In the last century, increasing knowledge about glial cells has revealed their importance in brain physiology and disease and an exciting potential for targeted therapeutic interventions (Hernández et al., 2021).

Ischemic stroke is the most common of all types of stroke. As the second leading cause of disability and mortality, ischemic stroke is a serious socioeconomic burden worldwide. Although it has been strongly associated with aging, during 2020 COVID-19 emerged as a new risk factor for ischemic stroke in younger patients (Cagnazzo et al., 2021). The only therapy or intervention currently available for humans is early reperfusion after the onset of the first symptoms. However, due to the high risk of brain hemorrhage, these treatments are suitable for only a small percentage of patients. Moreover, reperfusion does not prevent post-injury neurodegeneration, hence the importance of finding new therapeutic alternatives to ameliorate ischemic damage and improve post-stroke quality of life.

Upon ischemia, the reduced availability of oxygen, glucose, and some blood factors induce a metabolic failure in the cells of the damaged region, but not all neural cells are affected equally. This situation triggers the energetic failure of some neurons and their consequent death, loss of ionic gradients, excitotoxicity, oxidative stress, and finally the induction of apoptosis. The severity and extension of tissue damage and therefore the prognosis of the patient depends on the diameter of the affected artery and duration of occlusion. Two areas with a distinct degree of injury can be distinguished, namely a “core”, which is the most affected area, and the surrounding “penumbra”, the least affected region. While the effect of ischemia on neurons has been exhaustively characterized in the different stages of this pathology, the role of glial cells has been largely overlooked until recent years.

In the following sections, we will briefly present the current understanding of the role of each glial cell type upon brain ischemia and our particular view of the future directions of this exciting research field.

**Microglia:** Microglia account for 10–15% of the total number of brain cells. They are ubiquitous in the central nervous system and are the first line of defense against ischemic insults, responding from minutes to a few hours after the onset of the injury. This response includes microglial activation accompanied by deep morphological changes comprising hypertrophy of the cell body and shortening and thickening of its processes, which are associated with increased phagocytosis, cytokine and chemokine release, proliferation, and migration (Xue et al., 2021).

“Resting” microglia is the predominant phenotype under physiological conditions and these cells present low expression of some surface molecular markers, such as CD45, major histocompatibility complex-II, among other surface markers. Likewise, specific microglial markers such as Iba1 and IB4 have been described to increase in this context. In a similar way to macrophages, activated microglia have been classified into two groups on the basis of their inflammatory capacity, namely M1 (pro-inflammatory) and M2 (anti-inflammatory). Activated M1 microglia increase the levels of inflammatory cytokines, including tumor necrosis factor-α, interleukin (IL)-6, IL10, and interferon-γ, chemokines (CCL2 and CXCL10), metalloproteinases, including matrix metalloprotein (MMP)-3 and MMP-9, reactive oxygen species and reactive nitrogen species in the injured area, thus leading to cytotoxicity. Conversely, M2 cells secrete the protective molecules IL4, IL-10, IL13, transforming growth factor-β, insulin-like growth factor-1, and brain-derived neurotrophic factor, and promote the integrity of the blood-brain barrier (BBB) and angiogenesis, thereby ameliorating ischemic damage. The M1 to M2 ratio may determine the prognosis of ischemic stroke (Hernández et al., 2021). Far from this dual vision of microglia, several recent studies support the notion that there is greater heterogeneity in the activated status of microglial populations after cerebral ischemia, thus making the microglial response more complex. In this regard, several intermediate phenotypes have been described, including M2-a, M2-b, and M2-c. However, the contribution of each type to brain pathology versus repair is not yet understood (Lyu et al., 2021).

Moreover, some evidence supports the notion that microglia switch from one phenotype to another. In this regard, several “inducers” of each microglial phenotype have been described. Compounds that induce a switch from the M1 to the M2 profile with promising results in animal models include melatonin, a molecular cocktail from Ginkgo biloba, protocatechuic acid, and rapamycin. Minocycline, which has already given positive results in several clinical trials, leads to a decrease in the neuroinflammation induced by microglial activation, driving M1 to M2 (Figure 1) and thus reducing the secretion of pro-inflammatory molecules and neuronal death (Hernández et al., 2021).

**Astrocytes:** Accounting for approximately 50% of the total brain volume, astrocytes should be considered key players in ischemia/reperfusion injury. Furthermore, it is known that opposite active phenotypes exert notable effects in each main step of brain ischemia pathology (Liu and Chopp, 2016), thus resembling the previously mentioned property of microglia. Therefore, the A1 phenotype would be neurotoxic, whereas the A2 phenotype would be neuroprotective. However, the astrocytic response to ischemia is more complex.

Astrocytes show mechanisms directed to maintaining neuronal homeostasis after ischemia but they can become damaged enhancers. This cell population plays a pivotal role in glutamate uptake through specific transporters such GLUT-1 and GLAST, which is fundamental for maintaining normal synaptic function. Astrocytes are known to be altered under ischemic conditions, thereby contributing to excitotoxicity (Rao et al., 2001). Gap junctions and hemichannels formed by connexin 43 are also altered after an ischemic insult, driving the release of toxic molecules like ATP into the extracellular compartment. Under ischemic-reperfusion conditions, the energy supply to neurons is supported by astrocytes through the production and release of ketone bodies, which are used by these cells as precursor molecules for energy metabolism instead of lactate, substrate that they use under physiological conditions. At the same time, low O2 pressure prompts astroglia to secrete prostaeglandin E2, which induces vasodilation and therefore facilitates the diffusion of O2 and glucose into the brain parenchyma.

Another important feature of astrocytes is their ability to quench reactive oxygen species and reactive nitrogen species, thereby contributing to a less pro-inflammatory environment, mainly through the upregulation of antioxidant pathways and the secretion of molecules like adiponectin, ascorbic acid, and DJ-1. However, reactive oxygen...
species accumulation ultimately activates NLPR3 (nucleotide-binding-oligomerization domain-, leucine-rich-repeat- and pyrin domain-containing protein 3) inflammasome, forcing astrocytes to secrete pro-inflammatory molecules like IL-1β.

Astrocytic endfoot cover the cerebral blood vessels and are part of what is known as the neurovascular unit. This structure tightly regulates the permeability of the BBB and is severely disrupted upon ischemia. Astrocyte swelling due to excessive water intake and the acquisition of a pro-inflammatory morphology are the main causes of this phenomenon. In addition, the secretion of pro-inflammatory molecules, such as metalloproteinases (MMP-2 and MMP-9), nitric oxide, and endothelin, by activated astroglia enhances BBB disruption (Michinaga and Koyama, 2019). On the other hand, astrocyte-derived Shh, RALDH2, and insulin-like growth factor-1 have BBB protective properties.

A good example of the Janus-faced nature of these glial cells is the formation of the glial scar. This structure defines the frontier between penumbra and core. Scars help contain a priori unrepairable damage at early stages while impeding neuronal sprouting in later stages of the disease (Sofroniew, 2009). This same duality can be detected in the effect of astrocytes on neurogenesis and synaptogenesis. And the release of Ephrin-A5 inhibits axonal growth, while astrocyte-derived thrombospondins and activity-dependent neuroprotective protein are essential for synaptic plasticity and functional recovery. Interestingly, it was recently described that striatal astrocytes can differentiate into neurons after stroke and that glial scar astrocytes can be reprogrammed into neurons (Guo et al., 2014).

Finally, astrocytes are starting to gain visibility as preconditioning vehicles in the ischemic tolerance paradigm (Otsuka et al., 2019), which has been reported in animal models and humans. Therefore, these cells emerge as useful therapeutic targets to reduce ischemic brain damage (Figure 1).

Oligodendrocytes: Oligodendrocytes, which are responsible for axon myelination in the central nervous system, are the main cellular component of white matter. Ischemia induces white matter damage which has been correlated with the severity of the injury, thus having clinical relevance as a predictor. Ischemia induces white matter damage, which has been correlated with the severity of the injury and thus has clinical relevance. Preserving the integrity of white matter and therefore oligodendrocyte viability reduces neuronal injury and neurological symptoms (Dai et al., 2020).

In the adult brain, the oligodendrocyte population is highly heterogeneous, comprising both oligodendrocyte progenitor cells and various subpopulations corresponding to different developmental stages, ranging from immature to mature oligodendrocytes, the latter with the ability to form myelin sheaths. Olig2 is the specific transcription factor used to identify oligodendrocyte lineage. However, each subpopulation can be characterized using distinct specific markers (Hernández et al., 2021).

Oligodendrocyte progenitor cells, also called NG2+ cells, account for 5–8% of total glial cells in the adult brain and they can proliferate and differentiate into myelinating oligodendrocytes after ischemia (Figure 1). However, these cells can also acquire a pro-inflammatory phenotype after injury which may have a negative effect on neurological outcomes (Kirby et al., 2019). Recent data point to a time-dependent dual response of Olig2 cells after ischemia. In the acute phase, there is a reduction in the number of Olig2 cells in the injured area, possibly due to the high susceptibility of mature oligodendrocytes to ischemic insults. In contrast, in the chronic phase, the number of Olig2 cells in the ischemic area increases. Interestingly, these cells show high levels of the 3R-Tau isoform, whose function is still unknown (Villa González et al., 2020). Although the function of these newly generated cells is not well described, it is considered that they sustain white matter integrity and axon stability, thus ameliorating brain damage after stroke.

Perspectives: Although it is tempting to assert that any future success in treating stroke patients involves driving M1 microglia to M2, A1 astrocytes to A2, and oligodendrocyte progenitor cells to myelinating Olig2 cells, this interpretation would be an oversimplification. While the promotion of the beneficial effects of each glial cell type is interesting, we also know that a priori detrimental effects exerted by each type are also key on recovery after an ischemic insult. In this regard, M1 microglial cells may be crucial in early steps to activate the rest of the machinery and to promote the phagocytosis of cell debris, thereby reducing the toxic environment. Similarly, A1 astrocytes may be necessary for the some of the beneficial effects of damage through the formation of the glial scar. Given that there is a significant loss of gray matter after stroke, a greater understanding of how to induce the differentiation of glial cells into neurons may bring about effective therapies in the coming years, as well as promoting synaptogenesis, as, while stimulating the proliferation of myelinating oligodendrocytes could improve white matter regeneration. As we do not believe in a single “Holy Grail” treatment, we propose that research efforts be devoted to the combinatorial effect of the approaches available focused on single cell types. In line with this, we consider that early detection of at-risk individuals would allow preventive preconditioning treatment, which, in combination with developing glia-centered therapies and a proper post-stroke rehabilitation program (Figure 1), could have a significant impact on the prognosis of ischemic stroke patients. However, we are fully aware that at-risk individuals are difficult to identify and that the post-stroke therapies tested to date have shown limited positive impact. In this context, we firmly believe that greater insight into the plasticity of each glial phenotype in the different stages of ischemic stroke and the precise mechanisms underlying phenotype switching are key for any future successful treatment based on glial cells.

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