Review

Neuroinflammation and central nervous system regeneration in vertebrates

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Injuries in the central nervous system (CNS) are one of the leading causes of mortality or persistent disabilities in humans. One of the reasons why humans cannot recover from neuronal loss is the limited regenerative capacity of their CNS. By contrast, non-mammalian vertebrates exhibit widespread regeneration in diverse tissues including the CNS. Understanding those mechanisms activated during regeneration may improve the regenerative outcome in the severed mammalian CNS. Of those mechanisms, recent evidence suggests that inflammation may be important in regeneration. In this review we compare the different events following acute CNS injury in mammals and non-mammalian vertebrates. We also discuss the involvement of the immune response in initiating regenerative programs and how immune cells and neural stem/progenitor cells (NSPCs) communicate.

Inflammation during CNS regeneration: detriment or benefit?

Injuries of the CNS are one of the primary reasons for death and severe disabilities seen throughout the world. Despite many years of research, the therapeutic outcome of CNS injuries remains relatively poor. Non-physiological events taking place after injury, including stress responses, acute inflammation, and other mechanisms associated with wound healing, have detrimental effects on tissue regeneration [1]. Among these, inflammation – which manifests rapidly on injury [2,3] – has been regarded as a key regulator of regeneration because immunosuppression has repeatedly been shown to be permissive for tissue restoration [4–6].

On CNS injury in mammals, the immune response elicits an inflammatory cascade resulting in the secretion of many detrimental factors that may hinder successful regeneration. This negative effect of the inflammatory response has been observed in NSPCs. Neuroinflammation results in decreased reactive proliferation of NSPCs, reduced number of newborn neurons, poor survival, and ineffective integration into the circuitry [4–10]. These results suggested that inflammation can negatively regulate NSPCs leading to a reduced regenerative potential. However, in the past two decades reports suggesting a positive role for neuroinflammation during CNS regeneration in mammals have increased. Peripheral blood leukocytes (initially neutrophils and macrophages are chemically attracted to the wound site and subsequently cells of the lymphoid system; i.e., T cells and B cells) as well as resident microglia that invade the tissue can provide cues to enhance proliferation of progenitor cells and provide trophic support leading to higher neuronal survival rates [11–16]. Neuroinflammation has also been linked to better behavioral outcomes, because injection of monocyte-derived macrophages into the CNS resulted in improved hind-limb locomotor performance and hence better recovery after spinal cord injury [17]. Collectively, these findings pose a dilemma: is neuroinflammation detrimental or beneficial after CNS injury? It appears that the effect of inflammation depends on several parameters, including different cell types (e.g., leukocytes), signaling molecules (e.g., chemokines, cytokines), type and severity of injury (e.g., severe lesion, slight contusion), and timeline of the response (e.g., acute, subacute, chronic) [1]. Therefore, a useful way to solve this dilemma might be to develop a model where neuroinflammation does not display unfavorable effects and neuronal regeneration occurs naturally. The ideal candidates for such studies are non-mammalian vertebrates.

Unlike mammals that exhibit very poor regenerative ability, non-mammalian vertebrates have a tremendous capacity to regenerate damaged and/or lost tissues [18–20] despite initiating an acute inflammatory response after injury [21,22]. In particular, teleosts and amphibians are able efficiently to regenerate several tissues and organs including their extremities [23–28], heart [29–31], and CNS (brain, spinal cord, and retina) [32–35]. Various injury paradigms have been established over the past years including mechanical lesions [35–39], amputations [23,28,40], genetic ablations of specific cell populations [29,41,42], and administration of cytotoxic drugs [22,43] in both mammals and non-mammalian vertebrates that displayed a remarkable difference: whereas the tissue loss

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in mammals precedes formation of scar tissue, which is thought to be a major obstacle for regeneration [44–46], the lost tissue is restored completely in terms of morphology and function in teleost fish [29,35]. Given the high degree of conservation between zebrafish and mammals at the sequence and proteome level [47,48], understanding how zebrafish can couple inflammation to successful regeneration might open new avenues towards regenerative therapies in humans. However, it should not be disregarded that many open questions remain in the field of zebrafish immunology, especially on the similarities and differences of the features of the immune system between zebrafish and mammals. Some of the shared elements reported between zebrafish and mammalian immune systems are: leukocyte subtypes (granulocytes, eosinophils, macrophages, lymphocytes); the complement system, which is also very well developed in zebrafish; all three classes of the major histocompatibility complex; early tissue infiltration on wound or infection of granulocytes; secretion of well-conserved inflammatory proteins; and phagocytosis of debris and microbes [49,50]. However, despite the multiple common features there are others that have not yet been characterized in zebrafish. For example, dendritic cells and natural killer cells have not yet been identified in zebrafish. Similarly, although there is genetic evidence of the existence of all types of T cell in zebrafish, functional validation is still missing. In addition, and relevant to the regeneration studies, there remains no functional specialization of macrophages such as M1/M2 polarization [50,51]. All of the above illustrate that, despite the drawback of the as-yet incomplete characterization of its immune system, the zebrafish could serve as a model to study inflammatory processes and their crucial role in traumatic injuries.

Here, in light of recent results, we discuss how the immune system mediates the regenerative response via direct communication between the inflammatory cells and NSPCs and how such a response can lead to successful regeneration of the CNS tissues at least in non-mammalian vertebrates. We also provide a step-by-step comparison of the different molecular and cellular processes occurring after CNS injury in mammals and non-mammalian vertebrates. We believe that understanding the similarities and differences between these species may help translational applications for patients suffering from acute CNS injuries or neurodegenerative diseases.

Regeneration of the CNS

As reported by Cajal in the early 20th century, the brain was considered a tissue where new neurons could not be generated once development ended [52]. However, this dogma has been challenged by studies showing that newborn neurons can be generated throughout life in a process called 'adult neurogenesis'. Adult neurogenesis is the process of producing new neurons that integrate into the existing circuits after fetal and early postnatal development. In the mammalian brain this process predominantly takes place in two parts of the forebrain, the subventricular zone (SVZ) of the lateral ventricles in the telencephalon and the subgranular zone of the dentate gyrus in the hippocampus [53,54]. By contrast, zebrafish show profound neurogenesis in the adult brain, where many proliferative and neurogenic zones have been detected [55].

CNS reaction and regenerative responses differ according to the type of injury. This is generally due to the size of the area affected, the acuity of the subsequent inflammatory response, the degree of consequential damage, and the degree to which vascular supply is disrupted. One example of injury is traumatic brain injury (TBI) that affects a large region of the CNS. This type of injury has been extensively modeled in various organisms [56,57], including zebrafish [35,36,39,58]. Similar to mouse CNS injury, traumatic injury in zebrafish can be induced by pushing a small cannula through the nasal cavity and stabbing the dorsal part of one telencephalic hemisphere [35,37,38,59]. This particular lesion paradigm is similar in mouse and zebrafish because: (i) it spares the ventricular zone (VZ), the area where the NSPCs reside [radial glial cells (RGCs) in the adult fish brain and astrocyte-like cells of the SVZ in mouse brain]; (ii) it leaves the contralateral telencephalic hemisphere intact to serve as an internal control; and (iii) it exhibits very high survival rates (>95%). Furthermore, the subsequent events occurring shortly after such an injury – namely, primary cell death, acute inflammation, and proliferation of glial cells – are also comparable (Figure 1 and Table 1). However, unlike mammals following TBI, proliferating RGCs in zebrafish generate neurons that integrate into the existing circuitry of the zebrafish brain [35]. By contrast, in mouse, reactive astrocytes form scar tissue and remain in their lineage without apparent neurogenesis [59,60]. Therefore the mechanisms that enable glial cells to be neurogenic would be likely targets of regenerative medicine. These mechanisms might originate from the different processes occurring after CNS injury such as neuronal cell death, immune response, and activation of NSPCs. These are discussed in more detail below.

Cell death

At the cellular level, one of the first events following traumatic brain and spinal cord injuries in zebrafish is massive cell death, which peaks in the first few hours after the lesion develops and lasts up to several days later, albeit significantly declined [21,35,61]. Traumatic lesions in the mammalian brain and spinal cord also trigger massive neuronal cell death [62,63]. On injury, most apoptotic and necrotic cells initially appear within the first few hours. However, although this first wave of cell death resolves within a few hours, a second wave of cell death is observed several days or weeks after the primary damage, which is thought to be due to toxic metabolites secreted by reactive astrocytes and inflammatory cells [6]. Thus, it seems that cell death is more limited in regenerating organisms, suggesting a potential role of the rapid cessation of apoptosis for successful CNS regeneration. However, whether this cessation is the cause or the consequence of successful regeneration remains to be addressed.

Reactive proliferation

One of the most important cellular events to occur after TBI and neuronal cell death in zebrafish is so-called reactive proliferation. RGCs, oligodendrocytes, endothelial cells, leukocytes, and other uncharacterized (marker-negative)