and morphology, molecular expression, functions, and cellular interactions; permanent or long-lasting, and distinguish different types of cells

TLR

Toll-**L**ike **R**eceptors : family of cell membrane receptors recognizing molecules that can trigger innate immune signaling and activation

References

1. Sofroniew MV and Vinters HV (2010) Astrocytes: biology and pathology. Acta Neuropathol 119 (1), 7–35. [PubMed: 20012068]
2. Verkhratsky A and Nedergaard M (2018) Physiology of Astroglia. Physiol Rev 98 (1), 239–389. [PubMed: 29351512]
3. Magistretti PJ and Allaman I (2018) Lactate in the brain: from metabolic end-product to signalling molecule. Nat Rev Neurosci 19 (4), 235–249. [PubMed: 29515192]
4. MacVicar BA and Newman EA (2015) Astrocyte regulation of blood flow in the brain. Cold Spring Harb Perspect Biol 7 (5).
5. Plog BA and Nedergaard M (2018) The Glymphatic System in Central Nervous System Health and Disease: Past, Present, and Future. Annu Rev Pathol 13, 379–394. [PubMed: 29195051]
6. Allen NJ and Eroglu C (2017) Cell Biology of Astrocyte-Synapse Interactions. Neuron 96 (3), 697–708. [PubMed: 29096081]
7. Khakh BS (2019) Astrocyte-Neuron Interactions in the Striatum: Insights on Identity, Form, and Function. Trends Neurosci 42 (9), 617–630. [PubMed: 31351745]
8. Burda JE and Sofroniew MV (2014) Reactive gliosis and the multicellular response to CNS damage and disease. Neuron 81 (2), 229–48. [PubMed: 24462092]
9. Pekny M and Pekna M (2014) Astrocyte Reactivity and Reactive Astrogliosis: Costs and Benefits. Physiol Rev 94 (4), 1077–1098. [PubMed: 25287860]
10. Lu TY et al. (2017) Axon degeneration induces glial responses through Draper-TRAF4-JNK signalling. Nature Communications 8.
11. Bush TG et al. (1999) Leukocyte infiltration, neuronal degeneration, and neurite outgrowth after ablation of scar-forming, reactive astrocytes in adult transgenic mice. Neuron 23 (2), 297–308. [PubMed: 10399936]
12. Faulkner JR et al. (2004) Reactive astrocytes protect tissue and preserve function after spinal cord injury. J. Neurosci 24, 2143–2155. [PubMed: 14999065]
13. Wanner IB et al. (2013) Glial scar borders are formed by newly proliferated, elongated astrocytes that interact to corral inflammatory and fibrotic cells via STAT3-dependent mechanisms after spinal cord injury. J Neurosci 33 (31), 12870–86. [PubMed: 23904622]
14. Voskuhl RR et al. (2009) Reactive astrocytes form scar-like perivascular barriers to leukocytes during adaptive immune inflammation of the CNS. J. Neurosci 29 (37), 11511–11522. [PubMed: 19759299]
15. Haroon F et al. (2011) Gp130-dependent astrocytic survival is critical for the control of autoimmune central nervous system inflammation. J Immunol 186 (11), 6521–31. [PubMed: 21515788]
16. Spence RD et al. (2011) Neuroprotection mediated through estrogen receptor-{alpha} in astrocytes. Proc Natl Acad Sci U S A 108 (21), 8867–72. [PubMed: 21555578]
17. Itoh N et al. (2018) Cell-specific and region-specific transcriptomics in the multiple sclerosis model: Focus on astrocytes. Proc Natl Acad Sci U S A 115 (2), E302–E309. [PubMed: 29279367]
18. Wheeler MA and Quintana FJ (2019) Regulation of Astrocyte Functions in Multiple Sclerosis. Cold Spring Harb Perspect Med 9 (1).
19. Drogemuller K et al. (2008) Astrocyte gp130 expression is critical for the control of Toxoplasma encephalitis. J. Immunol 181 (4), 2683–2693. [PubMed: 18684959]
20. Soung A and Klein RS (2018) Viral Encephalitis and Neurologic Diseases: Focus on Astrocytes. Trends Mol Med 24 (11), 950–962. [PubMed: 30314877]
21. Geyer S et al. (2019) Immunity Against Bacterial Infection of the Central Nervous System: An Astrocyte Perspective. Front Mol Neurosci 12, 57. [PubMed: 30894799]
22. Priego N et al. (2018) STAT3 labels a subpopulation of reactive astrocytes required for brain metastasis. Nat Med 24 (7), 1024–1035. [PubMed: 29892069]

23. Henrik Heiland D et al. (2019) Tumor-associated reactive astrocytes aid the evolution of immunosuppressive environment in glioblastoma. Nat Commun 10 (1), 2541. [PubMed: 31186414]
24. O'Callaghan JP et al. (2014) Early Activation of STAT3 Regulates Reactive Astrogliosis Induced by Diverse Forms of Neurotoxicity. PLoS One 9 (7), e102003. [PubMed: 25025494]
25. Wheeler MA et al. (2019) Environmental Control of Astrocyte Pathogenic Activities in CNS Inflammation. Cell 176 (3), 581–596 e18. [PubMed: 30661753]
26. Rao KV et al. (2005) Astrocytes protect neurons from ammonia toxicity. Neurochem. Res 30 (10), 1311–1318. [PubMed: 16341593]
27. Shao W et al. (2013) Suppression of neuroinflammation by astrocytic dopamine D2 receptors via alphaB-crystallin. Nature 494 (7435), 90–4. [PubMed: 23242137]
28. Kraft AW et al. (2013) Attenuating astrocyte activation accelerates plaque pathogenesis in APP/PS1 mice. FASEB J 27 (1), 187–98. [PubMed: 23038755]
29. Katsouri L et al. (2020) Ablation of reactive astrocytes exacerbates disease pathology in a model of Alzheimer's disease. Glia 68 (5), 1017–1030. [PubMed: 31799735]
30. Sofroniew MV (2009) Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci. 32 (12), 638–647. [PubMed: 19782411]
31. Sofroniew MV (2015) Astrogliosis. Cold Spring Harb Perspect Biol 7 (2), a020420.
32. Sofroniew MV (2015) Astrocyte barriers to neurotoxic inflammation. Nat Rev Neurosci 16 (5), 249–63. [PubMed: 25891508]
33. Khakh BS and Sofroniew MV (2015) Diversity of astrocyte functions and phenotypes in neural circuits. Nat Neurosci 18 (7), 942–52. [PubMed: 26108722]
34. Zhang Y et al. (2016) Purification and Characterization of Progenitor and Mature Human Astrocytes Reveals Transcriptional and Functional Differences with Mouse. Neuron 89 (1), 37–53. [PubMed: 26687838]
35. Srinivasan R et al. (2016) New Transgenic Mouse Lines for Selectively Targeting Astrocytes and Studying Calcium Signals in Astrocyte Processes In Situ and In Vivo. Neuron 92 (6), 1181–1195. [PubMed: 27939582]
36. Chai H et al. (2017) Neural Circuit-Specialized Astrocytes: Transcriptomic, Proteomic, Morphological, and Functional Evidence. Neuron 95 (3), 531–549. [PubMed: 28712653]
37. Haim LB and Rowitch DH (2017) Functional diversity of astrocytes in neural circuit regulation. Nat Rev Neurosci 18 (1), 31–41. [PubMed: 27904142]
38. John Lin CC et al. (2017) Identification of diverse astrocyte populations and their malignant analogs. Nat Neurosci 20 (3), 396–405. [PubMed: 28166219]
39. Wu YE et al. (2017) Detecting Activated Cell Populations Using Single-Cell RNA-Seq. Neuron 96 (2), 313-+. [PubMed: 29024657]
40. Khakh BS and Deneen B (2019) The Emerging Nature of Astrocyte Diversity. Annu Rev Neurosci 42, 187–207. [PubMed: 31283899]
41. Batiuk MY et al. (2020) Identification of region-specific astrocyte subtypes at single cell resolution. Nat Commun 11 (1), 1220. [PubMed: 32139688]
42. Molofsky AV and Deneen B (2015) Astrocyte development: A Guide for the Perplexed. Glia 63 (8), 1320–9. [PubMed: 25963996]
43. Molofsky AV et al. (2014) Astrocyte-encoded positional cues maintain sensorimotor circuit integrity. Nature 509 (7499), 189–94. [PubMed: 24776795]
44. Anderson MA et al. (2014) Heterogeneity of reactive astrocytes. Neurosci Lett 565C, 23–29.
45. Benner EJ et al. (2013) Protective astrogenesis from the SVZ niche after injury is controlled by Notch modulator Thbs4. Nature 497 (7449), 369–73. [PubMed: 23615612]
46. Buffo A et al. (2008) Origin and progeny of reactive gliosis: A source of multipotent cells in the injured brain. Proc. Natl. Acad. Sci. USA 105 (9), 3581–3586. [PubMed: 18299565]
47. Bardehle S et al. (2013) Live imaging of astrocyte responses to acute injury reveals selective juxtavascular proliferation. Nat Neurosci 16, 580–586. [PubMed: 23542688]
48. Ren Y et al. (2017) Ependymal cell contribution to scar formation after spinal cord injury is minimal, local and dependent on direct ependymal injury. Sci Rep 7, 41122. [PubMed: 28117356]

49. Burda JE et al. (2016) Astrocyte roles in traumatic brain injury. Exp Neurol 275 Pt 3, 305–15. [PubMed: 25828533]
50. Zamanian JL et al. (2012) Genomic analysis of reactive astrogliosis. J Neurosci 32 (18), 6391–410. [PubMed: 22553043]
51. Wilhelmsson U et al. (2006) Redefining the concept of reactive astrocytes as cells that remain within their unique domains upon reaction to injury. Proc. Natl. Acad. Sci. USA 103 (46), 17513–17518. [PubMed: 17090684]
52. Tyzack GE et al. (2014) Astrocyte response to motor neuron injury promotes structural synaptic plasticity via STAT3-regulated TSP-1 expression. Nat Commun 5, 4294. [PubMed: 25014177]
53. Shandra O et al. (2019) Repetitive Diffuse Mild Traumatic Brain Injury Causes an Atypical Astrocyte Response and Spontaneous Recurrent Seizures. J Neurosci 39 (10), 1944–1963. [PubMed: 30665946]
54. Howarth C et al. (2017) A Critical Role for Astrocytes in Hypercapnic Vasodilation in Brain. J Neurosci 37 (9), 2403–2414. [PubMed: 28137973]
55. Sun SY et al. (2015) Translational profiling identifies a cascade of damage initiated in motor neurons and spreading to glia in mutant SOD1-mediated ALS. Proceedings of the National Academy of Sciences of the United States of America 112 (50), E6993–E7002. [PubMed: 26621731]
56. Sekar S et al. (2015) Alzheimer's disease is associated with altered expression of genes involved in immune response and mitochondrial processes in astrocytes. Neurobiol Aging 36 (2), 583–91. [PubMed: 25448601]
57. Anderson MA et al. (2016) Astrocyte scar formation aids central nervous system axon regeneration. Nature 532 (7598), 195–200. [PubMed: 27027288]
58. Diaz-Castro B et al. (2019) Astrocyte molecular signatures in Huntington's disease. Sci Transl Med 11 (514).
59. Schirmer L et al. (2019) Neuronal vulnerability and multilineage diversity in multiple sclerosis. Nature 573 (7772), 75–82. [PubMed: 31316211]
60. Al-Dalahmah O et al. (2020) Single-nucleus RNA-seq identifies Huntington disease astrocyte states. Acta Neuropathol Commun 8 (1), 19. [PubMed: 32070434]
61. Wheeler MA et al. (2020) MAFG-driven astrocytes promote CNS inflammation. Nature 578 (7796), 593–599. [PubMed: 32051591]
62. Kamphuis W et al. (2015) GFAP and vimentin deficiency alters gene expression in astrocytes and microglia in wild-type mice and changes the transcriptional response of reactive glia in mouse model for Alzheimer's disease. Glia 63 (6), 1036–56. [PubMed: 25731615]
63. Habib N et al. (2020) Disease-associated astrocytes in Alzheimer's disease and aging. Nat Neurosci.
64. Laug D et al. (2019) Nuclear factor I-A regulates diverse reactive astrocyte responses after CNS injury. J Clin Invest 129 (10), 4408–4418. [PubMed: 31498149]
65. Herrmann JE et al. (2008) STAT3 is a critical regulator of astrogliosis and scar formation after spinal cord injury. J. Neurosci 28 (28), 7231–7243. [PubMed: 18614693]
66. Liddelow SA et al. (2017) Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541 (7638), 481–487. [PubMed: 28099414]
67. Wu T et al. (2019) Complement C3 Is Activated in Human AD Brain and Is Required for Neurodegeneration in Mouse Models of Amyloidosis and Tauopathy. Cell Rep 28 (8), 2111–2123 e6. [PubMed: 31433986]
68. Smith HL et al. (2020) Astrocyte Unfolded Protein Response Induces a Specific Reactivity State that Causes Non-Cell-Autonomous Neuronal Degeneration. Neuron.
69. Sofroniew MV (2018) Dissecting spinal cord regeneration. Nature 557 (7705), 343–350. [PubMed: 29769671]
70. Wang X et al. (2013) Astrocytic Fas ligand expression is required to induce T-cell apoptosis and recovery from experimental autoimmune encephalomyelitis. European Journal of Immunology 43 (1), 115–124. [PubMed: 23011975]

71. Liu Z et al. (2014) Beneficial effects of gfap/vimentin reactive astrocytes for axonal remodeling and motor behavioral recovery in mice after stroke. Glia 62, 2022–33. [PubMed: 25043249]
72. Chen PC et al. (2009) Nrf2-mediated neuroprotection in the MPTP mouse model of Parkinson's disease: Critical role for the astrocyte. Proc. Natl. Acad. Sci. USA 106 (8), 2933–2938. [PubMed: 19196989]
73. Rojo AI et al. (2018) Deficiency in the transcription factor NRF2 worsens inflammatory parameters in a mouse model with combined tauopathy and amyloidopathy. Redox Biol 18, 173–180. [PubMed: 30029164]
74. Hartmann K et al. (2019) Complement 3(+)-astrocytes are highly abundant in prion diseases, but their abolishment led to an accelerated disease course and early dysregulation of microglia. Acta Neuropathol Commun 7 (1), 83. [PubMed: 31118110]
75. Oksanen M et al. (2020) NF-E2-related factor 2 activation boosts antioxidant defenses and ameliorates inflammatory and amyloid properties in human Presenilin-1 mutated Alzheimer's disease astrocytes. Glia 68 (3), 589–599. [PubMed: 31670864]
76. Anderson MA et al. (2018) Required growth facilitators propel axon regeneration across complete spinal cord injury. Nature 561 (7723), 396–400. [PubMed: 30158698]
77. Zukor K et al. (2013) Short hairpin RNA against PTEN enhances regenerative growth of corticospinal tract axons after spinal cord injury. J Neurosci 33 (39), 15350–61. [PubMed: 24068802]
78. Heimann G et al. (2017) Changes in the Proliferative Program Limit Astrocyte Homeostasis in the Aged Post-Traumatic Murine Cerebral Cortex. Cereb Cortex 27 (8), 4213–4228. [PubMed: 28472290]
79. Brambilla R et al. (2009) Transgenic inhibition of astroglial NF-kappaB improves functional outcome in experimental autoimmune encephalomyelitis by suppressing chronic central nervous system inflammation. J. Immunol 182 (5), 2628–2640. [PubMed: 19234157]
80. Argaw AT et al. (2012) Astrocyte-derived VEGF-A drives blood-brain barrier disruption in CNS inflammatory disease. J Clin Invest 122 (7), 2454–68. [PubMed: 22653056]
81. Kim RY et al. (2014) Astrocyte CCL2 sustains immune cell infiltration in chronic experimental autoimmune encephalomyelitis. J Neuroimmunol 274 (1–2), 53–61. [PubMed: 25005117]
82. Matias D et al. (2018) Microglia/Astrocytes-Glioblastoma Crosstalk: Crucial Molecular Mechanisms and Microenvironmental Factors. Front Cell Neurosci 12, 235. [PubMed: 30123112]
83. Guan X et al. (2018) Reactive Astrocytes in Glioblastoma Multiforme. Mol Neurobiol 55 (8), 6927–6938. [PubMed: 29363044]
84. Ceyzeriat K et al. (2018) Modulation of astrocyte reactivity improves functional deficits in mouse models of Alzheimer's disease. Acta Neuropathol Commun 6 (1), 104. [PubMed: 30322407]
85. Reichenbach N et al. (2019) Inhibition of Stat3-mediated astrogliosis ameliorates pathology in an Alzheimer's disease model. EMBO Mol Med.
86. Tong X et al. (2014) Astrocyte Kir4.1 ion channel deficits contribute to neuronal dysfunction in Huntington's disease model mice. Nat Neurosci 17 (5), 694–703. [PubMed: 24686787]
87. Brenner M et al. (2001) Mutations in GFAP, encoding glial fibrillary acidic protein, are associated with Alexander disease. Nat. Genet 27 (1), 117–120. [PubMed: 11138011]
88. Robel S and Sontheimer H (2016) Glia as drivers of abnormal neuronal activity. Nat Neurosci 19 (1), 28–33. [PubMed: 26713746]
89. Farina C et al. (2007) Astrocytes are active players in cerebral innate immunity. Trends Immunol. 28, 138–145. [PubMed: 17276138]
90. Gong T et al. (2019) DAMP-sensing receptors in sterile inflammation and inflammatory diseases. Nat Rev Immunol.
91. Rothhammer V et al. (2018) Microglial control of astrocytes in response to microbial metabolites. Nature 557 (7707), 724–728. [PubMed: 29769726]
92. Daniels BP et al. (2017) Regional astrocyte IFN signaling restricts pathogenesis during neurotropic viral infection. J Clin Invest 127 (3), 843–856. [PubMed: 28134626]
93. Horng S et al. (2017) Astrocytic tight junctions control inflammatory CNS lesion pathogenesis. J Clin Invest 127 (8), 3136–3151. [PubMed: 28737509]

94. Dahlgren MW and Molofsky AB (2019) Adventitial Cuffs: Regional Hubs for Tissue Immunity. Trends Immunol 40 (10), 877–887. [PubMed: 31522963]

95. Metcalf TU et al. (2013) Recruitment and retention of B cells in the central nervous system in response to alphavirus encephalomyelitis. J Virol 87 (5), 2420–9. [PubMed: 23255791]

96. Baxter VK and Griffin DE (2016) Interferon gamma modulation of disease manifestation and the local antibody response to alphavirus encephalomyelitis. J Gen Virol 97 (11), 2908–2925. [PubMed: 27667782]

97. Martin NM and Griffin DE (2018) Interleukin-10 Modulation of Virus Clearance and Disease in Mice with Alphaviral Encephalomyelitis. J Virol 92 (6).

98. Netea MG et al. (2016) Trained immunity: A program of innate immune memory in health and disease. Science 352 (6284), aaf1098. [PubMed: 27102489]

99. Rhett JM et al. (2008) Novel therapies for scar reduction and regenerative healing of skin wounds. Trends Biotechnol 26 (4), 173–80. [PubMed: 18295916]

100. Rog-Zielinska EA et al. (2016) The Living Scar--Cardiac Fibroblasts and the Injured Heart. Trends Mol Med 22 (2), 99–114. [PubMed: 26776094]

101. Gage FH (2000) Mammalian neural stem cells. Science 287, 1433–1438. [PubMed: 10688783]

102. Norenberg MD et al. (2004) The pathology of human spinal cord injury: defining the problems. J Neurotrauma 21 (4), 429–40. [PubMed: 15115592]

Box 1.

Reactive astrocytes are not scar tissue

There is a lingering historical practice of referring to astrocyte reactivity simply as “glial scar” formation. This practice is counter to other organ systems, where host tissue parenchymal cells are not considered to form scars. In other well-studied complex tissues such as skin and heart, scar tissue is defined as replacement of host organ parenchyma by mesenchymal (stromal) cells and fibrotic extracellular matrix [99, 100]. Astrocytes are neural parenchymal cells that derive from the same neural stem cells that give rise to neurons [101]. They are not mesenchymal or stromal cells. Astrocytes are critical for all neurological functions in health and disease [33]. When astrocytes proliferate, they replace CNS parenchyma and preserve CNS function [11–13]. Experts in human CNS pathology and astrocyte pathophysiology have previously emphasized that astrocytes responding to tissue damage should not be confused with true mesenchymal or stromal scar and have questioned the practice of referring to astrocyte responses as “scars” [102]. As discussed in this article, steadily mounting experimental evidence supports this view. Based on this evidence, we advocate that it is time to put the term “glial scar” to rest and restrict the term “scar” to non-neural stromal cells and fibrotic extracellular matrix in line with terminology in other tissues. The term “glial scar” has fostered a widespread negative culture in which students or new investigators entering the field become biased that glial cell responses to injury are detrimental in the first instance and therefore require attenuation. This is not the case and it is time to move on from this terminology.

Box 2:

Clinician's Corner

The presence of reactive astrocytes should no longer be regarded simply as a passive readout for CNS damage or disease. Astrocytes exert activities that are crucial for neurological functions in health and disease. Histopathological assessment methods are becoming increasingly sophisticated and automatable and can be applied to more refined evaluations of astrocytes and their cellular interactions in human pathological specimens. Astrocytes are increasingly implicated in influencing disorder outcome. As information grows, astrocytes will increasingly become targets for therapeutic interventions.

Outstanding Questions

How do starting conditions in healthy tissue, which may differ across CNS regions or at different ages, influence astrocyte reactivity?

What differences in specific astrocyte cellular functions are associated with differences in molecular expression in different reactivity states?

How do tumor cells co-opt reactive astrocytes to promote tumor growth and invasion?

How do past histories of prior exposure to insults alter astrocyte reactivity to new insult exposures via ‘innate immune memory’?

How do cell autonomous changes due to genetic mutations, polymorphisms, environmental toxins, infections or chronic inflammation change astrocyte reactivity states into astrocyte disease states?

How might astrocyte reactivity responses differ across species?

Highlights

Astrocyte reactivity in response to different CNS disorders is diverse, context dependent and can be functionally powerful.

Reactive astrocytes exhibit at least two broad overarching subtypes: (i) proliferative border-forming reactive astrocytes that surround areas of overt tissue damage, and (ii) non-proliferative reactive astrocytes that retain their basic structure and cellular interactions in essentially intact but reactive neural tissue.

Normal astrocyte reactivity is an evolutionarily ancient response that can protect neural tissue, maintain tissue homeostasis and preserve neurological functions after diverse CNS insults.

Dysfunction of reactive astrocytes can contribute to CNS disorders either through loss of normal functions, or through gain of abnormal functions.

Reactive astrocytes express DAMP and PAMP receptors and are emerging as integral and essential components of multicellular CNS innate immunity.

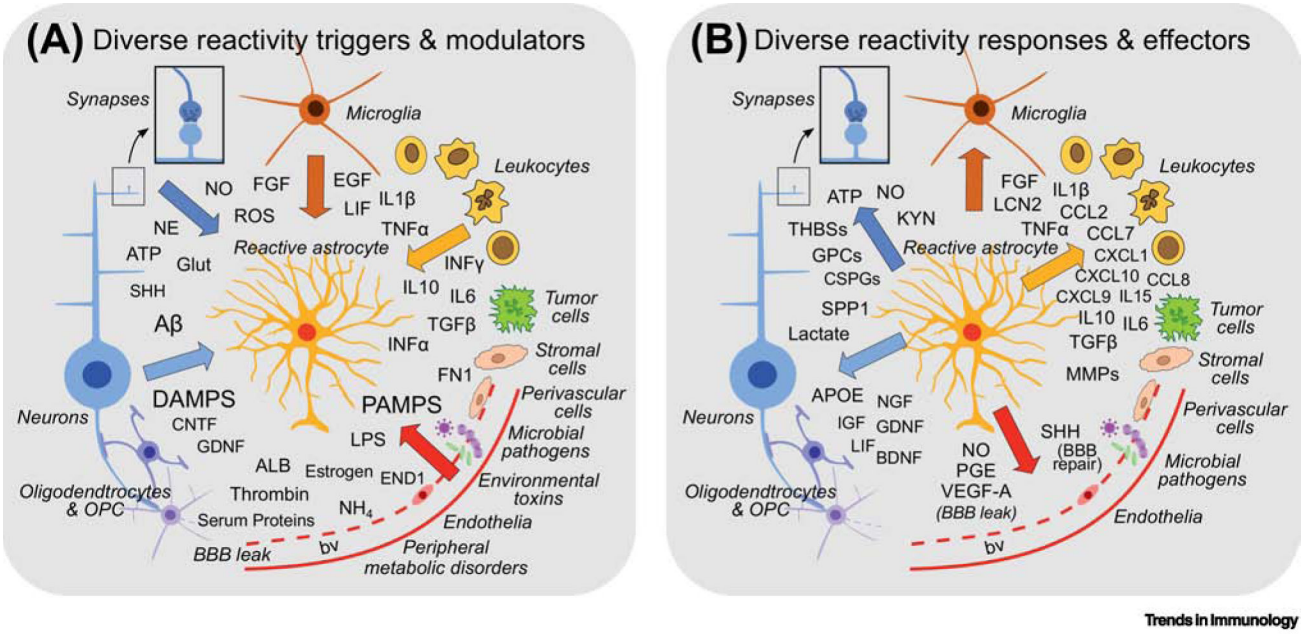

Figure. 1. Mammalian astrocytes respond to diverse reactivity-inducing triggers and produce diverse molecular effectors.

A. Astrocyte reactivity can be triggered by a wide variety of molecules that can derive from diverse sources, including any cell type in CNS tissue, as well as from microbial pathogens, circulating inflammatory cells, serum proteins, peripheral metabolic disorders or environmental toxins. **B.** Reactive astrocytes can exhibit diverse functional responses to these triggers and can elaborate a wide variety of effector molecules that can influence many different cell types in a context-specific manner. BBB blood-brain barrier, bv blood vessel, OPC oligodendrocyte progenitor cell. Protein abbreviations are per international guidelines (https://www.genecards.org/). Small molecule abbreviations: Aβ amyloid beta, Glut glutamate, NE norepinephrine, NO nitric oxide, ROS reactive oxygen species.

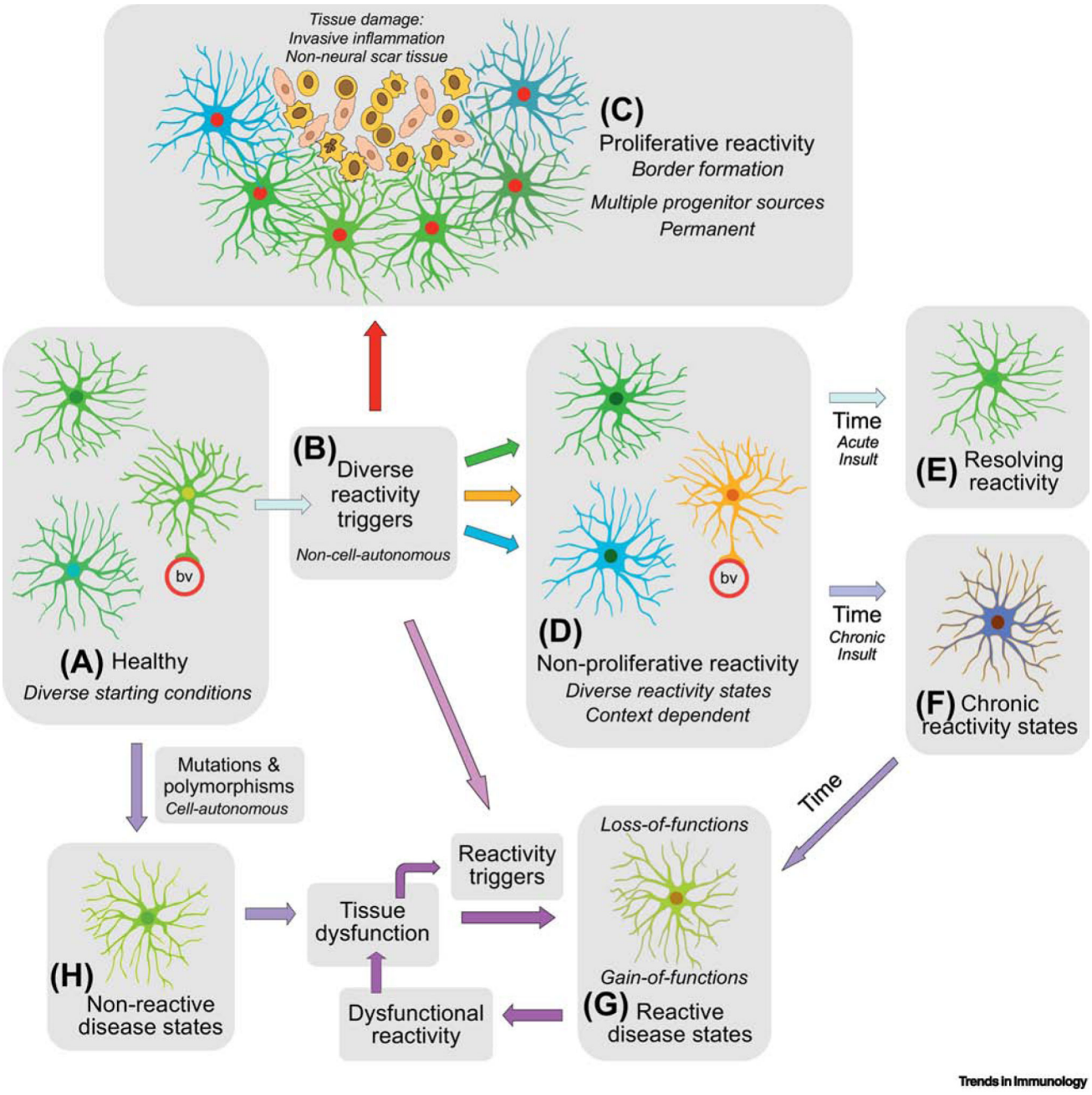

Figure 2. Key Figure – Working model of diverse astrocyte responses to CNS disorders.

A. In healthy tissue, astrocytes exhibit regional and local heterogeneity in gene expression and function. Diverse starting conditions can influence subsequent reactivity responses. **B.** Different forms of astrocyte reactivity are triggered by diverse non-cell-autonomous signals emanating from diverse CNS insults. **C.** Proliferative astrocyte reactivity occurs in response to tissue damage caused e.g. by traumatic or ischemic cell degeneration, blood-brain barrier leak, infection or autoimmune leukocytic inflammation. Newly proliferated astrocytes (red nuclei) form limitans borders that permanently separate and corral areas of damage, inflammation and non-neural scar tissue from adjacent viable neural tissue. **D.** Non-

proliferative astrocyte reactivity can exhibit diverse states with different changes in gene expression and functions that are context dependent as determined by astrocyte starting conditions and incoming reactivity triggers. Non-proliferative reactive astrocytes maintain but modify their interactions with surrounding cells in preserved tissue architecture. **E.** Non-proliferative astrocyte reactivity can resolve over time if acute triggers recede. **F.** Astrocyte reactivity can become chronic if triggers persist. **G.** Chronic astrocyte reactivity can lead to loss- or gain-of-functions resulting in disease states with dysfunctional reactivity that can exacerbate tissue pathologies and worsen disorder outcome. **H.** Genetic mutations and polymorphisms can lead to cell-autonomous dysfunctions in astrocytes that lead to non-reactive disease states. Such disease states can in the absence of astrocyte reactivity, cause or contribute to tissue dysfunctions that in turn lead to production of astrocyte reactivity triggers. The ensuing dysfunctional astrocyte reactivity exhibits gain- or loss-of-functions that can contribute to further tissue pathology in a vicious cycle. Details and literature references are in the main text.

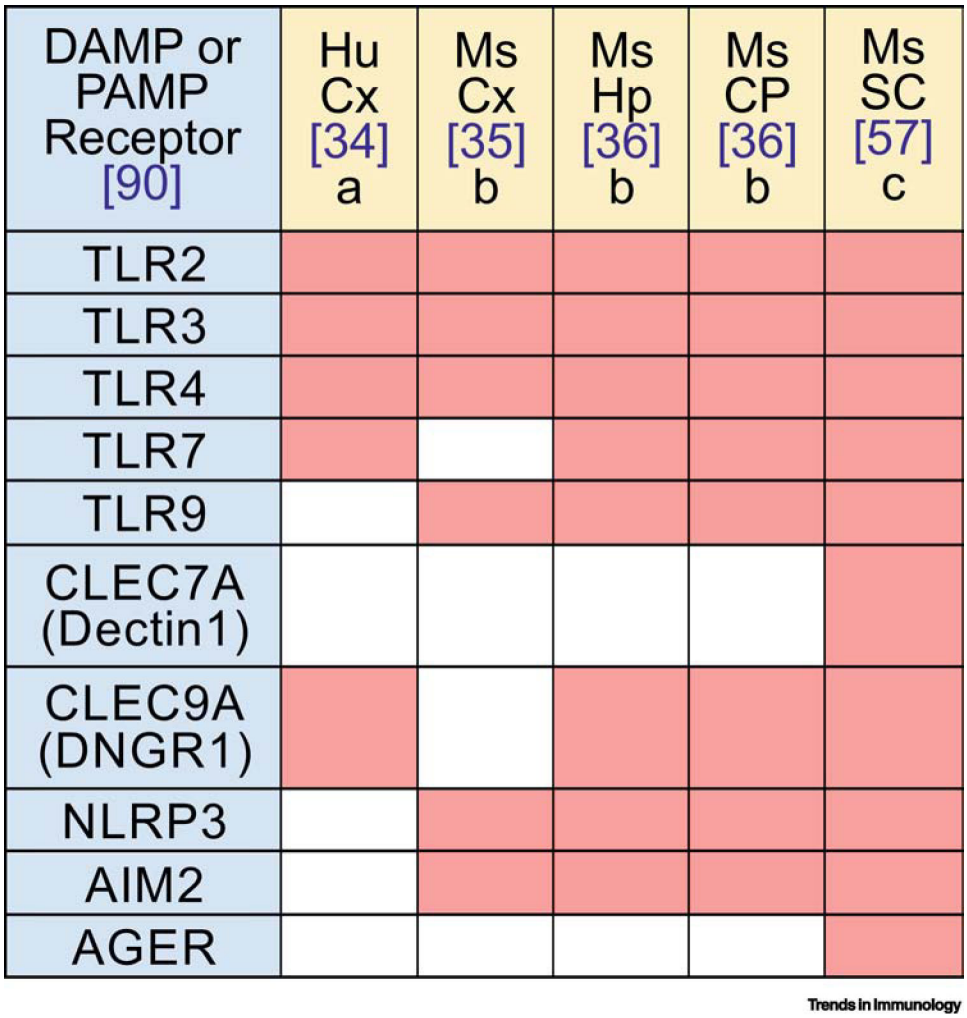

Figure 3. Astrocytes in healthy human and mouse tissue express multiple DAMP and PAMP receptors.

Pink boxes indicate significant receptor gene expression. DAMP and PAMP receptor information taken from reference [90]. CP, caudate putamen, Cx cerebral cortex, Hp hippocampus, Hu, human, Ms mouse, SC spinal cord. Expression data taken from references [34–36, 57]) and their associated open access databases: a http://www.brainrnaseq.org/ b http://astrocyternaseq.org/ c https://astrocyte.rnaseq.sofroniewlab.neurobio.ucla.edu./addgene?query=Clec9a