Published in final edited form as:
Arch Ophthalmol. 2010 August ; 128(8): 1059–1064. doi:10.1001/archophthalmol.2010.152.

Optic Nerve Regeneration

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Abstract

Retinal ganglion cells (RGCs) are normally unable to regenerate their axons after optic nerve injury or degenerative disorders, resulting in lifelong visual losses. This situation can be partially reversed by activating RGCs' intrinsic growth state, maintaining their viability, and counteracting inhibitory signals in the extracellular environment. Advances over the past few years continue to extend the amount of regeneration that can be achieved in animal models. These findings lend hope to the possibility that clinically meaningful regeneration may become a reality within a few years provided that regenerating axons can be guided to their appropriate destinations.

Keywords

optic nerve; regeneration; trophic factor; oncomodulin; PTEN; SOCS3; KIF4

As in most CNS pathways, axons that are injured in the mature optic nerve do not grow back, leaving victims of traumatic nerve injury or degenerative diseases such as glaucoma with lifelong losses in vision. The optic nerve has long been studied for insights into the causes of regenerative failure in the CNS, focusing on such issues as the inhibitory effects of CNS myelin and the glial scar, the absence of appropriate trophic factors, the immune response to injury, cell death pathways, and the decline in neurons' intrinsic growth capacity. The past 10–15 years have witnessed major advances in understanding why retinal ganglion cells (RGCs) normally fail to regenerate injured axons through the optic nerve and in devising ways to reverse this situation, lending hope to the possibility that functional repair might one day be possible.

Axon regeneration through the optic nerve

Under normal circumstances, damaged axons show a transient sprouting response following optic nerve injury but no long-distance regeneration. Tello, a student of Ramon y Cajal, found that if the optic nerve is cut and sutured to a segment of peripheral nerve (PN), axons will grow into the graft[1]. Aguayo and colleagues showed that some RGCs can regenerate axons through a PN graft that extends all the way from the cut end of the optic nerve to the superior colliculus and form synapses in the correct layer[2, 3].

The ability of RGCs to regenerate axons through a PN graft is likely to be related in part to higher levels of growth-permissive molecules (*e.g.*, laminin) and lower levels of growth-inhibitory molecules (*e.g.*, NogoA) in peripheral nerves vs. the optic nerve[4–6]. However, it is also possible that the two differ in their ability to provide essential trophic factors. To test

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this latter possibility, Berry and colleagues implanted a fragment of PN into the vitreous humor and found that this stimulated RGCs to regenerate lengthy axons beyond the site of an optic nerve crush injury[7]. Although this growth was initially attributed to trophic factors derived from Schwann cells, the grafts contained numerous macrophages, which can enhance axon regeneration when pre-activated and placed in the optic nerve[8, 9]. Other methods that induce intraocular inflammation, *i.e.*, injuring the lens or injecting the pro-inflammatory agent Zymosan into the eye, lead to even greater regeneration than PN implants (Fig. 1a,b)[10, 11, 91]. This regeneration is associated with a dramatic change in RGCs' intrinsic growth state, as evidenced by a marked upregulation of proteins such as GAP-43 and SPRR1A[12]. Although PN implants secrete ciliary neurotrophic factor (CNTF)[13], their primary effect *in vivo* is related to other factors associated with macrophages[14].

Oncomodulin is a potent growth factor for RGCs

Using dissociated retinal cell cultures as a bioassay, two molecules that are present in the eye were found to stimulate mature RGCs to regenerate their axons. One is man-nose, a simple sugar that is abundant in the vitreous. Mannose stimulates RGCs to extend moderately long axons provided that cells have sufficiently high levels of intracellular cAMP ($[cAMP]_i$)[15]. The second growth factor is oncomodulin (Ocm), a 12 kDa calcium-binding protein that is secreted by macrophages. Ocm accumulates rapidly in the eye following intravitreal inflammation and exhibits cAMP-dependent, high-affinity binding to a cell-surface receptor on RGCs[16, 17]. When released from polymeric beads placed into the vitreous, Ocm plus a cAMP analog induce nearly as much optic nerve axon regeneration as intraocular inflammation[16] (Fig. 1c). Conversely, either an Ocm peptide antagonist or a neutralizing anti-Ocm antibody dramatically suppresses inflammation-induced regeneration (Fig. 1d)[17]. Thus, Ocm appears to mediate most of the effect of intravitreal inflammation on optic nerve regeneration. However, additional factors derived from inflammatory cells or retinal glia also appear to play a role by causing an elevation of $[cAMP]_I$ and by enhancing RGC survival[17]. One group failed to detect an elevation of Ocm in the eye following inflammation[18] and reported that an anti-Ocm antibody does not diminish inflammation-induced regeneration[19]. The likely sources of these discrepant results are discussed elsewhere[17, 20]. Intraocular inflammation also enhances the ability of RGCs to regenerate their axons through a PN graft[11, 21], and this effect is likewise blocked by an Ocm antagonist peptide[17].

Altering intracellular signaling can promote optic nerve regeneration

The signaling pathways that enable RGCs to regenerate their axons are beginning to emerge. Mst3b, a purine-sensitive protein kinase, plays a central role in the signal transduction pathway through which trophic factors induce axon growth[22, 23]. Suppression of Mst3b expression blocks the axon-promoting effects of Ocm in culture and of inflammation-induced regeneration *in vivo*[23], whereas expression of a constitutively active form of Mst3b enables RGCs to regenerate axons even in the absence of growth factors[23]. The effects of Ocm can also be blocked by an inhibitor of CaM kinases or by combining inhibitors of the PI3K, MAPK, and Jak-STAT pathways[16]. Conversely, deleting genes that encode suppressors of these pathways stimulates axon regeneration *in vivo*. Appreciable optic nerve regeneration can be stimulated by deleting the gene for PTEN, a protein- and lipid phosphatase that suppresses signaling through the PI3 kinase-Akt pathway[24], and, to a somewhat lesser extent, by deleting the gene encoding SOCS3, a protein that suppresses signaling through the Jak-STAT pathway[25]. Deletion of either PTEN or SOCS3 leads to phosphorylation of the S6 kinase, implying that activation of the mTOR pathway plays an important role.

The intrinsic growth capacity of RGCs declines in the early postnatal period[26], and is accompanied by changes in the expression of Kruppel-Like Family (KLF) transcription factors. Overexpression of KLF-4 suppresses axon growth in immature RGCs, whereas diminished KLF-4 expression increases axon growth in mature RGCs and promotes a modest amount of regeneration *in vivo*[27]. It is not yet known whether the effects of intraocular inflammation, PTEN deletion, or SOCS deletion are mediated through changes in the expression of any KLF transcription factors.

The role of cAMP in optic nerve regeneration

The second messenger cAMP augments axon regeneration in multiple ways, including altering the response of growth cones to inhibitory signals[28, 29], stimulating the translocation of growth factor receptors to the cell surface[16, 30], and altering gene expression programs[31]. The latter effects include down-regulation of SOCS-3 expression[32] and upregulation of Arginase I (Arg I)[31]. Arg I is an enzyme involved in the biosynthesis of polyamines, which enhance the ability of neurons to extend axons over inhibitory substrates[31]. Spermidine stimulates a modest amount optic nerve regeneration[33], as does elevation of cAMP[16, 34, 35]. As noted above, cAMP strongly enhances the effects of Ocm[16], and increases the effects of intraocular inflammation[36]. A peptide that prevents Ocm from binding to its receptor eliminates the latter effects, showing that Ocm is the principal factor involved in both inflammation-induced regeneration and the enhancement of this phenomenon by cAMP[37].

RGC survival after optic nerve injury

RGCs begin to die a few days after their axons are injured, particularly if damage occurs close to the eye[38]. This death can be prevented almost completely by overexpressing the anti-apoptotic Bcl family proteins Bcl-2 or Bcl-xL in RGCs[39, 40]. However, although axon regeneration clearly requires RGCs to remain viable, axon outgrowth and cell survival utilize different intracellular signaling pathways. This dissociation is exemplified by the failure of RGCs overexpressing Bcl-2 or Bcl-xL to regenerate axons without additional growth factors[39, 41] and by the persistent enhancement of RGC survival seen after intraocular inflammation even when regeneration is suppressed by Ocm-blocking reagents[17].

RGC death can be slowed, but not stopped, with a number of trophic factors, including CNTF[35, 42, 43], brain-derived neurotrophic factor (BDNF)[42, 44, 45], neurotrophin-4/5 (NT-4/5)[46, 47], nerve growth factor (NGF)[48], insulin-like growth factor-1[49], granulocyte-colony stimulating factor[50], glial-derived neurotrophic factor (GDNF)[51, 52] and neurturin[53]. BDNF combined with GDNF, neurturin, or intraocular inflammation has additive effects on survival, although the latter combination suppresses axon regeneration[53, 54].

The death of axotomized RGCs can be slowed by preventing Caspase cleavage[55–58], blocking the nuclear enzyme poly(ADP-ribose) polymerase (PARP), a substrate for caspases[59], blocking nitric oxide synthase[60], introducing reducing agents[61] or inhibiting cell death via caspase-independent pathways[62–64]. Long-term prevention of RGC death after axotomy may require a combination of treatments.

Effects of other trophic factors on optic nerve regeneration

FGF2 stimulates some axon regeneration through the optic nerve[65]. NGF, NT-3, BDNF, and NT-4/5 do not[66, 67], although a combination of FGF2, NT-3 and NGF has been reported to induce substantial regeneration[67]. One group has argued that CNTF mediates the effects of intravitreal inflammation on optic nerve regeneration, based primarily on the outgrowth seen using concentrations of CNTF several orders of magnitude above the established ED_{50} value

and on the loss of axon regeneration and RGC survival seen when the genes encoding CNTF and LIF are deleted[19, 36, 68]. However, physiologically relevant concentrations of CNTF do not promote strong regeneration in culture[10, 16, 17, 46] and many labs have failed to find strong effects of CNTF *in vivo*[10, 25, 43, 54, 69]. In addition, CNTF inhibitors have no effect[10, 70] or only a mild effect[19] on inflammation-induced regeneration. CNTF enhances axon regeneration through a peripheral nerve graft[35, 71], but this effect is associated with intraocular inflammation and is eliminated when inflammation is suppressed[72]. Thus, the direct effect of CNTF on optic nerve regeneration is weak, although it may contribute to maintaining RGC survival. The axon-promoting effects of CNTF become strong when the gene that encodes SOCS3, the negative regulator of the jak-STAT pathway, is deleted[25]. However, optic nerve injury leads to an upregulation of SOCS in RGCs[12], which may help explain the low responsiveness of axotomized RGCs to CNTF[10, 25, 43, 54, 69], and intraocular inflammation amplifies axoto-my-induced SOCS upregulation greatly[12], further limiting any possible contribution of CNTF to inflammation-induced regeneration[20].

Growth-inhibitory signals in the optic nerve

The mature optic nerve contains many molecules that suppress axon growth, including the myelin-associated inhibitors NogoA, myelin-associated glycoprotein (MAG), and oligodendrocyte-myelin glycoprotein (OMgp); proteoglycans that accumulate in the scar at the injury site; and additional axon-repellants (*e.g.*, Semaphorins)[73–77]. Methods that counteract NogoA signaling do not lead to appreciable optic nerve regeneration on their own[78]. However, expression of a dominant-negative form of the nogo receptor strongly amplifies the axon-promoting effects of intraocular inflammation[79]. A more comprehensive way to counteract inhibition is by inactivating the small GTPase RhoA, a part of the intracellular pathway through which multiple signals inhibit axon growth. RhoA inhibition results in modest levels of axon regeneration in the injured optic nerve[80, 81], but increases the amount of regeneration associated with intraocular inflammation greatly[12] (Fig. 1e). Thus, although counteracting inhibitory signals is not sufficient to induce extensive optic nerve regeneration, treatments that simultaneously activate RGCs' growth state and counteract inhibition can have dramatic effects.

Transforming RGCs into an active growth state itself enables axons to partially overcome inhibitory signals. The scar that forms at the injury site contains basement membrane components[82] that are partially degraded by matrix metalloproteinases associated with growing axons[83]. However, as noted above, multiple other inhibitory signals remain in place, as evidenced by the dramatic effects seen when RhoA activity is suppressed in actively growing axons.

Axon guidance cues during development and regeneration

The initial development of retinal projections involves multiple cues that guide axons through the retina, optic disc, optic nerve, optic chiasm, diencephalon, and midbrain, and enable them to form a precise, topographically organized representation of visual space upon central target areas. The guidance of retinal axons during development involves many types of axon-guidance molecules, including netrins, Semaphorins, laminin, multiple members of the Ephrin/Eph families, Wnts, and Slits[84–86]. In view of the complex guidance mechanisms involved in the development of retinal projections, it will be important to determine whether the appropriate guidance cues persist in the mature brain to guide regenerating axons back to their correct destinations. There is evidence that at least some guidance cues remain in the mature CNS or become upregulated after optic nerve damage[87–89]. However, whether these cues will suffice to guide regenerating axons to their proper target areas and to re-form topographically organized maps remains to be determined.

Are we there yet?

In studies using the PN graft model, anterograde tracing and electrophysiology show that a small number of axons can regenerate all the way back to the superior colliculus[2, 90]. In the case of axon regeneration through the optic nerve, one group reported a re-mapping of the retina upon the SC[91], but the accompanying histology raised questions as to whether the axons had been severed in the first place. In view of the scientific and clinical importance of successful regeneration, it will be important to apply strict criteria to proving one's case, *e.g.*, evidence that connections are forming gradually over time and are not due to spared axons[92], and that any electrophysiological changes that are observed correlate with clear anatomical evidence of regeneration. In spite of these issues, the advances that have occurred over the past few years lend encouragement to the possibility that at least some RGCs will be able to regenerate their axons all the way to their central targets. The next challenges will include finding ways to optimize this regeneration and testing whether it restores functionally meaningful levels of vision.

Acknowledgments

We are grateful to the National Eye Institute of the NIH (EY05690 to LB), the Sheldon and Miriam Adelson Medical Research Foundation, and Alseres Pharmaceuticals for support of research from the authors' laboratory.

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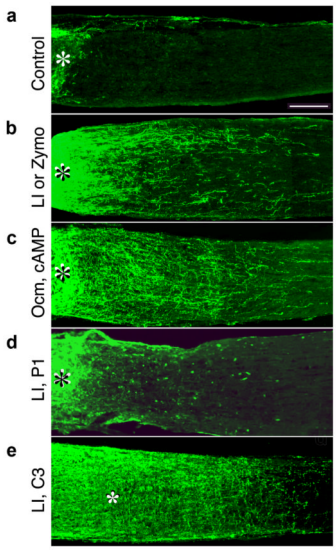

Figure 1.

Axon regeneration in the rat optic nerve. Longitudinal sections through the rat optic nerve were stained with antibodies to the protein GAP-43 2 weeks after optic nerve injury to visualize regenerating axons. Asterisks denote the injury site. (a) Almost no regeneration occurs in the absence of further stimulation. (b) Lens injury (LI) or Zy-mosan (Zymo) induces intraocular inflammation and enables RGCs to regenerate axons through the optic nerve[10, 11]. (c) Ocm plus a cAMP analog, when delivered from slow-release polymeric beads, mimic the effects of lens injury[16]. (d) P1, an Ocm receptor antagonist suppresses the effects of lens injury[17]. (e) Expression of the bacterial enzyme C3 ribosyltransferase (C3) in RGCs blocks the activity of RhoA and enables axons to ignore inhibitory signals in their environment. C3 expression by itself produces only modest levels of regeneration, but greatly enhances the effects of intraocular inflammation after LI[12].