CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

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All astrocytes are not created equal —the role of astroglia in brain injury

Darcie L. Moore & Sebastian Jessberger

Astrocytes have important roles in the brain, for example by regulating neurotransmitter clearance, controlling the formation and maintenance of synapses, and by contributing to the blood–brain barrier (BBB; for a review see [1]). In addition, astrocytes respond to acute and chronic injury by hypertrophy and induced proliferation. Notably, astrocytes in the mammalian brain represent a highly heterogeneous population and the exact cellular identity of the astrocytic response in the damaged brain remains largely unknown (for a review see [2]). Thus, live-imaging and single-cell studies are required to unravel the complexity of astrocyte behaviour and distinguish between the good and the bad effects of astrocytic activation on brain function and tissue homeostasis in response to acute and chronic injury.

It is thought that astrocytes respond to injury through hypertrophy of cell bodies and processes, upregulation of the intermediate filaments GFAP and vimentin, extension of processes, proliferation and gradual overlapping of astrocytic domains (for a review see [3]). Interestingly, it is known that although some aspects of the astrocyte response to injury can be detrimental—such as the formation of a glial scar—it can also be beneficial by limiting the invasion of immune cells into the brain parenchyma [4–6]. However, our understanding of the response of astrocytes to injury assumes a global homogeneous response, and an unawareness of the more complex and diverse *in vivo* situation. Two

papers from the group of Magdalena Götz, published in *Nature Neuroscience* and *Cell Stem Cell*, begin to unmask the heterogeneity of the astrocyte response to injury through *in vivo* live imaging after brain injury and by using multiple lesion models and comparing their effects on astroglial behaviour and properties within the injured brain.

In the first study, Bardehle *et al* used *in vivo* two-photon laser-scanning microscopy to monitor individual astrocytes for up to 28 days after a stab wound to the somatosensory cortex [7]. To visualize single cells, astrocytes were labelled using different lines: GLAST^{CreERT2}/eGFP or Confetti reporter, labelling 60–80% of all astrocytes; Aldh1l1-eGFP mice, labelling all astrocytes; and hGFAP-eGFP mice, labelling only those astrocytes with the highest GFAP expression. The authors found that most GFP⁺ astrocytes maintained their morphology after injury and that only subsets showed signs of hypertrophy and polarization towards the injury site. Interestingly, only a small population of astrocytes divided, all of which had their somata apposed to blood vessels (juxta-vascular) and depended on proper functioning of the small RhoGTPase Cdc42 for their proliferative response. Strikingly, none of the labelled astrocytes migrated towards the lesion site, suggesting that the increase in GFAP reactivity often seen at the site of injury is not due to astrocyte migration, but rather is due to increased GFAP expression through hypertrophy, an increased number of proliferative cells and the upregulation of

GFAP in cells that might not express detectable levels of GFAP before injury. Notably, migration of other glial cells (microglia and NG2⁺ glia) to the injury site was observed, suggesting that the migratory properties in response to injury in the brain might not be general to all glia. Thus, the contribution of activated astrocytes to the formation of a glial scar in the brain following injury might be limited and need to be reconsidered. In addition, the location of proliferating astroglial cells at juxta-vascular positions, and their limited movement, suggest that these proliferating astrocytes might be a subset that is responsible for the ‘beneficial’ astrocytic response to injury by tightening the BBB, preventing the invasion of cells into the lesioned brain parenchyma. Thus, observing the glial response after brain injury in real time within their *in vivo* environment identified a highly selective and cell-specific astrocyte response, challenging previously held concepts of astroglial migration and massive astrocyte proliferation after injury.

In the next study, Sirko *et al* analysed how the astroglial response varies between different types of acute or more chronic brain injury [8]. To this end the authors used four different models of injury: MCAo lesion (invasive), stab wound (invasive), APPPS1 mutation (non-invasive) and ectopic p25 activation in neurons (non-invasive). They analysed comparative data for reactive gliosis and induction of stem cell properties in activated astroglia found after brain injury (Figure 1). Interestingly, the two non-invasive, chronic lesion models induced the least response from astrocytes, with astrocytes undergoing hypertrophy but having low levels of proliferation and virtually no neurosphere-forming capacity, indicating that chronic injury in these models does not enhance astrocyte proliferation or acquisition of stem cell properties. In contrast, a much larger astrocytic response occurred in the invasive models, in which astrocytes not only underwent hypertrophy but also had a relatively high proliferative rate and formed multipotent and self-renewing neurospheres *in vitro*. The authors then showed that Sonic hedgehog (SHH) levels increased dramatically, but only in invasive models, and that SHH levels correlated with *in vivo* astrocyte proliferation rates and *in vitro* stem cell potential between injury conditions. By using pharmacological and genetic gain- and loss-of-function strategies, SHH signalling could indeed be identified as a crucial mediator of injury-induced acquisition of stem cell properties in

Fig 1 | Glial cell response, stem cell potential and extracellular Sonic hedgehog (SHH) levels vary depending on the type of brain injury. Astrocytes (yellow), NG2⁺ glial cells (blue) and microglia (red) reside in the uninjured intact brain, in which only NG2⁺ cells usually proliferate. When this tissue is studied *in vitro* to measure its stem cell potential, virtually no neurospheres are formed. After different types of injury, however, morphological and proliferative changes occur to all cells and their *in vitro* stem cell potential can be reactivated. In six-month-old APPSP1 mice, all glial cells change their morphology, with astrocytic and NG2⁺ hypertrophy of cell body and processes, and hypertrophy and reduction of processes in microglia. While few astrocytes proliferate, large amounts of proliferation occurs in both NG2⁺ glia and microglia. This tissue *in vitro* can form a few spheres that are self-renewing and multipotent, generating astrocytes, neurons and oligodendrocytes. In a model of neuronal death (CK/p25; overexpressing p25 in the postnatal forebrain), astrocytes and microglia change their morphology as described above. Astrocytes and NG2⁺ glia do not have any increase in proliferation rates, whereas microglia proliferate greatly. This tissue has little stem cell potential and makes only a few primary multipotent spheres. Finally, in the more invasive stab wound injury to the cortex, all glial cells become morphologically reactive, and astrocytes, NG2⁺ glia and microglia all proliferate in response. This tissue has the largest stem cell potential, capable of making both primary and secondary spheres with multipotent progeny. In each situation, the levels of SHH (green) can be correlated with the proliferation rates of astrocytes and *in vitro* stem cell potential, such that only in stab wound injury are SHH levels significantly upregulated. APPSP1, co-expresses mutated amyloid precursor protein 1 and mutated presenilin 1; NG2⁺, neuron-glia antigen 2.

astrocytes. Thus, Sirko *et al* identified substantial differences with respect to glial response between chronic and acute injury models and identified a molecular pathway (SHH) that at least partly accounts for enhanced astroglial response in invasive injury models.

The two papers by the Götz group shed new light on the *in vivo* response of glial cells to brain injury and characterize a highly

heterogeneous behaviour of astrocytes to chronic and acute brain injury. Surprisingly, only subsets of astrocytes proliferate or polarize, and none of them migrate towards the lesion. The juxtavascular position of proliferating astrocytes suggests that these cells might have access to the increase in SHH after invasive injury, which can regulate their division. However, it is not clear

whether this proliferation is through their de-differentiation and acquisition of neural stem cell potential, or whether it is a result of a mature astrocyte division. That the astrocyte progeny remains with the original cell at the juxtavascular location suggests that they might be acting in a positive way to limit the migration of invading immune cells into the brain. Further studies on whether the increase in juxtavascular, astroglial proliferation affects the BBB permeability or decreases the number of invading cells will be important to understand this effect. If it turns out that enhanced astroglial proliferation might be generally beneficial for the injured brain, it is also tempting to speculate that for other brain injuries where the proliferation rates and SHH levels are reduced, enhanced glial proliferation in close proximity to blood vessels might help to reduce tissue damage and to improve regeneration and repair. Thus, SHH could represent a future therapeutic target to activate glial proliferation in the context of non-invasive, chronic brain injury. In any case, the acquisition of stem cell properties allowing astrocytes to form neurospheres *in vitro* is not directly tied to the *in vivo* use of these stem cell properties (for a review, see [9]). Whether the de-differentiation of astrocytes and proliferation of stem cells *in vivo* is beneficial or detrimental remains unclear. However, the new data have set the cellular framework for future studies to understand injury-induced astroglial stem cell characteristics *in vivo* and whether this *in vitro* potential might be unleashed for regenerative strategies *in vivo*.

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