Neuroinflammation and optic nerve regeneration:

where do we stand in elucidating underlying cellular and molecular players?

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1 ABSTRACT

Neurodegenerative diseases and central nervous system (CNS) trauma are highly irreversible, in part because adult mammals lack a robust regenerative capacity. A multifactorial problem underlies the limited axonal regeneration potential. Strikingly, neuroinflammation seems to be able to induce axonal regrowth in the adult mammalian CNS. It is increasingly clear that both blood-borne and resident inflammatory cells as well as reactivated glial cells affect axonal regeneration. The scope of this review is to give a comprehensive overview of the knowledge that links inflammation (with a focus on the innate immune system) to axonal regeneration and to critically reflect on the controversy that still prevails about the cells, molecules and pathways that are dominating the scene. Also, a brief overview is given of what is already known about the crosstalk between and the heterogeneity of cell types that might play a role in axonal regeneration. Recent research indicates that inflammation-induced axonal regrowth is not solely driven by a single cell-population but probably relies on the crosstalk between multiple cell types and the strong regulation of these cell populations in time and space. Moreover, there is growing evidence that the different cell populations are highly heterogeneous and as such can react differently upon injury. This could explain the controversial results that have been obtained over the past years. The primary focus of this manuscript is the retinofugal system of adult mammals, however, when relevant, insights or examples of the spontaneous regenerating zebrafish model and spinal cord research are added.

2 Introduction

2.1 Failing regeneration in the adult mammalian central nervous system

The central nervous system (CNS) of adult mammals lacks the capacity to replace lost neurons or to regenerate its axons. Over the past decades, intensive research efforts have been focusing on different strategies to protect and/or repair the CNS, however, no successful therapies are available yet. As such, damage to the CNS, due to trauma or neurodegenerative disease, drastically diminishes life quality and forms a growing burden in our aging society. Complete functional recovery after CNS damage to the CNS injury is a complex matter and requires manipulations that focus on various aspects of the repair process: (1a) triggering endogenous stem cells to form new neurons (neurogenesis), (1b) protecting the (damaged) still surviving neurons from undergoing cell death (neuroprotection); (2) inducing a robust initiation of axonal outgrowth; (3) providing cues for axonal elongation and a correct navigation of the axons to their target neurons; and finally (4) resuming the formation of functional synapses. Thus, regardless of the first step being neurogenesis or neuroprotection, an essential part during this repair process is axonal (re)growth and synapse restoration to reestablish a functional neuronal circuit ¹.

In this review, we will compile the forthwith knowledge about the cells, molecules and pathways that determine inflammation-induced axonal regeneration in the visual system of adult mammals. The overview will be complemented with insights obtained within the spontaneously regenerating zebrafish and from spinal cord injury models since both are commonly used model systems that can help to understand the link between inflammation and axonal regeneration.

2.2 The retinofugal system as a model to study axonal regeneration

The retinofugal system is a powerful model system to investigate the mechanisms underlying (restricted) regenerative capacity, to study the molecules that affect axonal regeneration and to develop new strategies to stimulate axonal regeneration in the adult mammalian CNS^{2,3}. After all, (1) the optic nerve and retina are the most accessible regions of the CNS; (2) their morphology and function are well characterized; (3) the axons of the optic nerve originate from a single population of projection neurons, the retinal ganglion cells (RGCs); (4) the typical organization of the glial cells facilitates the study of glial reactivity; (5) RGCs can be manipulated with low-invasive techniques; and (6) the vitreous body can be used as a reservoir for the administration of drugs, molecules and recombinant genes^{3,4}.

The optic nerve crush and optic nerve transection paradigms are widely used in rodents to induce local axonal damage and investigate RGC survival and axonal regeneration⁴, as well as glial scar formation and inflammation². Importantly, optic nerve lesions not only induce acute inflammatory processes at the site of injury (e.g. formation of a glial scar) but also in the retina, where the RGC somata reside. Indeed, reactive microglia, astrocytes and infiltrating neutrophils and macrophages in the retina have been suggested to take part in the process of secondary degeneration, which contributes to spreading of damage beyond the initial (primary) lesion site^{5,6}. In rodents, although the damaged axons exhibit an early growth response, this response is abortive and is followed by axonal degeneration and ultimately neuronal cell death⁷. Onset of optic nerve crush-triggered retinal neurodegeneration is situated between 4 and 5 days after injury and progressively evolves until 21 days after injury, with an RGC survival rate that is strongly dependent on the experimental procedure (e.g. crush versus transection,

distance of injury to the optic nerve head, duration and force of the injury, visualization and quantification method)^{8,9}.

In contrast, in zebrafish subjected to optic nerve crush, spontaneous regeneration with full functional recovery takes place: almost all RGCs survive¹⁰ and, after an initial injury response of less than 1 day, RGCs prepare for axonal outgrowth (between 1 and 7 days after injury), elongate their axons towards their brain targets (day 5-18) and reestablish synaptic contacts in the optic tectum (day 7-25)^{2,11-13}.

2.3 Hurdles for axonal regeneration in the adult mammalian central nervous system

Over the past decades, several factors underlying the limited axonal regeneration capacity in the adult mammalian CNS have been identified. First of all, there is a strong extrinsic inhibitory environment in the injured CNS due to the myelin debris left behind after axonal degeneration and the production of myelin-derived inhibitory factors (e.g. neurite growth inhibitor (Nogo A), oligodendrocyte-myelin glycoprotein (OMgp) and myelin-associated glycoprotein (MAG))^{3,14,15}. Myelin debris is also an inflammatory modulator affecting the inflammatory response by directly influencing the phagocytosing macrophages and microglia. In most cases, it is proinflammatory, but the effect depends on the cellular and environmental conditions under which the debris is phagocytosed¹⁶. Indeed, secondly, a prominent reactivation of astrocytes and microglia and the influx of blood-borne inflammatory cells at the site of CNS damage, induce a neuroinflammatory response resulting in the formation of a glial scar, which represents an important barrier for axonal regrowth. The activated myeloid and glial cells express various cytokines and growth factors triggering the release of inhibitory molecules at the glial scar, such as Semaphorin 3A, Slit-1, Tenascin-R, chondroitin sulphate

proteoglycans (CSPGs), neurocan, versican, and neuron-glia antigen 2 (NG2))^{14,17–24}. A third process limiting axonal regeneration occurs in the retina, where neurotrophic factors, e.g. brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), glial-derived neurotrophic factor (GDNF), known to be important to support axonal regeneration, seem insufficiently expressed in the adult CNS^{25–29}. Finally, mature neurons have a low intrinsic growth potential due to a reduced expression of growth factors like growth-associated protein 43 (GAP-43), and small intracellular signaling molecules, e.g. cyclic adenosine monophosphate (cAMP))^{15,30}.

2.4 Strategies to induce long-distance axonal regeneration

Since the 80s, several strategies have been developed to induce axonal regeneration following optic nerve injury. First, researchers tried to achieve axonal regeneration by counteracting inhibitory ligands derived from myelin and the glial scar by e.g. administering antibodies against NogoA³¹, introducing a dominant-negative form of NgR³² or inactivating RhoA using C3 ribosyltransferase³³. Yet, these approaches had a relatively low success with a limited number of axons regenerating beyond the lesion site^{3,15,32,34}. More success has been attained through grafting of a peripheral nerve transplant at the optic nerve lesion site. The latter not only serves as a permissive substrate through which the axons can regenerate, the Schwann cells surrounding the peripheral nerve also produce neurotrophic factors, hence this approach tackles both extrinsic and intrinsic barriers to regeneration^{35,36}. Of note, also the inoculation of a peripheral nerve transplant in the vitreous cavity resulted in increased axonal regeneration in the optic nerve, possibly due to neurotrophic support that promotes RGC survival and axonal regrowth beyond the glial scar. A similar positive effect on RGC

regeneration has also been shown by neurotrophin administration, such as via CNTF gene-transfer in the retina ^{28,29}.

In an alternative approach, partial neuroprotection, axon outgrowth and elongation have been obtained via induction of a controlled retinal inflammation and associated glial reactivity ^{4,35,37-44}. This acute yet controlled inflammatory response in the retina has been induced through lens injury ⁴⁵⁻⁴⁷ or via pharmacological treatments ^{40,44,48}, and may also be important for inducing the regenerative response after transplantation of PNS segments.

In more recent years, researchers focused on methods to stimulate axonal regeneration via the activation of signaling pathways for intrinsic RGC growth control, e.g. JAK/STAT, PI3K/Akt and MAPK/ERK ^{49,50}. This can be achieved for example by deletion of Krüppel-like family of transcription factors (KLF) ⁵¹, osteopontin ⁵², phosphatase and tensin homolog (PTEN) ^{44,53,54} and/or suppressor of cytokine signaling 3 solution (SOCS3) ^{53,54}, activation of the mechanistic target of rapamycin (mTOR) signaling pathway ^{52,55}, administration of Rho kinase (ROCK) inhibitors ⁵⁶, overexpression of glycogen synthase kinase 3 (GSK3) ⁵⁷, c-myc ⁵⁸, neuritin-1 ⁵⁹, or thrombospondin-1 ⁶⁰. From recent studies, it is clear that multiple strategies that focus on both extrinsic and intrinsic factors should be combined to maximize axon outgrowth ^{37,38,44,61-64}. For example, intraocular inflammation, cAMP, and PTEN deletion were found to act synergistically to augment regeneration. The three-way combination more than doubled the number of regenerating axons close to the injury site, as compared to PTEN deletion alone ⁴⁴. Despite the above-mentioned strategies and limited successes, it is clear that many obstacles still must be overcome before regeneration in the CNS leads to full functional recovery. Notably, although these combined treatments can promote axonal extension,

navigation errors of the regrowing axons and correct synaptogenesis in the target brain areas are still unsolved and even largely unexplored^{56,65,66}, and hence functional recovery is still out of reach.

In this review manuscript, we will summarize the current knowledge that links inflammation, in particular, the innate immune system, to axonal regeneration in the optic nerve, and the molecules and pathways that connect these two processes. Whereas the inflammatory response has long been considered a harmful process in the past, it is now clear that both blood-borne and resident inflammatory cells contribute to and even trigger the regenerative growth state of axons^{52,58}. Indeed, where resident microglia, reactive macroglia (astrocytes and Müller glia) and infiltrating myeloid cells (neutrophils and monocyte-derived macrophages) were once considered dangerous players in CNS damage and disorders, we now know that these cells actually contribute to CNS recovery through their positive effects on neuronal survival, axonal regeneration, mobilization of precursor cells and/or remyelination^{67–70}. Of note, the advantageous effect of neuroinflammation on axonal regeneration (and neuroprotection) is not limited to the retinofugal system. It has also been observed after damage to the spinal cord and in other parts of the brain (e.g. hippocampus, cortex)^{3,41,42,47,71–74}.

3 Inflammation as modulator for axonal regeneration

Previous studies have shown that inflammatory stimulation by either lens injury or by intravitreal injection of toll-like receptor 2 (TLR2) agonists such as zymosan (pro-inflammatory glucan molecule from yeast cell wall) or Pam3Cys (synthetic lipopeptide) induces RGC axon growth in rodents^{45,47,48,63,75}. Moreover, inflammatory stimulation in the spontaneously regenerating zebrafish accelerates axonal regeneration after optic

nerve injury ^{2,76,77}. Notably, an intravitreal injection (injection through the sclera immediately posterior to the limbus, without damaging the lens) has different effects on RGC apoptosis and axonal regeneration compared to an intra-optic nerve injection (injection piercing the optic nerve pia mid-way between the lamina cribrosa and the site of optic nerve injury). Stimulation of inflammation both in the optic nerve and the vitreous protects RGCs from cell death, yet only vitreal inflammation promotes axonal regeneration, suggesting that this local inflammatory response stimulates axonal regeneration by activating certain signaling pathways in the RGC somata ⁴⁰. Moreover, since retinal glial cells get reactivated only upon vitreal inflammation, this also suggests that the regenerative growth response of RGCs is dependent on the factors expressed by retinal glia. In consequence, paradigms of inflammatory stimulation have focused on altering retinal events.

In general, the pro-regenerative effect of inflammatory stimulation can be attributed to either (1) infiltration of neutrophils and macrophages into the vitreous ⁵⁰, (2) glial reactivity of resident retinal micro- and macroglia, or crosstalk between certain of these cell populations (Figure 1). Either way, the molecules and pathways induced by these inflammatory/glial cells cause a delay of axotomy-induced RGC death and a transformation of the RGCs into an active growth state. In the next paragraphs, we provide an overview of the cell types and associated molecules that have been postulated to be involved in inflammatory-induced axonal regeneration.

3.1 Infiltrating inflammatory cells – neutrophils and macrophages

Granulocyte neutrophils are motile phagocytic leukocytes that are the first responders of the innate immune system at sites of acute inflammation. In the naive adult retina,

neutrophils reside in the circulation and almost none are found in the tissue. Upon optic nerve injury, however, neutrophils are recruited to the vitreous within 12 hours post injury, where they represent the major players during the early inflammatory stage, but are short-lived⁶¹. Similarly, almost no macrophages are present in the naive adult retina yet, following optic nerve injury, monocytes enter damaged tissue where they differentiate into macrophages. Monocyte-derived macrophages only first start to appear around 1 day post optic nerve crush, thus dominating the scene at a later inflammatory stage³⁹.

One of the first molecules found to play a central role in inflammation-induced axonal regeneration is oncomodulin. Initial reports showed that this small calcium-binding protein is secreted by macrophages during ocular inflammation, and binds to a high-affinity receptor on RGCs (Figure 1a). In the presence of mannose and elevated levels of cAMP, this induces axon outgrowth^{35,39}. Administration of a peptide competing for oncomodulin receptor binding was indeed found to prohibit axon growth after optic nerve injury in mice³⁵. However, although initial reports postulated that oncomodulin is abundantly expressed by activated macrophages, oncomodulin mRNA expression levels in the retina increase almost immediately after injury and peak within 24 hours after injury. Within this time window, the presence of macrophages is limited³⁵. This discrepancy led to the believe that neutrophils, which are infiltrating the eye before the macrophages, are the major source of oncomodulin (Figure 1a)^{61,78}. Indeed, immunodepletion of neutrophils was found to reduce the amount of oncomodulin and suppress the induction of axonal regeneration upon inflammatory stimulation as effectively as a peptide antagonist of oncomodulin, without having an effect on CNTF, leukemia inhibitory factor (LIF), or interleukin (IL) 6 levels⁶¹. Based on these data, and

the finding that macrophages are not capable of stimulating robust axonal regeneration in the absence of neutrophils ⁶¹, one may speculate that early production of oncomodulin by the neutrophils is necessary for axonal outgrowth and that prolonged expression of oncomodulin by the macrophages might be required for the maintenance of the regenerative response. However, peripheral macrophage depletion by clodronate liposome treatment did not have any effect on the regenerative capacity of RGCs upon optic nerve crush combined with lens injury ⁷⁹. Despite this evidence for a crucial role for neutrophils and oncomodulin, the exact role of the various infiltrating innate immune cells and their secretome is far from fully elucidated, and a conflicting report in which depleting neutrophils led to a more favorable outcome after optic nerve injury underlines their potentially dichotomous role ⁸⁰.

In mammals, interleukin-1 beta (IL-1 β) and tumor necrosis factor (TNF) have been suggested as important molecules during inflammatory-induced axonal regeneration in the spinal cord model, but this is not yet confirmed in the retinofugal system. IL-1 β deficient mice were characterized by a smaller lesion size, less astrogliosis and a higher number of regenerating axons after spinal cord compression injury, suggesting IL-1 β has a negative effect on axon outgrowth ^{81,82}. Moreover, after injury, the levels of TNF in IL-1 β deficient mice stayed significantly lower compared to wild-type mice ⁸², which suggests that IL-1 β contributes to the upregulation of TNF following the induction of a series of inflammatory events after injury. However, the effect of TNF on axon regeneration is not clear in mammals and may be either promoting ^{83,84} or inhibiting ⁸⁵. Transforming growth factor (TGF) α and β , on the other hand, have been suggested to promote axonal growth during the inflammatory response (Figure 1a) ^{24,86,87}.

More evidence for a tightly yet dynamic control of axonal regeneration by neutrophils and macrophages has been gathered in recent zebrafish studies using a spinal cord injury paradigm. These have shown that axonal regeneration is dependent on an initial transient upregulation of Il-1 β , followed by a later increase in Tnf⁸⁸. Strikingly, this biphasic response can be related to the timed influx of neutrophils and macrophages. Indeed, within their study, Tsarouchas et. al.⁸⁸ revealed that in a first phase (24 hours post injury in zebrafish larvae), axon outgrowth is promoted by neutrophils expressing Il-1 β . However, at later time points (48 hours post injury in zebrafish larvae), Il-1 β seems to be inhibitory for axonal regeneration. At this time, macrophages expressing Tnf (and low Il-1 β) become the major actors at the site of injury. Together with the reduced number of neutrophils, the macrophages seem to suppress Il-1 β expression in neutrophils, which results in diminished levels of Il-1 β at the injury site at later time points⁸⁸. These insights have been gathered in Irf8 mutant zebrafish larvae, which have a reduced number of macrophages and an increased number of neutrophils, and show a prolonged inflammatory response and a lower regenerative capacity after axonal injury. The latter has been related to the extended secretion of Il-1 β in these mutants. Decreasing the Il-1 β levels in the Irf8 mutant zebrafish, but not the Tnf levels, via pharmacological, morpholino or gene-editing techniques, restored axonal regeneration in these mutants. *Vice versa*, axonal regeneration was impaired in wild-type fish upon decreased Tnf levels, but not upon Il-1 β depletion, suggesting a pro-regenerative role for Tnf⁸⁸. Of note, similar to Tnf, expression levels of the anti-inflammatory cytokines Tgf β -1a and Tgf β -3 were low during the early and increased during late regeneration within the zebrafish spinal cord regeneration model⁸⁸. The levels of these cytokines were strongly reduced in the Irf8 mutant zebrafish, suggesting that these cytokines are

likely produced by macrophages and may, together with Tnf, be co-responsible for promoting axonal regeneration (Figure 1a)⁸⁸. These findings are in accordance with observations made in fin regeneration research, where a prolonged expression of IL-1 β leads to apoptosis of the regenerating cells and a proper regulation of IL-1 β levels regulated by macrophages is necessary for normal tissue regeneration⁹⁰. Moreover, preliminary data from our lab using a mouse optic nerve crush model combined with Pam3Cys-induced inflammatory stimulation, provide further evidence for the suggested working mechanism by Tsarouchas et. al.⁸⁸, *i.e.* that IL-1 β , whose levels are dynamically controlled by macrophages, seems to inhibit axonal regeneration at later time points, as opposed to its early promoting effect. Using Ccr2 deficient mice, which have a defective monocyte migration, we observed that a reduced number of macrophages after injury results in a higher number and a prolonged presence of infiltrating neutrophils. Similar as in zebrafish, this could mean that, also in mice, clearance of neutrophils is dependent on the macrophages. As such, the lack of macrophages prevents the neutrophil removal and hence the reduction of IL-1 β levels at later stages of inflammation. Since, at this time point, IL-1 β is inhibiting axonal regeneration, this would explain the reduced number of regenerating axons observed in Ccr2 deficient mice subjected to optic nerve crush and inflammatory stimulation, as compared to wild-type mice. These pilot data are currently being investigated in more detail and corroborated with cytokine expression analysis.

In conclusion, the data gathered up to now suggest that, for successful axonal regeneration, a biphasic inflammatory response with an initial pro-inflammatory phase followed by an anti-inflammatory phase is needed^{82,88}. The most important identified players related to neutrophils and macrophages are oncomodulin, IL-1 β and TNF. To

further uncover the importance of the different myeloid cell populations and the molecular pathways that are triggered in/between these cells, cell depletion studies combined with (single-cell) transcriptomic or proteomic profiling are needed. To this end, the optic nerve crush model combined with a pro-regenerative inflammatory stimulation can be further applied in e.g. *Ccr2* deficient mice (which have a defective macrophage migration)⁹¹, *Genista* mice (which have neutropenia)⁹², or mice treated with an anti-Ly6G antibody can be used to deplete neutrophils⁶¹.

3.2 Resident inflammatory cells – Microglia

Microglia are long-lived, resident macrophages that populate the retinal parenchyma. Ramified microglia with highly motile processes survey the naive adult retina⁹³ and act as phagocytic sentinels. Upon injury, microglia undergo a phenotypic transformation into so-called 'activated' microglia and start to release inflammatory mediators such as TNF, IL-1 β , IL-6, CCL (C-C Motif Chemokine Ligand) 2 and iNOS (Figure 1b)^{94,95}. Subsequently, activation is rapidly followed by a resolution and tissue repair phase where microglia start secreting anti-inflammatory molecules such as IL-4 and IL-10, IL-13 and TGF (Figure 1b), before returning to their surveilling state^{95–97}. These cytokines downregulate the expression of the pro-inflammatory molecules and induce tissue reconstruction and repair⁹⁵. In the case of an optic nerve injury, microglia density increases from 3 days after injury onwards and only returns to baseline numbers after 6 weeks⁹⁸. This sustained increase coincides with the period during which RGC death occurs, and microglia actively phagocytize debris from these RGCs and their axons^{98,99}. Notably, the increase in microglial cell number after optic nerve injury may not only originate from local proliferation but also from the infiltration of blood-derived macrophages⁹⁸. Since it has long been difficult to discriminate between resident

microglia and infiltrating macrophages¹⁰⁰, the relative contribution of these processes remains unclear.

In the mammalian visual system, microglia have more often been studied for their effect on RGC survival rather than axonal regeneration. Treatment with minocycline, a tetracycline derivative that suppresses microglial activation, improved RGC survival was observed at 4 weeks after optic nerve transection as well as in an experimental glaucoma model in mice^{101–103}. This suggests that microglia activation affects the number of surviving RGCs, and may thus indirectly affect the outcome of axonal regeneration. Contrarily, activated microglia have also been attributed pro-regenerative functions in the mammalian CNS. By phagocytosing non-functional structures, they make space for new connections and removes inhibitory factors, thereby stimulating axonal regeneration¹⁰⁴. Moreover, activated microglia have been found to produce anti-inflammatory factors that can activate RGCs to regenerate, and to ensheath neurons to provide trophic support under damaging or regenerating conditions of the CNS¹⁰⁵. Notably, inflammatory stimulation via intravitreal injection of a TLR2 agonist (e.g. zymosan) *versus* a TLR4 agonist (e.g. lipopolysaccharide (LPS)) induces a similar influx of inflammatory cells into the vitreous cavity¹⁰⁶ yet has a very distinct effect on axonal regeneration. Indeed, in contrast to TLR2 agonists, inflammatory stimulation with LPS does not induce optic nerve regeneration¹⁰⁶. Interestingly, microglia stimulated with LPS significantly increase their expression of TLR2, 4, 6, 8, and 9, cytokines such as interferon (IFN) α , IFN β , I-1 β , IL-6 and iNOS, and chemokines such as Ccl-2 and Ccl-5. In contrast, microglia stimulated with a TLR2 agonist do not increase expression of any of the TLRs and have much lower expression levels of these cytokines and chemokines¹⁰⁷.

The differential expression of cytokines and chemokines by activated microglia upon

TLR2- or TLR4-dependent inflammatory stimulation thus suggests that axonal regeneration is critically dependent on a balance of specific cytokines and chemokines and/or their engagement with specific immune receptors ¹⁰⁶. Of note, microglia activated by LPS have been shown to inhibit axonal growth and induced growth cone collapse of cultured cortical neurons via the expression of repulsive guidance molecule a (RGMa) upon direct contact between microglia and neurons. Treatment with RGMa-neutralizing antibodies, transfection of RGMa siRNA or administration of minocycline, impaired the inhibitory effects of microglia on axonal outgrowth (Figure 1b) ¹⁰⁸. Furthermore, recent findings show that zymosan does not only induce a controlled inflammatory response via TLR2 but also via dectin-1, which is expressed by infiltrating and resident inflammatory cells ¹⁰⁶.

Controversially, Hilla et al. recently posited that microglia are irrelevant for axonal regeneration in the adult mouse visual system. Treatment with a pharmacological colony stimulating factor 1 receptor inhibitor (PLX5622 or PLX3397), depleting microglia in the retina and optic nerve, did not affect the time course, the extent of RGC degeneration nor the induction of regeneration-associated genes after optic nerve crush and lens injury ⁷⁹. Only the combinatorial depletion of microglia and infiltrating macrophages via co-administration of the PLX5622 inhibitor and clodronate liposomes significantly decreased the number of regenerating axons ⁷⁹. Notably, microglia depletion resulted in a delayed population of the lesion site by astrocytes, which indicates that growing axons are not inhibited by the presence of astrocytes at the injury site ⁷⁹. Strikingly, also in *csfr1a/b* mutant zebrafish larvae, in which microglial cells are drastically reduced, axon regrowth was unaffected after spinal cord injury, suggesting that these cells are expendable for the spontaneous regeneration in these fish ⁸⁸.

359 However, contrasting studies question the above published data since, in mice suffering
360 from a hippocampal lesion, microglia elimination via PLX3397 facilitates functional
361 recovery, likely through a combination of a reduced secretion of pro-inflammatory
362 substances, a decrease in phagocytic activity, and by preventing microglia-induced
363 synaptic alterations ⁹⁷. Moreover, preliminary data from our lab, obtained from optic
364 nerve regeneration studies in the visual system of adult csfr1a/b mutant zebrafish, show
365 that elimination of microglia, which is accompanied by a reduced number of neutrophils,
366 improves spontaneous regeneration after injury. These findings are currently being
367 further and thoroughly explored.

368 Furthermore, even more discrepancies are found in studies using a spinal cord injury
369 model in adult mice, where the near-complete elimination of microglia via PLX5622
370 aggravated lesion pathology and impaired recovery. Microglia depletion led to an
371 abnormal astroglial scar and a greater lesion site. In addition, there was an increased
372 inflammatory response dominated by macrophages, an increased loss of neurons and
373 an enhanced demyelination, resulting in an impaired recovery upon delayed depletion
374 from 8 to 28 days after injury ^{109,110}. The latter surely indicates that microglia have an
375 essential role in tissue repair in the late phases of spinal cord recovery.

376 In conclusion, although microglia activation is among the first responses to
377 inflammatory stimulation, there is still a lot of controversy about their role in axonal
378 regeneration. No consensus can be found in literature and the role of microglia in axonal
379 regeneration remains under discussion. It seems that the question whether microglia
380 are just innocent bystanders or important promoting or inhibiting mediators of axonal
381 regeneration is highly species and model-dependent. While most recent data in the
382 mouse visual system suggest that microglia are not important for inflammation-induced

axonal regeneration, more research is clearly needed to elucidate their heterogeneity and possible differential roles after CNS injury. Further experiments trying to unravel the role of microglia during axonal regeneration should be performed using methods that clearly distinguish activated microglia from macrophages. For example, following up on the paper by Hilla et. al.⁷⁹, PLX5562 could be used in combination with optic nerve crush and inflammatory stimulation in Cx3cr1CreER x R26-stop-YFP mice, in which a temporary tamoxifen treatment can be applied to label the resident microglia but not the infiltrating macrophages¹¹¹.

3.3 Macroglia – Astrocytes and Müller glia

In the retina, two types of macroglia are present, namely astrocytes and Müller glia. While astrocytes are mostly located in the nerve fiber layer and surrounding the blood vessels in the inner nuclear layer, Müller glia span across all retinal layers with their cell bodies located in the inner nuclear layer. In mammals, macroglia respond to retinal injury in a variety of ways, including reactive gliosis, that can be either protective or detrimental to retinal function¹¹². In mice subjected to optic nerve crush, Glial fibrillary acidic protein (GFAP) expression, as a marker of reactive gliosis, was upregulated in astrocytes and Müller glia around 5 days after injury¹¹³.

Next to the myeloid cell populations, also these activated macroglia have been proposed to contribute to the increased axonal regeneration upon inflammatory stimulation. Reactive gliosis has historically been given a negative connotation due to the inhibition of axonal regeneration by glial scar formation, which physically and biochemically prevents axons from regrowing. However, gliosis has beneficial effects as well. The scarring around the CNS lesion site prevents pathogens from entering and enables restoration of the blood-brain barrier¹¹⁴.

Remarkably, macroglial cells express TLRs, suggesting that these cells can be directly stimulated by both zymosan, Pam3Cys and LPS^{115,116}. Consequently, the retinal astrocyte and Müller glia activation upon optic nerve injury may induce secretion of multiple glial-derived factors, including BDNF, CNTF, LIF, bone morphogenetic proteins (BMP), GDNF, FGF-2, and neurotrophin-4/5 (NT-4/5)^{86,117–121}. Three cytokines from the IL-6 superfamily, namely CNTF, LIF and IL-6, and their downstream signaling pathways, including JAK/STAT3 and PI3K/ATK/mTOR, have been proposed as key mediators of the stimulating effect of inflammation on optic nerve regeneration^{122,123}. Fischer *et al.* showed that optic nerve regeneration is stimulated by astrocyte-derived trophic factors, with astrocyte-derived CNTF as the principle mediator (Figure 1c)^{42,48,75}. Indeed, the neuroprotective and pro-regenerative effects of inflammatory stimulation are diminished in CNTF knockout mice, and abolished in CNTF/LIF double knockout mice, thereby ascribing a principal role to CNTF, with LIF as an additional contributing factor¹²². However, this reduction in axonal regeneration could also (in part) be a result of the reduced RGC viability as a consequence of the CNTF and LIF deletion^{31,42}. Also, and contrarily, other research has often failed to find any strong effect of CNTF deletion or overexpression on axonal regeneration *in vitro*¹²⁴ or *in vivo*^{35,44,45,72,125,126}. However, technological issues may underlie these discrepancies. Direct intravitreal injection of CNTF has only a limited effect on axonal regeneration^{48,113,127} due the short half-life of the protein in the vitreous^{29,31}, while continuous release of CNTF via adeno-associated viral (AAV)-mediated expression in RGCs or Müller glia elicits long-distance regrowth of axons up to the optic chiasm (but rarely beyond)^{28,65}. Furthermore, optic nerve injury combined with inflammatory stimulation induces the expression of IL-6 in the retina and this elicits a pro-regenerative response. Indeed,

431 adding IL-6 or specific IL-6 receptor agonists to the medium of cultured RGCs
432 significantly increases their neurite outgrowth *in vitro*^{123,128}, and RGCs, which express
433 the IL-6 receptor, are transformed into a regenerative state upon intravitreal injection
434 of IL-6 *in vivo*. Furthermore, axonal regeneration in the optic nerve was significantly
435 reduced in mice with IL-6 gene deletion. In addition, continuous AAV-driven expression
436 of hyper IL-6, a designer cytokine that consists of the covalently linked bioactive parts of
437 IL-6 and IL-6R α and acts upon the JAK/STAT pathway, in RGCs, has been recently
438 described as the most potent unifactorial treatment promoting axonal regeneration
439 known thus far¹²⁹. Altogether, these studies clearly indicate that this IL-6 related
440 cytokine group plays a determining role in optic nerve regeneration.

441 Notably, in zebrafish yet not in mammals, Müller glia undergo reprogramming and
442 obtain retinal stem cell characteristics upon retinal injury or inflammatory stimulation,
443 resulting in a proliferating population of progenitor cells that can regenerate all major
444 retinal cell types and restore vision¹³⁰. Whether this proliferation of Müller glia also
445 contributes to axonal regeneration remains unknown. Notably, recent pilot data from

446 our lab show a correlation between gliosis in Müller glia and an improved axonal
447 regeneration after injury in the visual system of adult zebrafish, findings that are
448 currently being investigated in more detail.

As such, it is tempting to speculate that
449 studies revealing the mechanisms that underlie Müller glia reprogramming in zebrafish
450 can lead to new strategies to induce *de novo* neurogenesis and axonal regeneration in
451 mammals¹¹². All in all, it is clear that the reactivation of macroglial cells can play a

452 prominent role during axonal regeneration. In order to define their exact contribution,

453 cell-specific ablation techniques could be used to deplete Müller glia and astrocytes, e.g.

454 using the R26-iDTR mouse line (expressing a tamoxifen-inducible diphtheria toxin

receptor) and macroglial cell-specific Cre recombinase mouse lines such as GLAST^{CreER} and Rlb^p^{CreER} ^{131,132}. Any effects of the cellular ablations on the regeneration outcome could be substantiated with further investigation of the underlying molecules and pathways using single-cell RNA sequencing.

3.4 Focus on the crosstalk between myeloid and macroglial cells

Compelling evidence exists for reciprocal interactions between micro- and macroglia shaping the response to injury and disease ^{133–136}. Hence one should keep in mind that the responses of neutrophils, macrophages, microglia, astrocytes and Müller glia are not independent parallel processes, but are highly intertwined and influence each other. Indeed, interestingly, a relatively recent RNA sequencing study of zebrafish macrophages/microglia transcripts during Müller glia-mediated regeneration at early time points following light damage found enrichment of genes categorized within cytokine signaling and immunity ¹³⁷, which indicated that macrophages/microglia can be found in regions containing regenerative Müller glia. This kind of sequencing studies will allow us to more accurately determine the function of microglia/macrophages during CNS regeneration. As such, glial-derived factors might promote RGC neuroprotection and axon growth by interacting with inflammatory cells or alternatively, glial cells may act as intermediaries, stimulated by the inflammatory cell-derived factors to produce pro-regenerative factors. The latter can be supported by the finding that zymosan-induced axonal regeneration seems to dominantly work in a dectin-1 dependent manner, which is found on the infiltrating and resident inflammatory cells but not on the macroglia ¹⁰⁴. For now, and based on many studies, it is tempting to speculate that inflammation-induced axonal regeneration is surely not dependent on a single myeloid/glial cell population. While only limited information about the crosstalk

between myeloid and macroglial cells is available in the optic nerve regeneration field, it has repeatedly been shown in other injury paradigms that myeloid and macroglial cells can activate/modulate each other^{117,138–140}. Although not the focus of this review, in the photoreceptor degeneration field, few studies show evidence for microglia-Müller glia crosstalk and their involvement in photoreceptor survival. For instance, in the rd10 retina, which is characterized by an increased microglial activity and by gliotic events prior to the onset of photoreceptor loss, it was found that Norgestrel (progesterone analog) mediated downregulation of IFN γ expression in the activated microglia causes a reduction of Müller gliosis¹⁴¹. Moreover, in a rat N-methyl-N-nitrosourea (MNU)-mediated photoreceptor loss model, Cx3cl1 secreted by Müller glia was shown to induce the migration of activated microglia to the site of injury¹⁴². In addition, several studies suggest Müller cells and microglia actively crosstalk to maintain constant levels of neurotrophic factors important for retinal physiology¹¹⁷. Microglia can directly trigger the release of several neurotrophic factors from Müller cells, including GDNF, LIF, CNTF and nerve growth factor (NGF)^{143–147}. Further emerging evidence for the bidirectional communication between microglia and astrocytes by releasing signaling molecules can be found in other CNS injury/disease models as reviewed by Jha et. al.¹⁴⁸. Indeed, activated microglia have been reported to induce astrocyte activation and determine the fate of astrocytes by secreting TNF, nitric oxide (NO), IFN, nicotinamide-adenine-dinucleotidephosphate (NADPH) oxidase-derived H₂O₂, IL-1 α , complement factor C1q and vascular endothelial growth factor (VEGF)-B (Figure 1). In a similar way, astrocytes do not only seem to be able to activate microglia but also to regulate their cellular functions by secreting orosomucoid (ORM) 2, lipocalin (LCN) 2, CCL2, C-X-C motif ligand (CXCL) 10, TGF- β , plasminogen activator inhibitor (PAI)-I, GDNF, pentraxin-related

protein (PTX) 3, complement factor C3, exosomal miR-9 (Figure 1). In axonal regeneration models, the crosstalk between the myeloid and macroglial cell populations has not been thoroughly studied yet. To uncover this, single-cell RNA sequencing studies combined with depletion of the specific cell populations (similar to what has been mentioned in section 3.1) could be employed to study how depletion of a specific cell-type affects cell number and activation status of the remainder populations.

3.5 Focus on cellular heterogeneity

Next to the question which cell types and which molecules are involved in inflammation-induced axonal regeneration of RGCs, an even more intriguing question that arises is whether and how the ambiguous role of the various inflammatory cells depends on the cellular heterogeneity within one population. Indeed, the cell types discussed here (*i.e.*, neutrophils, macrophages, microglia, astrocytes and Müller cells) are known to adopt different phenotypes, with more pro-inflammatory *versus* more anti-inflammatory properties, depending on the microenvironment and stimuli ^{149,150}. The ‘classically activated’ pro-inflammatory phenotype and the alternatively activated anti-inflammatory phenotype represent the extremes of a whole spectrum of activation states with overlapping properties ^{100,151–153}. Albeit unexplored in the context of optic nerve regeneration thus far, certain activation subtypes may be essential to drive inflammatory-induced regrowth potential while others may account for the negative effects on axonal regeneration ⁷⁸. Indeed, for macrophages/microglia, different activation states have been suggested to influence repair after spinal cord injury ¹⁵⁴. Pro-inflammatory M1-like macrophages/microglia have been associated with tissue damage, neuronal loss, axon retraction and demyelination while anti-inflammatory M2-like macrophages/microglia have been associated with neuroprotection and axon

growth^{155,156}. In the injured spinal cord, most macrophages/microglia display an M1-like activation state, with only a limited and transient presence of M2-like cells¹⁵⁷. However, adapting the environment after injury to shift macrophage/microglia activation towards a more M2-like state, *e.g.* via transplantation of *in vitro* cultured M2-like macrophages¹⁵⁸ or augmenting the number of M2-like macrophages/microglia via inhibition of the IL-6 receptor^{155,159}, has been shown to improve axonal regeneration¹⁶⁰.

Furthermore, there is also evidence that macrophage/microglia heterogeneity and more specifically the switch from a more M1-like to a more M2-like response is responsible for the spontaneous regeneration of zebrafish upon caudal fin amputation¹⁶¹. This mirrors successful wound healing in mammals and contrasts the overwhelming presence of pro-inflammatory M1-like macrophages/microglia after mammalian CNS injuries¹⁶¹. Analogous to macrophages/microglia, astrocytes may also adopt diverging activation states, and present with a destructive A1-like phenotype or a neuroprotective A2-like phenotype^{136,162}. For neutrophils, pro-inflammatory N1, as well as anti-inflammatory N2 subsets, have been identified in a cancer model, yet there are no reports thus far of neutrophil heterogeneity during CNS injury¹⁶³. For these cell types, it thus remains to be determined how their activation status affects the outcome after CNS injury.

Finally, related to this, another alluring question arises based on the fact that also RGCs form a very heterogeneous cell population. It is known that only a small percentage of RGCs regenerate upon axonal injury and specifically after inflammatory stimulation. A fascinating question is which characteristics are required for RGCs to respond to ocular inflammation and to provoke a regenerative response⁷⁸. Currently, 30-40 RGC subtypes have been defined, with diverging dendritic tree size, branching density and

stratification depth in the inner plexiform layer ¹⁶⁴. The answer to this question may come from the study of α RGCs, an RGC subtype with a great capacity to regenerate as compared to all other RGC subtypes ^{165,166}. A comprehensive analysis of the receptor expression profile of these and other RGC subtypes may aid to understand whether certain levels or combinations of receptors for regeneration-inducing factors underlie this differential regeneration potential.

4 Future explorations

Neuroinflammation has become an interesting aspect of inducing axonal regeneration in the adult mammalian CNS. It has become clear that both blood-borne and resident inflammatory cells and reactivated glial cells affect axonal regeneration. However, how these inflammatory cells and reactivated glial cells, and/or their crosstalk, impact on inflammation-induced axonal regeneration is ambiguous and a lot of controversies still exist. With the novel experimental techniques that are being developed and the new insights into the heterogeneity of the involved cell populations, many of these contradictions could be clarified in the future.

Recent progress in the field of next-generation sequencing-based technologies for genomics, transcriptomics and epigenomics are increasingly focusing on the characterization of individual cells. This 'single-cell revolution' can reveal complex and rare cell populations and can give us unprecedented insights into their heterogeneity and into the changes induced by injury. In addition, recent identification of microglia-specific genes such as P2y12r, Tmem119 and Sall1 has opened the possibility to more clearly distinguish between the resident microglia and the infiltrating macrophages

574 during injury in the CNS and forms a useful tool to study their respective roles ^{111,167}.

575 These novel experimental approaches hold exciting promise in future research.

5 Disclosure statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

6 References

1. Goldberg JL, Espinosa JS, Xu Y, Davidson N, Kovacs GTA, Barres BA. Retinal Ganglion Cells Do Not Extend Axons by Default: Promotion by Neurotrophic Signaling and Electrical Activity. *Neuron*. 2002 Feb 28;33(5):689–702.
2. Bollaerts I, Veys L, Geeraerts E, Andries L, De Groef L, Buyens T, Salinas-Navarro M, Moons L, Van Hove I. Complementary research models and methods to study axonal regeneration in the vertebrate retinofugal system. *Brain Struct Funct*. 2018 Mar 30;223(2):545–67.
3. Benowitz L, Yin Y. Rewiring the injured CNS: lessons from the optic nerve. *Exp Neurol*. 2008 Feb;209(2):389–98.
4. Berry M, Ahmed Z, Lorber B, Douglas M, Logan A. Regeneration of axons in the visual system. *Restor Neurol Neurosci*. 2008;26:147–74.
5. Li H-Y, Ruan Y-W, Ren C-R, Cui Q, So K-F. Mechanisms of secondary degeneration after partial optic nerve transection. *Neural Regen Res*. 2014 Mar 15;9(6):565–74.
6. Fitzgerald M, Bartlett CA, Harvey AR, Dunlop SA. Early Events of Secondary Degeneration after Partial Optic Nerve Transection: An Immunohistochemical Study. *J Neurotrauma*. 2010 Feb;27(2):439–52.
7. Berkelaar M, Clarke DB, Wang YC, Bray GM, Aguayo AJ. Axotomy results in delayed death and apoptosis of retinal ganglion cells in adult rats. *J Neurosci*. 1994 Jul 1;14(7):4368–74.
8. Dekeyser E, Geeraerts E, Buyens T, Van den Haute C, Baekelandt V, De Groef L, Salinas-Navarro M, Moons L. Tackling Glaucoma from within the Brain: An Unfortunate Interplay of BDNF and TrkB. *PLoS One*. 2015;10(11):e0142067.
9. De Groef L, Dekeyser E, Geeraerts E, Lefevere E, Stalmans I, Salinas Navarro M, Moons L. Differential visual system organization and susceptibility to experimental models of optic neuropathies in three commonly used mouse strains. *Exp Eye Res*. 2016;145:235–47.
10. Zou S, Tian C, Ge S, Hu B. Neurogenesis of retinal ganglion cells is not essential to visual functional recovery after optic nerve injury in adult zebrafish. *PLoS One*. 2013;8(2):e57280.
11. Becker CG, Becker T. Growth and pathfinding of regenerating axons in the optic projection of adult fish. *J Neurosci Res*. 2007 Sep 1;85(12):2793–9.
12. Becker T, Becker CG. Axonal regeneration in zebrafish. *Curr Opin Neurobiol*. 2014 Aug 1;27:186–91.
13. Van houcke J, Geeraerts E, Vanhunsel S, Beckers A, Noterdaeme L, Christiaens M, Bollaerts I, De Groef L, Moons L. Extensive growth is followed by neurodegenerative pathology in the continuously expanding adult zebrafish

retina. *Biogerontology*. 2019 Feb;20(1):109–25.

14. Filbin MT. Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS. *Nat Rev Neurosci*. 2003 Sep;4(9):703–13.
15. Benowitz L, Yin Y. Combinatorial Treatments for Promoting Axon Regeneration in the CNS: Strategies for Overcoming Inhibitory Signals and Activating Neurons' Intrinsic Growth State. *Dev Neurobiol*. 2007 May 30;1148–65.
16. Kopper TJ, Gensel JC. Myelin as an inflammatory mediator: Myelin interactions with complement, macrophages, and microglia in spinal cord injury. *J Neurosci Res*. 2018 Jun;96(6):969–77.
17. Goldberg JL, Klassen MP, Hua Y, Barres B a. Amacrine-signaled loss of intrinsic axon growth ability by retinal ganglion cells. *Science*. 2002 Jun 7;296(5574):1860–4.
18. Benowitz L, Yin Y. Optic Nerve Regeneration. *Arch Ophtalmol*. 2010;128(8):1059–64.
19. McKeon RJ, Schreiber RC, Rudge JS, Silver J. Reduction of neurite outgrowth in a model of glial scarring following CNS injury is correlated with the expression of inhibitory molecules on reactive astrocytes. *J Neurosci*. 1991 Nov 1;11(11):3398–411.
20. Niederöst BP, Zimmermann DR, Schwab ME, Bandtlow CE. Bovine CNS Myelin Contains Neurite Growth-Inhibitory Activity Associated with Chondroitin Sulfate Proteoglycans. *J Neurosci*. 1999 Oct 15;19(20):8979–89.
21. Hagino S, Iseki K, Mori T, Zhang Y, Hikake T, Yokoya S, Takeuchi M, Hasimoto H, Kikuchi S, Wanaka A. Slit and glypican-1 mRNAs are coexpressed in the reactive astrocytes of the injured adult brain. *Glia*. 2003 Apr 15;42(2):130–8.
22. Tang X, Davies JE, Davies SJA. Changes in distribution, cell associations, and protein expression levels of NG2, neurocan, phosphacan, brevican, versican V2, and tenascin-C during acute to chronic maturation of spinal cord scar tissue. *J Neurosci Res*. 2003 Feb 1;71(3):427–44.
23. Kaneko S, Iwanami A, Nakamura M, Kishino A, Kikuchi K, Shibata S, Okano HJ, Ikegami T, Moriya A, Konishi O, et al. A selective Sema3A inhibitor enhances regenerative responses and functional recovery of the injured spinal cord. *Nat Med*. 2006 Dec 12;12(12):1380–9.
24. Lagord C, Berry M, Logan A. Expression of TGF β 2 but Not TGF β 1 Correlates with the Deposition of Scar Tissue in the Lesioned Spinal Cord. *Mol Cell Neurosci*. 2002 May 1;20(1):69–92.
25. Van de Velde S, De Groef L, Stalmans I, Moons L, Van Hove I. Towards axonal regeneration and neuroprotection in glaucoma: Rho kinase inhibitors as promising therapeutics. *Prog Neurobiol*. 2015 Aug;131:105–19.

26. Zhang C-W, Lu Q, You S-W, Zhi Y, Yip HK, Wu W, So K-F, Cui Q. CNTF and BDNF Have Similar Effects on Retinal Ganglion Cell Survival but Differential Effects on Nitric Oxide Synthase Expression Soon after Optic Nerve Injury. *Investig Ophthalmology Vis Sci*. 2005 Apr 1;46(4):1497.
27. Dalkara D, Kolstad KD, Guerin KI, Hoffmann N V, Visel M, Klimczak RR, Schaffer D V, Flannery JG. AAV mediated GDNF secretion from retinal glia slows down retinal degeneration in a rat model of retinitis pigmentosa. *Mol Ther*. 2011 Sep;19(9):1602–8.
28. Pernet V, Joly S, Dalkara D, Jordi N, Schwarz O, Christ F, Schaffer D V, Flannery JG, Schwab ME. Long-distance axonal regeneration induced by CNTF gene transfer is impaired by axonal misguidance in the injured adult optic nerve. *Neurobiol Dis*. 2013 Mar;51:202–13.
29. Leaver SG, Cui Q, Plant GW, Arulpragasam A, Hisheh S, Verhaagen J, Harvey a R. AAV-mediated expression of CNTF promotes long-term survival and regeneration of adult rat retinal ganglion cells. *Gene Ther*. 2006 Oct;13(18):1328–41.
30. Cai D, Qiu J, Cao Z, McAtee M, Bregman BS, Filbin MT. Neuronal cyclic AMP controls the developmental loss in ability of axons to regenerate. *J Neurosci*. 2001 Jul 1;21(13):4731–9.
31. Weise J, Isenmann S, Klöcker N, Kügler S, Hirsch S, Gravel C, Bähr M. Adenovirus-Mediated Expression of Ciliary Neurotrophic Factor (CNTF) Rescues Axotomized Rat Retinal Ganglion Cells But Does Not Support Axonal Regeneration in Vivo. *Neurobiol Dis*. 2000 Jun 1;7(3):212–23.
32. 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 Feb 18;24(7):1646–51.
33. Lehmann M, Fournier A, Selles-Navarro I, Dergham P, Sebok A, Leclerc N, Tigyi G, McKerracher L. Inactivation of Rho Signaling Pathway Promotes CNS Axon Regeneration. *J Neurosci*. 1999 Sep 1;19(17):7537–47.
34. Benowitz LI, Carmichael ST. Promoting axonal rewiring to improve outcome after stroke. *Neurobiol Dis*. 2010 Feb;37(2):259–66.
35. Yin Y, Cui Q, Gilbert H, Yang Y, Yang Z, Berlinicke C, Li Z. Oncomodulin links inflammation to optic. *Proc Natl Acad Sci U S A*. 2009;106(46):19587–92.
36. Berry M, Carlile J, Hunter A. Peripheral nerve explants grafted into the vitreous body of the eye promote the regeneration of retinal ganglion cell axons severed in the optic nerve. *J Neurocytol*. 1996;29:753–64.
37. de Lima S, Habboub G, Benowitz LI. Combinatorial therapy stimulates long-distance regeneration, target reinnervation, and partial recovery of vision after optic nerve injury in mice. *Int Rev Neurobiol*. 2012 Jan 1;106:153–72.

38. Lorber B, Guidi A, Fawcett JW, Martin KR. Activated retinal glia mediated axon regeneration in experimental glaucoma. *Neurobiol Dis.* 2012 Jan;45(1):243–52.
39. Yin Y, Henzl MT, Lorber B, Nakazawa T, Thomas TT, Jiang F, Langer R, Benowitz LI. Oncomodulin is a macrophage-derived signal for axon regeneration in retinal ganglion cells. *Nat Neurosci.* 2006 Jun;9(6):843–52.
40. Ahmed Z, Aslam M, Lorber B, Suggate EL, Berry M, Logan A. Optic nerve and vitreal inflammation are both RGC neuroprotective but only the latter is RGC axogenic. *Neurobiol Dis.* 2010 Feb;37(2):441–54.
41. Cui Q, Yin Y, Benowitz LI. The role of macrophages in optic nerve regeneration. *Neuroscience.* 2009 Feb 6;158(3):1039–48.
42. Leibinger M, Müller A, Andreadaki A, Hauk TG, Kirsch M, Fischer D. Neuroprotective and axon growth-promoting effects following inflammatory stimulation on mature retinal ganglion cells in mice depend on ciliary neurotrophic factor and leukemia inhibitory factor. *J Neurosci.* 2009 Nov 11;29(45):14334–41.
43. Lorber B, Berry M, Douglas MR, Nakazawa T, Logan A. Activated retinal glia promote neurite outgrowth of retinal ganglion cells via apolipoprotein E. *J Neurosci Res.* 2009 Sep;87(12):2645–52.
44. Kurimoto T, Yin Y, Omura K, Gilbert H, Kim D, Cen L-P, Moko L, Kügler S, Benowitz LI. Long-distance axon regeneration in the mature optic nerve: contributions of oncomodulin, cAMP, and pten gene deletion. *J Neurosci.* 2010 Nov 17;30(46):15654–63.
45. Leon S, Yin Y, Nguyen J, Irwin N, Benowitz LI. Lens injury stimulates axon regeneration in the mature rat optic nerve. *J Neurosci.* 2000 Jun 15;20(12):4615–26.
46. Fischer D, Heiduschka P, Thanos S. Lens-injury-stimulated axonal regeneration throughout the optic pathway of adult rats. *Exp Neurol.* 2001 Dec;172(2):257–72.
47. Lorber B, Berry M, Logan A. Lens injury stimulates adult mouse retinal ganglion cell axon regeneration via both macrophage- and lens-derived factors. *Eur J Neurosci.* 2005 Apr;21(7):2029–34.
48. Hauk TG, Müller A, Lee J, Schwendener R, Fischer D. Neuroprotective and axon growth promoting effects of intraocular inflammation do not depend on oncomodulin or the presence of large numbers of activated macrophages. *Exp Neurol.* 2008;209(2):469–82.
49. Leibinger M, Andreadaki A, Fischer D. Role of mTOR in neuroprotection and axon regeneration after inflammatory stimulation. *Neurobiol Dis.* 2012;46(2):314–24.
50. Fischer D, Leibinger M. Promoting optic nerve regeneration. *Prog Retin Eye Res.*

2012;31(July):1–15.

51. Apará A, Goldberg JL. Molecular mechanisms of the suppression of axon regeneration by KLF transcription factors. *Neural Regen Res.* 2014;9(15):1418–21.
52. Duan X, Qiao M, Bei F, Kim I-J, He Z, Sanes JR. Subtype-Specific Regeneration of Retinal Ganglion Cells following Axotomy: Effects of Osteopontin and mTOR Signaling. *Neuron.* 2015;85(6):1244–56.
53. Sun F, Park KK, Belin S, Wang D, Lu T, Chen G, Zhang K, Yeung C, Feng G, Yankner BA, et al. Sustained axon regeneration induced by co-deletion of PTEN and SOCS3. *Nature.* 2011 Dec 15;480(7377):372–5.
54. Li S, He Q, Wang H, Tang X, Ho KW, Gao X, Zhang Q, Shen Y, Cheung A, Wong F, et al. Injured adult retinal axons with Pten and Socs3 co-deletion reform active synapses with suprachiasmatic neurons. *Neurobiol Dis.* 2015;73:366–76.
55. Diekmann H, Kalbhen P, Fischer D. Active mechanistic target of rapamycin plays an ancillary rather than essential role in zebrafish CNS axon regeneration. *Front Cell Neurosci.* 2015;9(July):1–11.
56. Pernet V, Joly S, Jordi N, Dalkara D, Guzik-Kornacka A, Flannery JG, Schwab ME. Misguidance and modulation of axonal regeneration by Stat3 and Rho/ROCK signaling in the transparent optic nerve. *Cell Death Dis.* 2013 Jan;4(7):e734.
57. Gobrecht P, Leibinger M, Andreadaki A, Fischer D. Sustained GSK3 activity markedly facilitates nerve regeneration. *Nat Commun.* 2014 Jan 31;5:4561.
58. Belin S, Nawabi H, Wang C, Tang S, Latremoliere A, Warren P, Schorle H, Uncu C, Woolf CJ, He Z, et al. Injury-induced decline of intrinsic regenerative ability revealed by quantitative proteomics. *Neuron.* 2015 May;86(4):1000–14.
59. Sharma TP, Liu Y, Wordinger RJ, Pang I-H, Clark AF. Neuritin 1 promotes retinal ganglion cell survival and axonal regeneration following optic nerve crush. *Cell Death Dis.* 2015 Jan 26;6:e1661.
60. Bray ER, Yungher BJ, Levay K, Ribeiro M, Dvoryanchikov G, Ayupe AC, Thakor K, Marks V, Randolph M, Danzi MC, et al. Thrombospondin-1 Mediates Axon Regeneration in Retinal Ganglion Cells. *Neuron.* 2019 Jun;
61. Kurimoto T, Yin Y, Habboub G, Gilbert H-Y, Li Y, Nakao S, Hafezi-Moghadam A, Benowitz LI. Neutrophils express oncomodulin and promote optic nerve regeneration. *J Neurosci.* 2013 Sep 11;33(37):14816–24.
62. Benowitz LI, Popovich PG. Inflammation and axon regeneration. *Curr Opin Neurol.* 2011 Dec;24(6):577–83.
63. Leibinger M, Andreadaki A, Gobrecht P, Levin E, Diekmann H, Fischer D. Boosting Central Nervous System Axon Regeneration by Circumventing Limitations of Natural Cytokine Signaling. *Mol Ther.* 2016 Oct 16;24(10):1712–25.

64. Hung SSC, Chrysostomou V, Li F, Lim JKH, Wang J-H, Powell JE, Tu L, Daniszewski M, Lo C, Wong RC, et al. AAV-Mediated CRISPR/Cas Gene Editing of Retinal Cells In Vivo. *Investig Ophthalmology Vis Sci*. 2016 Jun 29;57(7):3470.
65. Pernet V, Schwab ME. Lost in the jungle: new hurdles for optic nerve axon regeneration. *Trends Neurosci*. 2014 Jul 1;37(7):381–7.
66. Luo X, Salgueiro Y, Beckerman SR, Lemmon VP, Tsoulfas P, Park KK. Three-dimensional evaluation of retinal ganglion cell axon regeneration and pathfinding in whole mouse tissue after injury. *Exp Neurol*. 2013 Sep;247:653–62.
67. Kigerl K a, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG. Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. *J Neurosci*. 2009;29(43):13435–44.
68. Kyritsis N, Kizil C, Brand M. Neuroinflammation and central nervous system regeneration in vertebrates. *Trends Cell Biol*. 2014 Feb;24(2):128–35.
69. Miron VE, Boyd A, Zhao J-W, Yuen TJ, Ruckh JM, Shadrach JL, van Wijngaarden P, Wagers AJ, Williams A, Franklin RJM, et al. M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. *Nat Neurosci*. 2013;16(9):1211–8.
70. Sierra A, Abiega O, Shahraz A, Neumann H. Janus-faced microglia: beneficial and detrimental consequences of microglial phagocytosis. *Front Cell Neurosci*. 2013 Jan;7:6.
71. Okada T, Ichikawa M, Tokita Y, Horie H, Saito K, Yoshida J, Watanabe M. Intravitreal macrophage activation enables cat retinal ganglion cells to regenerate injured axons into the mature optic nerve. *Exp Neurol*. 2005 Nov;196(1):153–63.
72. Pernet V, Polo A Di. Synergistic action of brain-derived neurotrophic factor and lens injury promotes retinal ganglion cell survival , but leads to optic nerve dystrophy in vivo. *Brain*. 2006;129:1014–26.
73. Barrette B, Hébert M-A, Filali M, Lafortune K, Vallières N, Gowing G, Julien J-P, Lacroix S. Requirement of myeloid cells for axon regeneration. *J Neurosci*. 2008;28(38):9363–76.
74. Lalancette-Hébert M, Gowing G, Simard A, Weng YC, Kriz J. Selective ablation of proliferating microglial cells exacerbates ischemic injury in the brain. *J Neurosci*. 2007;27(10):2596–605.
75. Hauk TG, Leibinger M, Müller A, Andreadaki A, Knippschild U, Fischer D. Stimulation of axon regeneration in the mature optic nerve by intravitreal application of the toll-like receptor 2 agonist Pam3Cys. *Invest Ophthalmol Vis Sci*. 2010;51(1):459–64.

76. Bollaerts I, Van houcke J, Beckers A, Lemmens K, Vanhunsel S, De Groef L, Moons L. Prior Exposure to Immunosuppressors Sensitizes Retinal Microglia and Accelerates Optic Nerve Regeneration in Zebrafish. *Mediators Inflamm.* 2019 Feb 10;2019:1–16.
77. Bollaerts I, Van Houcke J, Andries L, De Groef L, Moons L. Neuroinflammation as Fuel for Axonal Regeneration in the Injured Vertebrate Central Nervous System. *Mediators Inflamm.* 2017;2017:9478542.
78. Singh B, Plemel JR. Neutrophil contribution in facilitating optic nerve regeneration. *J Neurosci.* 2014 Jan 22;34(4):1081–2.
79. Hilla AM, Diekmann H, Fischer D. Microglia Are Irrelevant for Neuronal Degeneration and Axon Regeneration after Acute Injury. *J Neurosci.* 2017 Jun 21;37(25):6113–24.
80. Taoka Y, Okajima K, Uchiba M, Murakami K, Kushimoto S, Johnno M, Naruo M, Okabe H, Takatsuki K. Role of neutrophils in spinal cord injury in the rat. *Neuroscience.* 1997 Jun 6;79(4):1177–82.
81. Boato F, Rosenberger K, Nelissen S, Geboes L, Peters EM, Nitsch R, Hendrix S. Absence of IL-1 β positively affects neurological outcome, lesion development and axonal plasticity after spinal cord injury. *J Neuroinflammation.* 2013 Dec 14;10(1):792.
82. Sato A, Ohtaki H, Tsumuraya T, Song D, Ohara K, Asano M, Iwakura Y, Atsumi T, Shioda S. Interleukin-1 participates in the classical and alternative activation of microglia/macrophages after spinal cord injury. *J Neuroinflammation.* 2012 Dec 7;9(1):553.
83. Schwartz M, Solomon A, Lavie V, Ben-Bassat S, Belkin M, Cohen A. Tumor necrosis factor facilitates regeneration of injured central nervous system axons. *Brain Res.* 1991 Apr 5;545(1–2):334–8.
84. Saleh A, Smith DR, Balakrishnan S, Dunn L, Martens C, Tweed CW, Fernyhough P. Tumor necrosis factor- α elevates neurite outgrowth through an NF- κ B-dependent pathway in cultured adult sensory neurons: Diminished expression in diabetes may contribute to sensory neuropathy. *Brain Res.* 2011 Nov 14;1423:87–95.
85. Kato K, Liu H, Kikuchi S, Myers RR, Shubayev VI. Immediate anti-tumor necrosis factor- β (etanercept) therapy enhances axonal regeneration after sciatic nerve crush. *J Neurosci Res.* 2010 Feb 1;88(2):360–8.
86. White RE, Yin FQ, Jakeman LB. TGF- α increases astrocyte invasion and promotes axonal growth into the lesion following spinal cord injury in mice. *Exp Neurol.* 2008 Nov 1;214(1):10–24.
87. Schwob JE. Neural regeneration and the peripheral olfactory system. *Anat Rec.* 2002 Feb 15;269(1):33–49.

88. Tsarouchas TM, Wehner D, Cavone L, Munir T, Keatinge M, Lambertus M, Underhill A, Barrett T, Kassapis E, Ogryzko N, et al. Dynamic control of proinflammatory cytokines Il-1 β and Tnf- α by macrophages in zebrafish spinal cord regeneration. *Nat Commun.* 2018 Dec 7;9(1):4670.
89. Tsarouchas TM, Wehner D, Cavone L, Munir T, Keatinge M, Lambertus M, Underhill A, Barrett T, Kassapis E, Ogryzko N, et al. Dynamic control of proinflammatory cytokines Il-1 β and Tnf- α by macrophages in zebrafish spinal cord regeneration. *Nat Commun.* 2018 Dec 7;9(1):4670.
90. Hasegawa T, Hall CJ, Crosier PS, Abe G, Kawakami K, Kudo A, Kawakami A. Transient inflammatory response mediated by interleukin-1 β is required for proper regeneration in zebrafish fin fold. *Elife.* 2017;6.
91. Kurihara T, Warr G, Loy J, Bravo R. Defects in macrophage recruitment and host defense in mice lacking the CCR2 chemokine receptor. *J Exp Med.* 1997 Nov 17;186(10):1757–62.
92. Ordoñez-Rueda D, Jönsson F, Mancardi DA, Zhao W, Malzac A, Liang Y, Bertosio E, Grenot P, Blanquet V, Sabrautzki S, et al. A hypomorphic mutation in the Gfi1 transcriptional repressor results in a novel form of neutropenia. *Eur J Immunol.* 2012 Sep 1;42(9):2395–408.
93. Ginhoux F, Lim S, Hoeffel G, Low D, Huber T. Origin and differentiation of microglia. *Front Cell Neurosci.* 2013 Apr 17;7:45.
94. Rathnasamy G, Foulds WS, Ling E-A, Kaur C. Retinal microglia – A key player in healthy and diseased retina. *Prog Neurobiol.* 2019 Feb 1;173:18–40.
95. Colton CA, Wilcock DM. Assessing Activation States in Microglia. *CNS Neurol Disord - Drug Targets.* 2010 Apr 1;9(2):174–91.
96. Harry GJ, Kraft AD. Neuroinflammation and microglia: considerations and approaches for neurotoxicity assessment. *Expert Opin Drug Metab Toxicol.* 2008 Oct;4(10):1265–77.
97. Rice RA, Spangenberg EE, Yamate-Morgan H, Lee RJ, Arora RPS, Hernandez MX, Tenner AJ, West BL, Green KN. Elimination of Microglia Improves Functional Outcomes Following Extensive Neuronal Loss in the Hippocampus. *J Neurosci.* 2015;35(27):9977–89.
98. Cen LP, Han M, Zhou L, Tan L, Liang JJ, Pang CP, Zhang M. Bilateral retinal microglial response to unilateral optic nerve transection in rats. *Neuroscience.* 2015 Dec 17;311:56–66.
99. Sobrado-Calvo P, Vidal-Sanz M, Villegas-Pérez MP. Rat retinal microglial cells under normal conditions, after optic nerve section, and after optic nerve section and intravitreal injection of trophic factors or macrophage inhibitory factor. *J Comp Neurol.* 2007 Apr 20;501(6):866–78.
100. Shechter R, London A, Varol C, Raposo C, Cusimano M, Yovel G, Rolls A, Mack M,

- Pluchino S, Martino G, et al. Infiltrating Blood-Derived Macrophages Are Vital Cells Playing an Anti-inflammatory Role in Recovery from Spinal Cord Injury in Mice. Graeber MB, editor. PLoS Med. 2009 Jul 28;6(7):e1000113.
101. Rong Fan, Feng Xu, Mary Lou Previti, Judianne Davis, Alicia M. Grande, John K. Robinson, and William E. Van Nostrand. Minocycline Reduces Microglial Activation and Improves Behavioral Deficits in a Transgenic Model of Cerebral Microvascular Amyloid. J Neurosci. 2007 Mar 21;27(12):2057–63.
 102. Levkovitch-Verbin H. Animal models of optic nerve diseases. Eye (Lond). 2004 Nov;18(11):1066–74.
 103. Bosco A, Inman DM, Steele MR, Wu G, Soto I, Marsh-Armstrong N, Hubbard WC, Calkins DJ, Horner PJ, Vetter ML. Reduced Retina Microglial Activation and Improved Optic Nerve Integrity with Minocycline Treatment in the DBA/2J Mouse Model of Glaucoma. Invest Ophthalmology Vis Sci. 2008 Apr 1;49(4):1437.
 104. Hanisch U-K, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci. 2007 Nov 26;10(11):1387–94.
 105. Streit WJ. Microglia and the Response to Brain Injury. In: Neuroinflammation — From Bench to Bedside. Berlin, Heidelberg: Springer Berlin Heidelberg; 2002 [cited 2019 May 15]. p. 11–24.
 106. Baldwin KT, Carbajal KS, Segal BM, Giger RJ. Neuroinflammation triggered by β -glucan/dectin-1 signaling enables CNS axon regeneration. Proc Natl Acad Sci U S A. 2015 Feb 24;112(8):2581–6.
 107. Olson JK, Miller SD, Hickey WF, Sedgwick JD. Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. J Immunol. 2004 Sep 15;173(6):3916–24.
 108. Kitayama M, Ueno M, Itakura T, Yamashita T. Activated Microglia Inhibit Axonal Growth through RGMa. Minami M, editor. PLoS One. 2011 Sep 21;6(9):e25234.
 109. Brennan FH, Hall JCE, Guan Z, Popovich PG. Microglia limit lesion expansion and promote functional recovery after spinal cord injury in mice. bioRxiv. 2018 Sep 6;410258.
 110. Bellver-Landete V, Bretheau F, Mailhot B, Vallières N, Lessard M, Janelle M-E, Vernoux N, Tremblay M-È, Fuehrmann T, Shoichet MS, et al. Microglia are an essential component of the neuroprotective scar that forms after spinal cord injury. Nat Commun. 2019 Dec 31;10(1):518.
 111. O’Koren EG, Mathew R, Saban DR. Fate mapping reveals that microglia and recruited monocyte-derived macrophages are definitively distinguishable by phenotype in the retina. Sci Rep. 2016 Feb 9;6:20636.
 112. Goldman D. Müller glial cell reprogramming and retina regeneration. Nat Rev Neurosci. 2014 Jul 4;15(7):431–42.

113. Müller A, Hauk TG, Fischer D. Astrocyte-derived CNTF switches mature RGCs to a regenerative state following inflammatory stimulation. *Brain*. 2007;130(12).
114. Fawcett JW, Asher RA. The glial scar and central nervous system repair. *Brain Res*. 1999;49(6):1–15.
115. Esen N, Tanga FY, DeLeo JA, Kielian T. Toll-like receptor 2 (TLR2) mediates astrocyte activation in response to the Gram-positive bacterium *Staphylococcus aureus*. *J Neurochem*. 2003 Dec 23;88(3):746–58.
116. Gorina R, Font-Nieves M, Márquez-Kisinousky L, Santalucia T, Planas AM. Astrocyte TLR4 activation induces a proinflammatory environment through the interplay between MyD88-dependent NFκB signaling, MAPK, and Jak1/Stat1 pathways. *Glia*. 2011 Feb 1;59(2):242–55.
117. Harada T, Harada C, Kohsaka S, Wada E, Yoshida K, Ohno S, Mamada H, Tanaka K, Parada LF, Wada K. Microglia–Müller Glia Cell Interactions Control Neurotrophic Factor Production during Light-Induced Retinal Degeneration. *J Neurosci*. 2002 Nov 1;22(21):9228–36.
118. Seki M, Tanaka T, Sakai Y, Fukuchi T, Abe H, Nawa H, Takei N. Müller Cells as a Source of Brain-derived Neurotrophic Factor in the Retina: Noradrenaline Upregulates Brain-derived Neurotrophic Factor Levels in Cultured Rat Müller Cells. *Neurochem Res*. 2005 Sep;30(9):1163–70.
119. Gómez-Pinilla F, Vu L, Cotman CW. Regulation of astrocyte proliferation by FGF-2 and heparan sulfate in vivo. *J Neurosci*. 1995 Mar 1;15(3 Pt 1):2021–9.
120. Chang M-Y, Son H, Lee Y-S, Lee S-H. Neurons and astrocytes secrete factors that cause stem cells to differentiate into neurons and astrocytes, respectively. *Mol Cell Neurosci*. 2003 Jul 1;23(3):414–26.
121. Faijerson J, Tinsley RB, Apricó K, Thorsell A, Nodin C, Nilsson M, Blomstrand F, Eriksson PS. Reactive astrogliosis induces astrocytic differentiation of adult neural stem/progenitor cells in vitro. *J Neurosci Res*. 2006 Nov 15;84(7):1415–24.
122. Leibinger M, Andreadaki A, Diekmann H, Fischer D. Neuronal STAT3 activation is essential for CNTF- and inflammatory stimulation-induced CNS axon regeneration. *Cell Death Dis*. 2013 Sep 19;4(9):e805–e805.
123. Leibinger M, Müller A, Gobrecht P, Diekmann H, Andreadaki A, Fischer D. Interleukin-6 contributes to CNS axon regeneration upon inflammatory stimulation. *Cell Death Dis*. 2013 Apr 25;4(4):e609.
124. Cohen A, Bray GM, Aguayo AJ. Neurotrophin-4/5 (NT-4/5) increases adult rat retinal ganglion cell survival and neurite outgrowth in vitro. *J Neurobiol*. 1994 Aug 1;25(8):953–9.
125. Lingor P, Tönges L, Pieper N, Bermel C, Barski E, Planchamp V, Bähr M. ROCK inhibition and CNTF interact on intrinsic signalling pathways and differentially

- regulate survival and regeneration in retinal ganglion cells. *Brain*. 2008 Jan 1;131(1):250–63.
126. Smith PD, Sun F, Park KK, Cai B, Wang C, Kuwako K, Martinez-Carrasco I, Connolly L, He Z. SOCS3 deletion promotes optic nerve regeneration in vivo. *Neuron*. 2009 Dec 10;64(5):617–23.
 127. Müller A, Hauk TG, Leibinger M, Marienfeld R, Fischer D. Exogenous CNTF stimulates axon regeneration of retinal ganglion cells partially via endogenous CNTF. *Mol Cell Neurosci*. 2009 Jun 1;41(2):233–46.
 128. Fischer D. Hyper-IL-6: a potent and efficacious stimulator of RGC regeneration. *Eye*. 2016 Nov 25;
 129. Morgan-Warren PJ, Berry M, Ahmed Z, Scott RAH, Logan A, N. S, BL. T, JL. G, O. M, KH. W, et al. Exploiting mTOR Signaling: A Novel Translatable Treatment Strategy for Traumatic Optic Neuropathy? *Investig Ophthalmology Vis Sci*. 2013 Oct 23;54(10):6903.
 130. Gorsuch RA, Hyde DR. Regulation of Müller glial dependent neuronal regeneration in the damaged adult zebrafish retina. *Exp Eye Res*. 2014 Jun;123:131–40.
 131. Wohl SG, Reh TA. The microRNA expression profile of mouse Müller glia in vivo and in vitro. *Sci Rep*. 2016 Dec 14;6(1):35423.
 132. Va'zquez-Chona FR, Clark AM, Levine EM. *R/bp1* Promoter Drives Robust Müller Glial GFP Expression in Transgenic Mice. *Investig Ophthalmology Vis Sci*. 2009 Aug 1;50(8):3996.
 133. Wang M, Wong WT. Microglia-Müller cell interactions in the retina. *Adv Exp Med Biol*. 2014;801:333–8.
 134. Gao Z, Zhu Q, Zhang Y, Zhao Y, Cai L, Shields CB, Cai J. Reciprocal modulation between microglia and astrocyte in reactive gliosis following the CNS injury. *Mol Neurobiol*. 2013 Dec;48(3):690–701.
 135. Karve IP, Taylor JM, Crack PJ. The contribution of astrocytes and microglia to traumatic brain injury. *Br J Pharmacol*. 2016 Feb;173(4):692–702.
 136. Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, Bennett ML, Münch AE, Chung W-S, Peterson TC, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature*. 2017 Jan 18;541(7638):481–7.
 137. Mitchell DM, Sun C, Hunter SS, New DD, Stenkamp DL. Regeneration associated transcriptional signature of retinal microglia and macrophages. *Sci Rep*. 2019 Dec 18;9(1):4768.
 138. Batlle M, Ferri L, Andrade C, Ortega F-J, Vidal-Taboada JM, Pugliese M, Mahy N, Rodríguez MJ. Astroglia-Microglia Cross Talk during Neurodegeneration in the

Rat Hippocampus. Biomed Res Int. 2015 Apr 21;2015:102419.

139. Kirkley KS, Popichak KA, Afzali MF, Legare ME, Tjalkens RB. Microglia amplify inflammatory activation of astrocytes in manganese neurotoxicity. J Neuroinflammation. 2017;14(1):99.
140. Chen S-H, Oyarzabal EA, Sung Y-F, Chu C-H, Wang Q, Chen S-L, Lu R-B, Hong J-S. Microglial regulation of immunological and neuroprotective functions of astroglia. Glia. 2015 Jan 1;63(1):118–31.
141. Roche SL, Ruiz-Lopez AM, Moloney JN, Byrne AM, Cotter TG. Microglial-induced Müller cell gliosis is attenuated by progesterone in a mouse model of retinitis pigmentosa. Glia. 2018 Feb 1;66(2):295–310.
142. Zhang S, Zhang S, Gong W, Zhu G, Wang S, Wang Y, Halim M, Wang K, Zhou G, Liu Q. Müller Cell Regulated Microglial Activation and Migration in Rats With N-Methyl-N-Nitrosourea-Induced Retinal Degeneration. Front Neurosci. 2018 Dec 3;12:890.
143. Wang M, Ma W, Zhao L, Fariss RN, Wong WT. Adaptive Müller cell responses to microglial activation mediate neuroprotection and coordinate inflammation in the retina. J Neuroinflammation. 2011 Dec 7;8(1):173.
144. Joly S, Lange C, Thiersch M, Samardzija M, Grimm C. Leukemia Inhibitory Factor Extends the Lifespan of Injured Photoreceptors In Vivo. J Neurosci. 2008 Dec 17;28(51):13765–74.
145. Carwile ME, Culbert RB, Sturdivant RL, Kraft TW. Rod Outer Segment Maintenance Is Enhanced in the Presence of bFGF, CNTF and GDNF. Exp Eye Res. 1998 Jun;66(6):791–805.
146. Harada C, Harada T, Quah H-MA, Maekawa F, Yoshida K, Ohno S, Wada K, Parada LF, Tanaka K. Potential role of glial cell line-derived neurotrophic factor receptors in Müller glial cells during light-induced retinal degeneration. Neuroscience. 2003;122(1):229–35.
147. Arroba AI, Álvarez-Lindo N, van Rooijen N, de la Rosa EJ. Microglia-Mediated IGF-I Neuroprotection in the *rd10* Mouse Model of Retinitis Pigmentosa. Investig Ophthalmology Vis Sci. 2011 Nov 24;52(12):9124.
148. Jha MK, Jo M, Kim JH, Suk K. Microglia-Astrocyte Crosstalk: An Intimate Molecular Conversation. Neuroscientist. 2019;25(3):227–40.
149. Stout RD, Suttles J. Functional plasticity of macrophages: reversible adaptation to changing microenvironments. J Leukoc Biol. 2004 Sep;76(3):509–13.
150. Van Hove H, Martens L, Scheytlens I, De Vlaminck K, Pombo Antunes AR, De Prijck S, Vandamme N, De Schepper S, Van Isterdael G, Scott CL, et al. A single-cell atlas of mouse brain macrophages reveals unique transcriptional identities shaped by ontogeny and tissue environment. Nat Neurosci. 2019 Jun 6;22(6):1021–35.

151. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol.* 2008 Dec 1;8(12):958–69.
152. David S, Greenhalgh AD, Kroner A. Macrophage and microglial plasticity in the injured spinal cord. *Neuroscience.* 2015 Oct 29;307:311–8.
153. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, Gordon S, Hamilton JA, Ivashkiv LB, Lawrence T, et al. Macrophage Activation and Polarization: Nomenclature and Experimental Guidelines. *Immunity.* 2014 Jul 17;41(1):14–20.
154. Gensel JC, Zhang B. Macrophage activation and its role in repair and pathology after spinal cord injury. *Brain Res.* 2015 Sep 4;1619:1–11.
155. Lee-Liu D, Edwards-Faret G, Tapia VS, Larraín J. Spinal cord regeneration: Lessons for mammals from non-mammalian vertebrates. *genesis.* 2013 Aug 1;51(8):529–44.
156. David S, Kroner A. Repertoire of microglial and macrophage responses after spinal cord injury. *Nat Rev Neurosci.* 2011 Jul 15;12(7):388–99.
157. Davis MJ, Tsang TM, Qiu Y, Dayrit JK, Freij JB, Huffnagle GB, Olszewski MA. Macrophage M1/M2 polarization dynamically adapts to changes in cytokine microenvironments in *Cryptococcus neoformans* infection. *MBio.* 2013 Jun 18;4(3):e00264-13.
158. Ma S-F, Chen Y-J, Zhang J-X, Shen L, Wang R, Zhou J-S, Hu J-G, Lü H-Z. Adoptive transfer of M2 macrophages promotes locomotor recovery in adult rats after spinal cord injury. *Brain Behav Immun.* 2015 Mar 1;45:157–70.
159. Klimczak RR, Koerber JT, Dalkara D, Flannery JG, Schaffer D V. A novel adeno-associated viral variant for efficient and selective intravitreal transduction of rat Müller cells. *PLoS One.* 2009 Jan 14;4(10):e7467.
160. Guerrero AR, Uchida K, Nakajima H, Watanabe S, Nakamura M, Johnson WE, Baba H. Blockade of interleukin-6 signaling inhibits the classic pathway and promotes an alternative pathway of macrophage activation after spinal cord injury in mice. *J Neuroinflammation.* 2012 Dec 27;9(1):533.
161. Nguyen-Chi M, Laplace-Builhe B, Travnickova J, Luz-Crawford P, Tejedor G, Phan QT, Duroux-Richard I, Levraud J-P, Kissa K, Lutfalla G, et al. Identification of polarized macrophage subsets in zebrafish. *Elife.* 2015 Jul 8;4:e07288.
162. Miller SJ. Astrocyte Heterogeneity in the Adult Central Nervous System. *Front Cell Neurosci.* 2018;12:401.
163. Beyrau M, Bodkin JV, Nourshargh S. Neutrophil heterogeneity in health and disease: a revitalized avenue in inflammation and immunity. *Open Biol.* 2012 Nov;2(11):120134.
164. Rheaume BA, Jereen A, Bolisetty M, Sajid MS, Yang Y, Renna K, Sun L, Robson P,

- Trakhtenberg EF. Single cell transcriptome profiling of retinal ganglion cells identifies cellular subtypes. *Nat Commun.* 2018 Dec 17;9(1):2759.
165. Watanabe M, Sawai H, Fukuda Y. Number, distribution, and morphology of retinal ganglion cells with axons regenerated into peripheral nerve graft in adult cats. *J Neurosci.* 1993 May 1;13(5):2105–17.
 166. Berry M, Ahmed Z, Logan A. Return of function after CNS axon regeneration: Lessons from injury-responsive intrinsically photosensitive and alpha retinal ganglion cells. *Prog Retin Eye Res.* 2018 Nov 17;
 167. Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, Gabriely G, Koeglsperger T, Dake B, Wu PM, Doykan CE, et al. Identification of a unique TGF- β -dependent molecular and functional signature in microglia. *Nat Neurosci.* 2014 Jan 8;17(1):131–43.

Figure 1: Overview of the molecular pathways possibly involved in inflammation-induced axonal regeneration within/between the different glial/inflammatory cell populations, as gathered from research performed in rodents and fish. The neuroinflammatory response involves different cellular processes. a) Blood-borne myeloid cells are infiltrating, with neutrophils invading first at early time points after injury (early), followed by macrophages at later time points after injury (late). Neutrophils and macrophages have been known to induce axonal regeneration via oncomodulin. Moreover, an early upregulated expression of IL-1 β followed by an increase of TNF expression has been suggested to play an important role in axonal regeneration in spinal cord injury models. b) In addition, resident myeloid microglia get reactivated which are known to be pro-inflammatory at first (early) and switch to an anti-inflammatory phenotype at later stages (late). So, they express both pro- and anti-inflammatory cytokines in a time-dependent manner which can influence CNS recovery. c) Moreover, also macroglial cells, such as astrocytes and Müller glia, get reactivated. They mainly affect axonal regeneration via the production of CNTF, LIF and IL-6. Furthermore, crosstalk between the different cell types could be necessary to induce axonal regeneration. Of note, in some cases, molecular signaling is timed and restricted to either early or later time points after injury, as indicated with the designations 'early' or 'late'.