Inflammation and axon regeneration
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Introduction
Following injury to the central nervous system (CNS), neurons are generally unable to regenerate damaged axons over long distances and have only a limited capacity to extend axon collaterals to help form compensatory circuits. These limitations have dire consequences for victims of traumatic brain injury, stroke, spinal cord injury (SCI) or neurodegenerative disease. Even in the peripheral nervous system (PNS) in which appreciable regeneration can occur, it is often incomplete and in some cases results in neuropathic conditions. Consequently, there has been a great deal of interest in identifying factors that limit axon regeneration and collateral sprouting in the injured nervous system, with recent attention focused on cell-extrinsic inhibitors of axon growth \([1–3]\) and in regulating neurons’ intrinsic growth state \([4–7]\). However, another important and often overlooked issue is the role that innate immune cells play in regulating axon regeneration.

The interactions between the immune system and the nervous system are extensive and complex. We will not attempt to review all of these interactions here, but will focus on what has been learned about the role of inflammation on axon regeneration since the appearance of a review with a similar title some years ago \([8]\). We refer the reader to excellent recent reviews for other topics such as neuroimmune interactions in neurodegenerative diseases (e.g. Parkinson’s, Alzheimer’s, ALS) \([9]\), cognitive disorders and depression \([10]\), pain \([11,12]\), multiple sclerosis \([13]\) and neurogenesis \([14]\).

Nerve injury invariably elicits inflammation, a complex series of molecular and cellular events that culminates in the recruitment of circulating proteins and white blood cells (leukocytes) to the injury site \([15]\). Typically, innate immune cells (e.g. neutrophils, monocytes, dendritic cells) are recruited within hours to days after injury, with adaptive immune cells (e.g. T and B lymphocytes) infiltrating after several days. Neutrophils are potent bactericidal cells that also secrete chemokines that recruit monocytes. How or if neutrophils influence axon regeneration is unknown. Monocytes differentiate into macrophages, which are potent secretory immune cells that regulate additional leukocyte recruitment, revascularization and extracellular matrix remodeling, that is, functions that are important for successful...
nerve repair. Macrophages also produce cytokines that influence the recruitment, activation and survival of lymphocytes. Like neutrophils, the precise role played by lymphocytes in nerve regeneration is unclear; however, genetic deletion of T or B lymphocytes impairs neuron survival and regeneration of injured peripheral nerves [16,17].

Peripheral nervous system regeneration
Unlike the situation in the CNS, neurons are able to regenerate injured axons within the PNS. This process results in near-complete restoration of function in mice and rats, but is often incomplete in man [18]. Therefore, understanding the mechanisms that underlie PNS regeneration could have significant clinical implications.

Inflammation plays an essential role in peripheral nerve regeneration. Following injury to a peripheral nerve, axons distal to the injury site begin to degenerate, inflammatory cells accumulate at the injury site and along the nerve and myelin debris is removed, clearing a path for new axons to regenerate [19]. Myelin clearance fails if circulating macrophages are prevented from reaching the damaged nerve [20]. The link between inflammation and peripheral nerve regeneration is further strengthened by a recent study in which cells of the myeloid lineage were genetically altered in mice to express a 'suicide gene', viral thymidine kinase [21]. Thymidine kinase phosphorylates the prodrug ganciclovir, creating a toxic product that kills thymidine kinase-positive cells. Infusion of ganciclovir nearly eliminated microglia, neutrophils and macrophages in this study, and as a result, myelin debris was not removed efficiently distal to the injury site and regeneration was severely attenuated. In addition, revascularization of the distal nerve was inefficient and injury-induced expression of several neurotrophic factors [NGF, BDNF, neurotrophin-3 (NT-3), NT-4/5] was attenuated at the injury site. These trophic factors could contribute to the ability of peripheral neurons to regenerate their axons. On the contrary, several changes in gene expression that normally accompany axon regeneration, including increased expression of GAP-43, CAP-23 and Tu.1 tubulin, were seen even in the absence of a frank regenerative response. Regeneration of injured peripheral nerves is also influenced by cells and humoral mediators of adaptive immunity. In mice deficient in B lymphocytes and, therefore, antibodies, macrophage recruitment, myelin clearance and axon regeneration were delayed after a crush injury of the sciatic nerve [17]. Together, these studies demonstrate that myeloid and lymphoid cells collaborate to positively influence many aspects of peripheral nerve repair.

Within the CNS, although most neurons cannot regenerate axons through or around sites of injury, some can regenerate injured axons through a peripheral nerve graft [22,23]. This phenomenon is also blocked by suppressing myeloid cells [21].

In contrast to the ability of sensory neurons to regenerate their peripherally directed axons after injury, they cannot regenerate the branch of their axons that projects through the dorsal roots into the spinal cord. However, if the peripheral branch is injured prior to injuring the central branch, sensory neurons are transformed into an active growth state, augmenting their ability to regenerate their centrally directed axons [24]. Injecting a pro-inflammatory agent into dorsal root ganglia (DRG) mimics the effects of a peripheral conditioning lesion [25,26]. In addition, if intraganglionic inflammation is combined with enzymatic digestion of chondroitin sulfate proteoglycans (CSPGs) at the dorsal root entry zone, sensory neurons can regenerate dorsal root axons into appropriate areas of the spinal cord and restore spinal reflexes [26]. The signals that mediate the growth-enhancing effects of intraganglionic inflammation are unknown, but could include some of the same molecules through which inflammation normally enables DRG neurons to regenerate their peripheral axons. The effects of intraganglionic inflammation are not entirely positive, however, and also cause a decrease in the number of neurons within the DRG [27].

Optic nerve
The optic nerve is an anatomically simple, experimentally accessible pathway that has long been studied for insights into the factors that limit regeneration in the CNS. Under normal circumstances, retinal ganglion cells (RGCs), the projection neurons of the eye, undergo a transient sprouting reaction at the site of nerve injury, but cannot extend their axons over long distances [28]. Beginning shortly after injury, a zone surrounding the crush site loses its astrocytes and oligodendrocytes and becomes filled with macrophages that express EphB3 [29,30]. EphB3 acts upon a cognate ephrin, EprinB3, located on the terminals of RGC axons, to stimulate local sprouting [30]. Both EphB3 and EprinB3 can act as either ligands or receptors, and in this instance, EphB3 serves as a ligand and EprinB3 as a receptor.
It is unclear why infiltrating macrophages do not clear axonal debris and myelin distal to the injury site as efficiently in the optic nerve as in peripheral nerves. In general, slow myelin clearance is found throughout the CNS, and has been proposed as being one of the factors that contributes to the difference in regeneration between the CNS and PNS. In unfixed longitudinal sections through the optic nerve, the vicinity around the injury site is permissive for neurite outgrowth, and the remainder of the nerve becomes permissive if macrophages are applied to it, presumably by removing myelin debris [31]. When macrophages that had been pre-exposed to peripheral nerve were placed in the cavity created by a complete transection of the optic nerve, RGCs were reported to regenerate axons beyond the gap [32]. However, these findings were not based upon anterograde labeling or direct visualization of regenerating axons traversing the injury site, but instead used retrograde labeling with a dye that may have diffused from the injection site to label axons that remained proximal to the injury site.

Although studies going back to the early 20th century showed that RGCs can regenerate severed axons through a peripheral nerve graft [28,33,34], regeneration through the optic nerve itself was long considered to be impossible. However, in 1996, Berry et al. [35] found that implanting a fragment of peripheral nerve into the posterior chamber of the eye enabled RGCs to regenerate axons for considerable distances down the injured optic nerve. This effect was initially attributed to trophic factors secreted by Schwann cells, but the PN grafts also contained high levels of inflammatory cells, and subsequent studies have shown that the induction of intraocular inflammation by other means is sufficient to stimulate appreciable levels of regeneration [36–39]. Oncomodulin (Ocm), a small calcium-binding protein, plays a major role in mediating this phenomenon. Ocm was first shown to be an axon-promoting factor for RGCs that is secreted by macrophages in culture [38,40] and was then found to be expressed at high levels by blood-borne cells that infiltrate the posterior chamber of the eye upon the induction of inflammation [41]. Ocm diffuses from the posterior chamber of the eye into the retina, wherein it binds to a high-affinity receptor on RGCs in a cAMP-dependent fashion [40,42*]. When released from a slow-release polymer together with a cAMP analogue, Ocm produces nearly as much regeneration as intraocular inflammation; conversely, blocking the effects of Ocm immunologically or with a peptide antagonist suppresses the effect of intravitreal inflammation on axon regeneration, although not on RGC survival (Fig. 1) [36,40,41,43]. These studies show that Ocm is integral for inflammation-induced regeneration, and that additional factors also play a role by increasing intracellular cAMP levels and enhancing RGC survival [41,42*]. The latter include ciliary neurotrophic factor and leukemia inhibitory factor [39,44,45].

The rise in Ocm levels that occurs shortly after inducing intraocular inflammation appears to be due to the influx of both neutrophils and macrophages into the eye [46*]. When intraocular inflammation is combined
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with treatments that enhance regeneration by other mechanisms, many RGCs are able to regenerate axons through the glial scar and myelin debris at the injury site, with some extending axons as far as the thalamus (Fig. 1) [42^,43,47]. T lymphocytes may also contribute to inflammation-induced axon regeneration, as T-cell depletion (via thymectomy) increases RGC survival after nerve crush but diminishes zymosan-induced macrophage invasion and the ability of RGCs to regenerate injured axons through a peripheral nerve graft [48].

Spinal cord

The effects of inflammation on axon regeneration in the spinal cord are highly complex and include effects that are largely destructive near the epicenter of inflammation but proregenerative at greater distances. These divergent effects are consistent with the profile of molecules expressed by innate immune cells, including factors that are toxic to neurons (e.g. reactive oxygen and nitrogen species, interleukin-1, tumor necrosis factor-α) and multiple trophic factors.

Rabchevsky and Streit [49] showed that brain microglia embedded in a Gelfoam matrix promote the ingrowth of axons. Growing axons were associated with the infiltration of Schwann cells and laminin deposition. On the contrary, injection of zymosan into the CNS parenchyma activates resident microglia and induces an influx of blood-borne macrophages, leading to a withdrawal of astrocytes, axonal damage, cavitation and deposition of growth-inhibitory CSPGs [50,51]. These toxic effects appear to be mediated by soluble factors derived from macrophages as well as direct physical interactions between macrophages and dystrophic axons [52]. Accordingly, macrophage depletion diminishes astrocyte withdrawal, cavitation and axon die-back after zymosan injection or SCI [27,53]. Under normal circumstances, the negative effects of inflammation on axon sprouting appear to be limited in extent by NG2^+ progenitor cells that enter the area vacated by other cells and provide a favorable substrate for axon sprouting [54^].

Injecting zymosan into the parenchyma of the spinal cord also has potent trophic effects, enabling DRG neurons implanted several millimeters away from the injection site to extend lengthy axons toward it (Fig. 2) [27]. However, as axons approach the site of inflammation, they atrophy and are engulfed by macrophages [27]. These results imply the simultaneous existence of macrophage-derived trophic factors that either act directly on axons over long distances or induce other cells to produce growth-promoting factors, together with cytotoxic factors and direct macrophage–axon interactions that lead to axonal degeneration close to the site of inflammation [27]. In most inflamed tissues, activated macrophages exhibit at least two phenotypes, an M1 or classically activated phenotype that produces bactericidal and neurotoxic reactive oxygen and nitrogen species, and an M2 or ‘alternatively’ activated phenotype that is associated with wound healing and immune regulation. After SCI, the M2 response occurs early and is transitory, whereas the M1 response persists indefinitely [55]. In cell culture, M2 macrophages produce factors that promote the formation of long axons, whereas M1 macrophages produce factors that are cytotoxic to neurons and cause them to extend short neuritic sprouts [55]. The sequence of macrophage activation seen after SCI is different from what is typically seen in wound healing, in which M1 macrophages appear first followed by M2 macrophages that promote healing.

Macrophages preexposed to injured peripheral nerve, when injected into the lesion site and distal parenchyma of transected rat spinal cord, have been reported to promote the regrowth of severed axons across the gap and partially restore hindlimb function after 19 weeks [56]. These gains were eliminated when the same area was relesioned, suggesting that recovery was not due to spared tissue or local circuit activity. However, a second surgery might also eliminate nerve fibers that had been spared initially and cause sufficient trauma to reduce the effects of local circuit activity. A single attempt to replicate this approach in a dog model failed to demonstrate axon growth-promoting effects of autologous macrophage transplantation [57]. In a phase I clinical trial begun in 2000, surgical implantation of autologous macrophages was found to be feasible in acute SCI patients and without adverse effects [58]. A phase II trial was initiated in 2003, but was suspended prematurely 3 years later, reportedly because of financial limitations. A recent
The identity of key toxic and trophic molecules involved, may prove to be in its infancy, as we are only first learning regeneration. Understanding the interactions among cells and others promoting cell survival, axon sprouting and components causing tissue damage and neural death, the nervous system is highly complex, with some As reviewed here, the immune response to injury in Conclusion

Inflammation can augment the ability of exogenous trophic factors to promote axon growth after SCI. Following a unilateral transection of the corticospinal tract (CST), overexpression of NT-3 by motor neurons on the denervated side of the spinal cord promotes the growth of axon collaterals from the intact CST over to the denervated side [64]. This effect is seen if NT-3 is overexpressed during the time at which inflammation was occurring at the injury site, but not later. Depletion of innate and adaptive immune cells eliminates the ability of NT-3 to promote sprouting at early time points, whereas reactivation of an inflammatory response restores the effectiveness of NT-3 beyond the period in which it is otherwise effective [64].

Acknowledgments

Conflicts of interest

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The authors declare that they do not have competing financial interests.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

•• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).


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This article demonstrates a role for the adaptive immune system in modulating macrophage invasion and peripheral nerve repair.


David S, Bouchard C, Tsatas O, Giftochristos N. Macrophages can modify the growth capacity of sensory neurons combined with the degradation of inhibitory proteoglycans allows functional regeneration of sensory axons through the dorsal root entry zone in the mammalian spinal cord. J Neurosci 2000; 20:4615–4626.


Fischer D, He Z, Benowitz LI. Counteracting the Nogo receptor enhances optic nerve regeneration if retinal ganglion cells are in an active growth state. J Neurosci 2004; 24:1646–1651.


This article shows that neutrophils are a major source of the growth-promoting molecule Ocm.


This article demonstrates that the local effects of inflammation on axon atrophy are normally countered by NG2-positive glial precursor cells.
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An up-to-date review on the effects of current therapies for SCI on the immune system.
Please check the authors’ affiliation details for correctness. - correct

Please check the corresponding author’s name and the correspondence details for correctness. – please add Dr. Popovich as a second corresponding author as follows:
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Please provide the full form of the following acronyms: ALS, NGF, BDNF, GAP-43, CAP-23, EphB3, PN, NG2, NogoA, NgR, EGFP (now corrected in text)
ALS, Amyotrophic lateral sclerosis
NGF, Nerve growth factor
BDNF, Brain-derived neurotrophic factor
GAP-43: This is the name that is used in the literature. However, if you require the full name, it is “Growth-associated protein-43”
CAP-23: As above, this is the common name for it in the literature and it is not spelled out. However, if you require the full name, it is “Cortical cytoskeletal associated protein-23”
EphB3: this is the official name of the protein
PN, peripheral nerve
NG2: this is the full name of the cell type.
NogoA, (full name)
NgR, Nogo receptor
EGFP, enhanced green fluorescent protein

Please check the section heading for correctness. Pls change to “Conclusions”

Please check the editors name and provide the published location in Ref. [5]. Editors’ names should be corrected to: Kordower JH and Tuszynski MH. The book was published in San Diego, CA

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46. (this is the correct form of the citation)

As the following references are outside the review period, bullets and annotations have been deleted as per style. Refs. [27,41,55,60]. OK