Corrigendum: Purinergic signaling systems across comparative models of spinal cord injury

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Due to the editorial office’s error, a number of corrections were not made to the article prior to its publication; the publisher wishes to apologize to all concerned. The corrected version of the article appears in full below.

Review

Purinergic signaling systems across comparative models of spinal cord injury

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Introduction

Following spinal cord injury (SCI), functional recovery is extremely limited in adult mammals. This lack of recovery is reflective of the collective failure of lesioned axons to re-grow and damaged neurons to regenerate in the spinal cord following the primary trauma. The result is a debilitating reduction in sensorimotor function and quality of life in approximately 770,000 people of all ages, genders, ethnicities, and socioeconomic backgrounds each year (Kumar et al., 2018). In contrast to the devastating consequence of spinal cord injury in mammals, several non-mammalian species within the vertebrate subphylum have a much higher regenerative capacity within the central nervous system (CNS) and can undergo functional recovery in adulthood. Although the underlying source of the regenerative differences between regenerating and non-regenerating species is not fully understood, landmark work studying these comparisons has begun to shed light on the defining features and characteristics of successful regeneration and functional recovery. While all vertebrates have a certain degree of regenerative capacity in the CNS during development, by adulthood, this plasticity is highly restricted in birds and mammals. Limitations to regeneration are largely attributed to a secondary cellular insult involving acute and chronic processes of neuroinflammation, cellular death, reactive gliosis, and axonal degeneration, within the spinal cord following injury. The species who maintain some regenerative ability beyond development appear to be members of the more primitive classes of vertebrates including Agnatha (jawless fish), Reptilia, Amphibia, and Osteichthyae (teleost fish), suggesting that regeneration is an ancestral trait that is diminished over evolution. Indeed, zebrasfish (Danio rerio) (Reimer et al., 2008; Hui et al., 2010; Goldshmit et al., 2012), sea lamprey (Petromyzon marinus) (Herman et al., 2018), axolotl (Ambystoma mexicanum) (Sabin et al., 2019), knifefish (Apteronotus leptorhynchus) (Vitalo et al., 2016), goldfish (Carassius auratus) (Takeda et al., 2015, 2017), and newts (Notophthalmus viridescens) (Zukar et al., 2011) are all non-mammalian vertebrate species with a preserved high degree of CNS regeneration capacity. One particularly distinct difference between these select species and mammals is the ability to undergo injury-induced neurogenesis following SCI (Reimer et al., 2008; Takeda et al., 2008; Sirbulescu et al., 2009; Hui et al., 2010; Goldshmit et al., 2012; Joven and Simon, 2018). In adult zebrafish for instance, newly generated motoneurons are integrated into the existing circuitry to restore locomotion below the level of the lesion within 6 weeks post SCI (Reimer et al., 2008). A further understanding of the neuroprotective and regenerative mechanisms in these species will help to uncover the molecular cues associated with limiting secondary injury and promoting regeneration and recovery.

One evolutionarily conserved family of signaling factors involved in various aspects of secondary responses to injury in both regenerative and non-regenerative species is the purinergic signaling system. Purinergic signaling...
has been associated with regulating cellular proliferation, migration, differentiation, and survival during development and in adulthood (Burnstock and Ulrich, 2011; Gomez-Villafuertes, 2016; Oliveira et al., 2016; Ribeiro et al., 2016). Purines and pyrimidines, such as adenosine triphosphate (ATP) and uridine triphosphate (UTP) respectively, along with their metabolites, function as extracellular signaling molecules that mediate cellular communication via purinergic receptors (Burnstock, 2018). These receptors have extensive protein families, including metabotropic adenosine A1, A2a, A2b, A3, ionotropic nucleotide P2X, and metabotropic nucleotide P2Y receptors (Burnstock, 2015). Following mammalian SCI, Wang et al. (2004) found that extracellular ATP is elevated within the lesion site for several hours, thus suggesting a role for injury-induced purinergic signaling. Since then, it has been proposed that ATP may function as an early distress signal within the tissue that is involved in the chemoattraction of various cell types and induction of cell reactivity and proliferation (Haynes et al., 2006; Saudacini et al., 2006; Dou et al., 2012; Shinozaki et al., 2017; Quintas et al., 2018; Kobayakawa et al., 2019). While accumulating evidence supports a role for purinergic signaling in the CNS injury response of mammals, how this complex system may be adapted in regeneratively competent, non-mammalian species is less clear. Here, we review on the role of purinergic signaling during each phase of the secondary injury [(1) cellular death, (2) neuroinflammation, (3) reactive gliosis, and (4) axonal degeneration] and examine the variations of this signaling system in regenerative and non-regenerative species.

Search Strategy and Selection Criteria

Bibliographic citation databases searched include PubMed and Google Scholar. Search terms include: P1 receptors, P2X receptors, P2Y receptors, teleost spinal cord injury, mammalian spinal cord injury, P1/P2X/P2Y and inflammation, P1/P2X/P2Y and cell death, P1/P2X/P2Y and gliosis, P1/P2X/P2Y and neurogenesis, P2Y/P2X/P2Y axonal regeneration. All searches were completed between 2020–2021.

Cell Death

Following an initial mechanical trauma to the spinal cord, secondary injury is associated with a wave of cell death that exacerbates damage and expands the lesion size (Figure 1). In rodent models of SCI, the predominant mechanism of neuronal cell death is necrosis. This begins almost immediately, peaks at 1–2 days, and continues for months (Hassannejad et al., 2018; Kwiecien et al., 2020). Glial cells similarly reach peak levels of necrotic cell death between 1–2 days following SCI (Kwiecien et al., 2020). Meanwhile, apoptosis of neurons peaks first around 4–8 hours and then again at 3 days (Hassannejad et al., 2018). In oligodendrocytes, it peaks within hours and continues for weeks (Crowe et al., 1997), and in microglia, it peaks at 1 day (Bellver-Landete et al., 2019). Additional mechanisms of secondary cell death include intracellular calcium dysregulation, excitotoxicity, and mitochondrial dysfunction/oxidative stress (Ohishi et al., 2016; Reigada et al., 2017).

In contrast, regeneratively competent species predominantly display apoptotic, as opposed to necrotic, cell death following a CNS injury (Figure 2) (Sirbulescu et al., 2009). Notably, necrosis stimulates a pro-inflammatory immune response that leads to further damage, whereas apoptosis is typically anti-inflammatory (Kono and Rock, 2008). The presence of secondary cell death in regenerative species suggests that some degree of cell death following injury may be tolerated, and perhaps essential, for successful CNS regeneration. Indeed, inhibition of apoptosis following caudal fin amputation in adult zebrafish reduced blastema formation and sensory neuron regrowth (Rampon et al., 2014). Similarly, inhibition of caspase-mediated cell death within the first 24 hours post injury was found to prevent tail regeneration following amputation in the African clawed frog tadpole (Xenopus laevis) (Tseng et al., 2007). However, the duration of cell death differs significantly between regenerative and non-regenerative species. In teleosts, apoptosis peaks between 15–24 hours post SCI and then declines sharply after 3–5 days (Sirbulescu et al., 2009; Hui et al., 2010, 2014). The sustained expression of apoptotic markers observed 3 weeks post SCI in rodents (Crowe et al., 1997) and 8 weeks in humans (Emery et al., 1998) suggests that regenerative failure in mammals may not be caused by the presence of secondary cell death, but by its necrotic and prolonged nature.

Activation of purinergic signaling is kicked off very early and known to promote cell death in the damaged mammalian spinal cord (Figure 3). Apoptosis stimulates damaged cells to release nucleotides into the extracellular space, further stimulating additional ATP release from nearby cells (Elliott et al., 2009; Chekeni et al., 2010). In addition, excess glutamate within the lesion site induces increased Ca2+ influx in adjacent neurons and glia that also stimulates ATP release (Sieger et al., 2012). As a result, an elevated concentration of extracellular ATP in and around the lesion is sustained for several hours following injury (Wang et al., 2004). Local activation of both adenosine and P2X receptors have been implicated in dysregulation of intracellular calcium homeostasis and cell death induction. In particular, activation of both A1 and P2X, enables rapid cytosolic Ca2+ overload and subsequent cell death soon after mouse SCI (Wang et al., 2004; Paterniti et al., 2011; Leeson et al., 2018). Moreover, P2X receptor activation potentiates P2X-mediated cell death (Kawano et al., 2012b). Purinergic activation of microglia and macrophages within the lesion site also facilitates P2X-mediated cytotoxic cell death of oligodendrocytes (Lopez-Serrano et al., 2019) and apoptosis of many other potential cell types expressing P2X5 receptors, including spinal neurons, ependymal cells, astrocytes, and inflammatory cells (Wang et al., 2012; Gandelman et al., 2013; Marichal et al., 2016; He et al., 2017; Bidula et al., 2019). Interestingly, P2X2 is conserved and expressed ubiquitously within most tissues of zebrafish and other bony fish species (Ogryzko et al., 2019; Jumpy et al., 2020). However, the exact role of P2X2 in mediating injury-induced cell death within the spinal cord of these species has not been explored.

Figure 1 | Timeline depicting the secondary cellular injury response following mammalian spinal cord injury.

The primary mechanical trauma is exacerbated by prolonged cell death, widespread inflammation, reactive gliosis, and axonal degeneration. These events prevent successful regeneration and limit sensorimotor recovery. Created with BioRender.com with permissions and publication license.
Figure 2 | Timeline depicting the secondary cellular injury response following non-mammalian spinal cord injury.

The primary mechanical trauma induces transient cell death, controlled inflammation, reactive gliosis, neurogenesis, and axonal regeneration. These events conclude within weeks and facilitate recovery and restoration of locomotor function in non-mammalian vertebrates. Created with BioRender.com with permissions and publication license.

Figure 3 | Purinergic signaling within the spinal cord microenvironment during the early injury response.

The first several days following mammalian SCI are characterized by widespread cell death, migration of various cell types to the lesion, inflammation, and reactive gliosis. Identified roles for purinergic receptors in these processes is summarized. Created with BioRender.com with permissions and publication license. ADP: adenosine diphosphate; ATP: adenosine triphosphate; CD39: cluster of differentiation 39; Ca²⁺: calcium; CREB: cAMP response element-binding protein; ERK1/2: extracellular signal-regulated kinases 1/2; IL: interleukin; NGF2: nerve growth factor-2; NLRP1/3: NLR family pyrin domain containing 1/3; NT3: neurotrophin-3; P2X7: signal transducer and activator of transcription 3; TNF-α: tumor necrosis factor alpha; UDP: uridine diphosphate; UTP: uridine triphosphate. Created with BioRender.com with permissions and publication license.
While purines may exacerbate the initial injury by inducing cell death, there is evidence that they may also induce cell proliferation and neuroprotection. Following caudal fin amputation in zebrafish, activation of A$_2$A receptors via stimulation to inhibit regeneration cell death, which results in increased cell proliferation within the lesion beyond spontaneous levels (Rampon et al., 2014). In mammals, microglia P2Y$_2$ receptor stimulation enhanced neuronal survival following transient forebrain ischemic injury (Fukumoto et al., 2019). Interestingly, astroglial and neuronal P2$_Y$ receptors stimulation was associated with the inhibition of apoptosis through activation of ERK1/2 and Akt signaling pathways and upregulation of neurotrophins, growth factors, and anti-apoptotic genes (Chorna et al., 2004; Arthurs et al., 2008). Furthermore, recruitment of macrophages with dibutyryl tetraphosphate prior to strong ATP stimulation decreased excitotoxicity (Reigada et al., 2017). This reduced not only P2X$_4$, but also P2X$_2$, P2Y$_1$, and P2Y$_2$ expression, probably through activation of P2Y$_1$ receptors (Reigada et al., 2017). These results demonstrate how macrophages modulated by neuronal purine immune cells and is upregulated from 4–28 days following injury (Rodriguez-Zayas et al., 2010). Interestingly, stimulation of astrocyte P2X$_2$ receptors induces phosphorylation of ERK1/2, which is only increased in astrocytes expressing IL-6 (O’Almond et al., 2007). Whether P2Y$_2$, and P2Y$_2$, play similar roles in regeneratively competent vertebrates following SCI remains unknown.

**Immunne Response**

Shortly after the primary trauma, the microenvironment of the injured mammalian spinal cord is occupied and dominated by inflammatory phagocytic cells (Figure 1). Microglia, CNS-derived macrophages, undergo cell death within the first 24 hours post injury like other cell types; however, residual microglia also undergo activation and proliferation to phagocytose the spinal cord debris (Bellver-Landete et al., 2019). Indeed, rapidly proliferating microglia are the predominant phagocytes of cellular and myelin debris within the first weeks after injury (Beller-Landete et al., 2019). By 7 days, microglia proliferation peaks, and by 14 days, macrophage recruitment by neutrophils to the lesion core reaches maximal levels corresponding to the transformation of the area into a ‘lesion cavity’ (Beller-Landete et al., 2019; Kwicien et al., 2020). While few peripheral macrophages and fibroblasts occupy the lesion site at 72 hours post injury, these cells rapidly infiltrate over the course of the following week (Zhu et al., 2015) and soon outnumber microglia (Greenhalgh and David, 2014). The occurrence of phagocytic activity within the spinal cord appears immediate, and the recruitment of spinal cord microglia results in delayed myeloid cell recruitment, increased lesion size, enhanced neuronal and oligodendrocyte cell death, and exacerbated locomotor dysfunction (Bellver-Landete et al., 2019). However, harmful inflammatory cells have been found to decrease the phagocytic activity of microglia beyond the spinal cord lesion site within 1 hour post injury (Bellver-Landete et al., 2019). During early glial scar formation (5–7 days), newly recruited astrocytes as they form a ‘dense meshwork’ of intersecting and overlapping radial-like morphology, and GFAP spanning the lesion site consists of increasing astroglial proliferation, density, and eventually outnumber microglia (de la Rosa et al., 2020), and A$_2$A receptors, which similarly decrease TNF-α release (Cohen et al., 2013). Lastly, stimulation of microglial P2Y$_2$, receptors also appears to induce IL-1β, IL-6, and TNF-α release (Uli et al., 2012). In addition to P2X$_7$ and P2Y$_2$, several other purinergic receptors have also been implicated in driving cytokine release in mammals. These include macrophage P2Y$_2$, receptors, which induce an increase in IL-1β, IL-10, and a decrease in TNF-α (de la Rosa et al., 2020), and A$_2$A receptors, which also correspond with a longer anti-inflammatory response characterized by elevated expression of transforming growth factor-β (TGF-β1a) and fibroblast growth factor-β3 (TGF-β3) by 3 days following injury (Hui et al., 2014; Tsarouchas et al., 2018). This supports the view that an early pro-inflammatory response is adaptive and required for regeneration, but sustained immune responses can be detrimental to recovery.

**Reactive Gliosis**

The migration of macrophages and microglia to the lesion core is known to recruit other cell types, including perivascular fibroblasts and astrocytes, that form a multi-layer ‘glial scar’ in mammalian SCI (Figure 1). Peripheral macrophages appear to be largely responsible for the formation of an inner fibrotic scar around the core of the lesion site that is fully encircled by reactive microglia. Within the first week post SCI, astrocytes and microglia fuse to form a ‘dense meshwork’ of intersecting and overlapping radial-like morphology, and GFAP spanning the lesion site consists of increasing astroglial proliferation, density, radial-like morphology, and GFAP expression. During chronic gliosis, the astrocyte scar forms around the lesion core and promote regeneration early on, is it equally important to limit this process in chronic conditions.
Both purinergic and cytokine-mediated signaling are involved in astrocyte reactivity and proliferation (Figure 3). How factors of these signaling families contribute to astroglial scar formation appears to be part of a delicate balance of local interactions between astrocytes and microglia. Purinergic signaling via P2Y receptors has been shown to play a significant role in astrocytic proliferation (Quintas et al., 2018). However, both the presence of microglia and IL-1α/ TNF-α prevented P2Y2, activation and astrocyte proliferation in response to injury. Importantly, authors propose that this loss of P2Y2 receptors may result in the release of IL-1α and TNF-α, inhibition of astrocyte P2Y2, receptors, and prevention of astrocyte proliferation. It is likely that while microglia draw astrocytes to the lesion site, they also act to modulate the degree of astrocyte proliferation in response to elevated proinflammatory levels. Indeed, P2Y2 receptor proliferation following SCI peaks within 3–5 days (Wanner et al., 2013): a point in time when proinflammatory IL-1α and TNF-α expression is also strongly upregulated (Kwicien et al., 2020).

In conjunction with proliferation, cytokine signaling is also involved in regulating astrocyte reactivity and cell survival. Microglial secretion of cytokines IL-6, IL-1α, and TNF-α promotes astrocyte transformation from a basal to a reactive phenotype by the concurrent downregulation of astrocyte P2Y2 expression and upregulation of STAT3 phosphorylation (Shinozaki et al., 2017). In this study, P2Y2 knockdown (KD) in astrocytes promotes astrocyte proliferation. GFAP expression, and STAT3 phosphorylation, as a smaller injury core, decreased neuronal cell death, and decreased immune cell infiltration (Shinozaki et al., 2017). In contrast, others have demonstrated that astrocyte P2Y2 upregulation protects against oxidative stress induced by traumatic brain injury (TBI) in mice (Fujita et al., 2009; Oh et al., 2010). It is likely that the apparent conflicting roles of P2Y2 stem from the action of additional P2Y receptors also expressed within the glial scar.

In addition to other proinflammatory cytokines, IL-1β expression is also elevated within the first week following SCI in mice (Kwicien et al., 2020) and treatment of mouse primary striatal astrocytes or mouse primary cortical neurons with IL-1β increases P2Y2, mRNA expression (Stella et al., 1997; Peterson et al., 2013). Similar to P2Y2, P2Y1 receptor stimulation has also been shown to induce astrocyte proliferation and STAT3 activation (Chorna et al., 2004; Wu et al., 2018). Thus, P2Y2 may work to compensate for P2Y1-mediated astrocyte proliferation, in myelina knockouts, to promote astroglial injury. Interestingly, treatment with pan P2Y antagonists, which target both P2Y1 and P2Y2 receptors, significantly reduce GFAP expression within the lesion site and exacerbate secondary cell death (Rodriguez-Zayas et al., 2012), demonstrating the overall neuroprotective role of these factors and the astroglial scar.

While reactive glia in regeneratively competent teleosts is reflective of that in mammals, key differences do exist (Figure 2). Similar to mammals, goldfish and zebrafish generate a fibrinous scar within 1–3 weeks following a spinal cord hemisection. Unlike in mammals, IL-18 increases P2Y1, mRNA expression (Stella et al., 2012). Glial cells with elevated GFAP expression surround the fibrinous scar but do not display a hypertrophied morphology (Goldshmidt et al., 2012; Takeda et al., 2015; Herman et al., 2018). In mammals, reactive gliosis and scar formation is observed in all species (Goldshmit et al., 2012). RG cells are present in the zone of goldfish fibrinous scar at 3 weeks post injury but its reduction by 6 weeks aligns with the increase in axon infiltration (Takeda et al., 2015).

Filibrast growth factor (FGF) signaling appears to be particularly important for the formation of the glial bridge. Inhibition of FGF in zebrafish following SCI results in significant reductions to GFAP expression, as well as a decrease in axonal regeneration and functional recovery (Goldshmidt et al., 2012). Treatment of primary astrocyte cultures derived from postnatal day 14 marmoset (Callithrix jacchus) cerebral cortex with human recombinant FGF2 promotes glial cell closure in an in vitro assay by increasing proliferation, migration, and astrocyte transformation from a stellate/multipolar to an elongated/bipolar morphology (Goldshmidt et al., 2012). These findings were replicated in a mouse model of SCI where application of exogenous FGF2 dampened the neuroinflammatory response, decreased levels of CSPG, and promoted the phenotypic transformation of astrocytes into a bipolar morphology resembling the glial bridge in teleost species (Goldshmidt et al., 2012). Interestingly, exogenous FGF2 also induces STAT3 phosphorylation and hippocampal induced astroglia. On one hand, P2X2 activation enhances proliferation of astrocytes induced by FGF2; while P2X2 stimulation inhibits proliferation by inducing a state of growth arrest (Neary et al., 2008). In addition to FGF2, P2X2 also enhances astrocytic reactive tissue growth following SCI, accelerates glial bridge formation, enhances axonogenesis, and promotes functional recovery in zebrafish (Mokalled et al., 2016). Purinergic-mediated CTGFα secretion via P2X2 receptors also supports a role for purines in promoting tissue repair (Chopp and Wang, 2007) and thus, understanding purinergic influences in mammalian and non-mammalian injury-induced glial.

Neurogenesis and Axonal Regeneration

In the mid 1990s, a latent neural stem cell niche lining the rodent central canal was discovered. It was found that within this niche, mammalian spinal epidural cells give rise to new neurons and glia in vitro: a feat not normally present in vivo. The exception to this is the result of a direct injury, but even then, migration is almost absent, proliferation is minimal, and excitability testing for a week or two is consistent with that of very poorly derived astrocytes (Ren et al., 2017). Yet transplantation of spinal epidural cells into the dentate gyrus of the hippocampus, a neurogenic niche within the adult mammalian brain, induced differentiation of these cells into neurons (Shinogawa et al., 2015). This demonstrated the existence of a permissive microenvironment and prompted much work into identifying potential cues responsible for determining the cell fate of endogenous spinal epidural cells (Becker et al., 2018; Liu and Chen, 2019).

Apart from other mammalian species, the existence of injury-induced spinal progenitors in humans has been recently challenged. One study examining the spinal cord in human embryos that contain spinal cord segments in rodents, the central canal closes caudal to the brainstem beyond 18 years of age (Garcia-Ovejero et al., 2015). The authors show that the ependymal layer is replaced by a dense accumulation of GFAP astroglial processes, ependymocytes, and perivascular pseudorosettes. Furthermore, expression of stem cell markers is highly limited within this region (Garcia-Ovejero et al., 2015). In conjunction, a post-mortem study of human SCI patients (aged 33–88) found no evidence for injury-induced proliferation of cells within the ependymal region (Paniagua-Torija et al., 2018). Together, these studies suggest that morphological and molecular profiles in adult humans may be quite unique compared to other vertebrate species, and approaches to inducing endogenous neural progenitor cells in mammals should be more applicable to both younger individuals and those with higher level injuries.

Another regenerative strategy to encourage functional recovery following SCI is the re-growth of damaged axons or compensatory axonal sprouting. Unfortunately, axonal regeneration and sprouting are also highly restricted in mammalian species (Figure 1). Within 30 minutes following injury, neurons undergo rapid and symmetrical proximal and distal axon end fragmentation, which is followed by a period of slow axonal retraction until ~30 hours post transection (Kerschensteiner et al., 2005). After this point the injury extends beyond the proximal axon ends. The distal axon ends begin to undergo Wallerian degeneration mediated by infiltrating peripheral macrophages for several days to weeks (Evans et al., 2014). Although limiting macrophage migration to the injury site does not promote axonal survival, restricting these cells to the distal axon ends in rats (Kerschensteiner et al., 2019). Interestingly, approximately 30% of transected axons do undergo compensatory sprouting at their proximal ends (Kerschensteiner et al., 2005). While these sprouts grow relatively rapidly and straight within the first two weeks, they quickly lose directional and are unable to grow across the injury site. This is largely due to the presence of numerous growth inhibitory environmental cues at the injury site that lead to dystrophic growth cone formation and prevent axonal regeneration (Filouš and Schwab, 2018).

In contrast to mammals, adult teleost fish and urodèles amphibians achieve functional recovery by not only successfully replacing lost neurons, but also by regenerating damaged axons. In zebrafish (Figure 1), in situ hybridization of injury marker mRNAs demonstrates that in contrast to mammals, spinal cord transection in newts results in axonal retraction and dystrophic growth cone formation following Mauthner cell axotomy, which is followed by additional retraction and further degeneration of the axon (Evans et al., 2014). Although limiting macrophage migration to the injury site does not promote axonal survival, restricting these cells to the distal axon ends in rats (Kerschensteiner et al., 2019). Interestingly, approximately 30% of transected axons do undergo compensatory sprouting at their proximal ends (Kerschensteiner et al., 2005). While these sprouts grow relatively rapidly and straight within the first two weeks, they quickly lose directional and are unable to grow across the injury site. This is largely due to the presence of numerous growth inhibitory environmental cues at the injury site that lead to dystrophic growth cone formation and prevent axonal regeneration (Filouš and Schwab, 2018).

The regeneration of axons across the lesion site in these non-mammalian species occurs simultaneously with injury-induced neurogenesis. In larval zebrafish, acute axonal degeneration occurs within the first 30 minutes following Mauthner cell axotomy, which is followed by additional retraction and Wallerian degeneration that concludes by 24 hours (Hu et al., 2010). Remarkably, they found that by 96 hours post injury axons are sprouting (Paniagua-Torija et al., 2019), and salamanders (Joven and Simon, 2018). Morphologically, they line the central canal and extend radial processes out to the pial surface. Functionally, they regenerate axons beyond the proximal axon ends (Barry and McDermott, 2005; Xing et al., 2018). However, they undergo terminal differentiation into astrocytes during early postnatal development and are relatively absent from the adult spinal cord (Barry and McDermott, 2005).

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Identifying the factors and conditions present in regeneratively competent species, particularly at the later time points; therefore, may be of most use in promoting axonal regeneration in mammals.

Regardless of their ability to achieve functional recovery, non-mammalian vertebrate CNS regeneration does not result in a return to uninjured conditions. First, newly generated interneurons in larval zebrafish appear morphologically and functionally distinct from their uninjured counterparts following SCI (Vasudevan et al., 2021). In addition to having a smaller soma, they have a higher input resistance, a more depolarized resting membrane potential, lower firing frequencies, and lower spike frequencies; however, they are sufficient to restore function. Second, only a subset of neurons undergo axonal regeneration in non-mammalian species studied to date, including newts (Zukor et al., 2011), goldfish (Takeda et al., 2015), and zebrafish (Goldshmit et al., 2012). Finally, while some regenerating axons travel along their original trajectories, as observed in newts (Zukor et al., 2011) and larval zebrafish (Hu et al., 2018), many take novel routes as seen in adult zebrafish (Becker and Becker, 2001). Notably, regenerative ability appears to decline with age in both mammals (Geoffroy et al., 2016) and non-mammalian vertebrates (Edelmann et al., 2013). Further examination of the post injury cytoarchitecture, the regulatory cues present in the microenvironment, and the changes to both over time are all important considerations in understanding regeneration in these species.

The role of purinergic signaling in injury-induced neurogenesis and axonal regeneration is quite limited (Figure 4). Expression of several purinergic receptors have been identified in mammalian spinal ependymal cells, including P2Y1, P2Y2, P2X3, and P2X2 (Gomez-Villafuertes et al., 2015). In response to SCI, the expression of P2Y7 receptors are downregulated, whereas expression of P2Y2, P2X3, and P2X2 receptors is upregulated (Gomez-Villafuertes et al., 2015). How the differential expression of these subtypes in ependymal cells influences their response to injury is largely unknown, but has been shown to play roles in both proliferation and differentiation of other cell types. For instance, P2Y2 receptor activation induces proliferation of astrocytes, RG, and tanyocytes within the ependymal cell lining of the third ventricle (Weissman et al., 2004; Quintas et al., 2018; Recabal et al., 2021). Similarly, P2X2 receptor expression has been associated with enhanced growth factor expression (e.g. brain-derived neurotrophic factor) and indirectly promoting ependymal cell proliferation (Xu et al., 2018; Ma et al., 2019; Su et al., 2019). In terms of differentiation, P2Y13 expression accompanies glutamatergic differentiation of embryonic stem cells, and P2X7 stimulation regulates neuronal differentiation of both embryonic and adult neural progenitor cells (Tsao et al., 2013; Glaser et al., 2014; Uda et al., 2016; Leeson et al., 2018). It is likely that the balance and timing of purinergic receptor expression influences the response of ependymal cells to SCI, but further work is needed to determine their roles.

In addition to those mentioned above, purinergic receptors P2Y2, P2Y13, and A2A have also been shown to regulate progenitor cell proliferation, prevent secondary injury, or promote functional recovery. In a recent study of P2Y2 knockout mice, there was a significant reduction in the number of proliferating neural progenitor cells within the subgranular zone of the dentate gyrus of the hippocampus and the subventricular zone of the lateral ventricles, demonstrating that P2Y2 receptor expression is important for progenitor cell proliferation within the adult mammalian brain (Ali et al., 2021). Meanwhile, P2Y2 knockout mice displayed enhanced progenitor cell proliferation within the dentate gyrus of the hippocampus in early and late adulthood, as well as an increase in neurogenesis (Stefani et al., 2018). Interestingly, inhibition of vesicular nucleotide transporter in postnatal cerebellar cells inhibited quiescence and self-renewal to drive cell-cycle exit toward a neurogenic fate (Paniagua-Herranz et al., 2020). Mechanistically, vesicular nucleotide transporter is responsible for promoting vesicular ATP storage; therefore, inhibition of this transporter decreases ATP signalling and suggests that variation in purinergic ligand alone is sufficient to influence neurogenesis (Paniagua-Herranz et al., 2020). Following SCI, activation of the A2A receptor increases Wnt3a and β-catenin mRNA expression in mammals resulting in reductions of inflammation and neuronal cell death (Irrera et al., 2018). While a general reduction in Wnt/β-catenin signaling is typically observed with mammalian SCI (Gonzalez-Fernandez et al., 2014; Irrera et al., 2018), this pathway appears upregulated following SCI in zebrafish and in sea lamprey (Briona et al., 2015; Strand et al., 2016). Various interventions targeting the upregulation of canonical Wnt/β-catenin signaling, including A2A antagonism, following mammalian CNS injury have been widely successful in limiting neuroinflammation and secondary cell death, and enhancing functional recovery (Shruster et al., 2012; Irrera et al., 2018; Xu et al., 2019).

Figure 4 | Purinergic signaling within the spinal cord microenvironment during the chronic injury response. After the first week following mammalian SCI, reactive astrocytes become scar forming, ependymal and neural progenitor cells fail to undergo injury-induced proliferation and neuronal differentiation, and astrocytes continue to degenerate. Identified and hypothesized roles for purinergic receptors in these processes is summarized. Created with BioRender.com with publication permissions and publication license.

CD39: cluster of differentiation 39; Ca²⁺: calcium; CREB: cAMP response element-binding protein; ERK1/2: extracellular signal-regulated kinases 1/2; IL: interleukin; NGF2: nerve growth factor-2; NLRP1/3: NLR family pyrin domain containing 1/3; NT3: neurotrophin-3; STAT3: signal transducer and activator of transcription 3; TNF-α: tumor necrosis factor alpha; UDP: uridine diphosphate; UTP: uridine triphosphate; Wnt: wingless-related integration site. Created with BioRender.com with permissions and publication license.
Corrigendum

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