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Research article

The challenge of regenerative therapies for the optic nerve in glaucoma☆

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ABSTRACT

This review arose from a discussion of regenerative therapies to treat optic nerve degeneration in glaucoma at the 2015 Lasker/IRRF Initiative on Astrocytes and Glaucomatous Neurodegeneration. In addition to the authors, participants included Jonathan Crowston, Andrew Huberman, Elaine Johnson, Richard Lu, Hemai Phatnami, Rebecca Sappington, and Don Zack. Glaucoma is a neurodegenerative disease of the optic nerve, and is the leading cause of irreversible blindness worldwide. The disease progresses as sensitivity to intraocular pressure (IOP) is conveyed through the optic nerve head to distal retinal ganglion cell (RGC) projections. Because the nerve and retina are components of the central nervous system (CNS), their intrinsic regenerative capacity is limited. However, recent research in regenerative therapies has resulted in multiple breakthroughs that may unlock the optic nerve's regenerative potential. Increasing levels of Schwann-cell derived trophic factors and reducing potent cell-intrinsic suppressors of regeneration have resulted in axonal regeneration even beyond the optic chiasm. Despite this success, many challenges remain. RGC axons must be able to form new connections with their appropriate targets in central brain regions and these connections must be retinotopically correct. Furthermore, for new axons penetrating the optic projection, oligodendrocyte glia must provide myelination. Additionally, reactive gliosis and inflammation that increase the regenerative capacity must be outweigh pro-apoptotic processes to create an environment within which maximal regeneration can occur.

1. Introduction

Glaucoma causes blindness through degeneration of the retinal ganglion cell (RGC) projection to the brain, which effectively separates the visual cortex from its sensory input. Degeneration arises in most forms of the disease from chronic (e.g., progressive) stress due to sensitivity to intraocular pressure (IOP). This stress is conveyed or transduced to the unmyelinated RGC axon segment as it passes through the optic nerve head in complex ways that include mechanical, inflammatory, and bioenergetic components. In exiting the nerve head, the axon becomes myelinated by oligodendrocytes in forming the remainder of the optic nerve. Both the pressure-dependent nature of this stress and its origin at the nerve head are considered defining features of glaucoma in its many forms. Even so, signs of RGC degeneration in glaucoma can be found early in progression at distal sites in the projection. This observation underscores that glaucoma is like other neurodegenerative disorders in that neuronal stress at one site can be manifest pathogenically at quite another.

The distal RGC projection is important in the context of regeneration or repair for a number of reasons. One of the hallmarks of glaucoma is RGC axonal dysfunction early in progression (Libby et al., 2005; Nickells, 2007; Calkins, 2012; Howell et al., 2013).

☆ This article summarizes the results of a targeted session on this topic at the March 2015 conference Astrocytes and Glaucomatous Neurodegeneration. This meeting was a follow-up to the 2010 meeting on the same topic, both of which were conducted as part of The Lasker/IRRF Initiative for Innovation in Vision Science. For more information about this conference, its participants and other review articles that originated from it see Tamm and Dowling, 2016.

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One index of this dysfunction is depletion of anterograde intra-axonal transport, that is, transport from the retina to central projection targets in the brain (Crish et al., 2010). Degradation of anterograde axonal transport to the brain marks the beginning of an interventional window, defined by the period between the onset of axon dysfunction and actual degeneration of RGC axons in the optic projection and RGC bodies in the retina, which occur later. Neuroprotective interventions that rescue axon transport to the brain also prevent axon degeneration in the optic nerve and subsequent RGC body loss in the retina. These include systemic delivery of brimonidine tartrate, an alpha-adrenergic receptor agonist (Lambert et al., 2011), and topical delivery of a selective p38 MAP kinase inhibitor (Dapper et al., 2013). Conversely, conditions that accelerate axon dysfunction also accelerate degeneration in the nerve and RGC loss in the retina (Ward et al., 2014). The bottom line is this: if axon function can be repaired early enough, either by reducing IOP-related stress on the axon or by ameliorating the influence of this stress, loss of tissue in glaucoma can be avoided – at least in experimental models. That the same might hold true for human glaucoma is gleaned from results showing reversal of physiological deficits with early IOP-lowering interventions (Sehi et al., 2010).

Unfortunately, loss of vision in glaucoma is difficult to detect early on (Crabb et al., 2013), so by the time patients are identified they often have substantial visual field deficits, corresponding to loss of RGC axons in the optic nerve. Since the nerve and retina are integral components of the central nervous system (CNS), their intrinsic (or spontaneous) capacity for regeneration in adult tissue is severely limited by the same multitude of factors that limit regeneration in other CNS tissues, such as the spinal cord. Several factors compound the problem. RGC axon degeneration in the optic nerve is not confined to a discrete locus, nor does it follow a single mechanistic process. Rather, evidence combined from chronic and inducible models suggests that axon degeneration in glaucoma involves components of both early and progressive distal axonopathy and more rapid Wallerian degeneration, involving disassembly of the axon at multiple points along the nerve (Calkins, 2012). Thus, regenerative strategies either have to replace entire lengths of axon or repair axons at multiple break points. In either case, the task is daunting, as the optic nerve in the human brain stretches some 5 cm in total length.

2. A brief history of optic nerve regeneration

As recently as 20 years ago, most observers considered the possibility of optic nerve regeneration to be remote. Although rodent RGCs show robust axon outgrowth through the late-embryonic or early postnatal period when placed in culture and *in vivo*, this growth capacity is lost shortly after birth due to cell-cell contacts that occur during the formation of retinal circuitry (Chen et al., 1995; Goldberg et al., 2002a). Nonetheless, in the early twentieth century, Cajal's student Tello found that a few RGCs could regenerate axons into a peripheral nerve graft sutured to the cut end of the optic nerve, and several decades later, Aguayo and his colleagues investigated this phenomenon in considerable depth (Ramon y Cajal, 1991; Aguayo et al., 1991).

Axon regeneration through the optic nerve itself was thought to be impossible until 1996, when Berry et al. discovered that implanting a peripheral nerve graft into the back of the eye – with the intention of providing Schwann cell-derived trophic factors – enabled many RGCs to regenerate axons well into the optic nerve itself. While testing whether this latter phenomenon might be mediated by a glial cell-derived factor that they were studying at the time, the Benowitz lab discovered that several manipulations that induce intraocular inflammation, including lens injury or injection of Zymosan, a yeast cell wall preparation, was a sufficient stimulus to induce regeneration (Leon et al., 2000; Yin et al., 2003). The primary mediator of this phenomenon was identified as Oncomodulin (Ocm), an atypical growth factor that is heavily expressed by both neutrophils and macrophages and that binds to a high-affinity receptor on RGCs in a cAMP-dependent manner (Yin et al., 2006; Yin et al., 2009; Kurimoto et al., 2010; Kurimoto et al., 2013). The effects of Zymosan are strongly enhanced when it is combined with manipulations that counteract cell-extrinsic or cell-intrinsic suppressors of axon growth. Whereas counteracting cell-extrinsic suppressors of axon growth by itself results in only modest levels of regeneration, combining any of these treatments with Zymosan increases the effects of the latter several-fold (Lehmann et al., 1999; Fischer et al., 2004a,b; Stiles et al., 2013).

Some of the most potent cell-intrinsic suppressors of regeneration increase during the course of development and account in part for the decline in regenerative capacity in the early postnatal period noted earlier (Wang et al., 2007; Park et al., 2009). These include the transcription factor Klf-4 (Moore et al., 2009), PTEN, a suppressor of cell signaling through the PI3 kinase-Akt pathway (Park et al., 2008), and SOCS3, which suppresses signaling through the Jak-STAT pathway and prevents agents such as CNTF from having a major effect (Smith et al., 2009). Other Klf family transcription factors that promote regeneration decline during development (Moore et al., 2009). Combining Zymosan, a cAMP analog, and *pten* gene deletion results in approximately 10 times more regeneration than any of these treatments in isolation and enables some RGCs to regenerate axons into central target areas (Fig. 1), with a partial return of simple visual responses (Kurimoto et al., 2010; De Lima et al., 2012). Combining double-deletion of *pten* and *socs3* with CNTF also has strongly synergistic effects, though not many axons extend past the optic chiasm unless surgery is done in the distal optic nerve, in which case there is an eventual innervation of the suprachiasmatic nucleus (Sun et al., 2011; Li et al., 2014a,b). Another major factor that suppresses optic nerve regeneration is the massive elevation of ionic zinc that occurs in synaptic contacts between amacrine cells and RGCs within an hour after nerve injury (Li et al., 2014a,b). Finally, at least one strain of mice has been identified that shows considerably greater optic nerve regeneration in response to fzymosan than the highly inbred strains that are more commonly used for this type of research (Omura et al., 2015).

3. Challenges and future directions

3.1. Specificity of connections

In order for regenerated (or replaced) axons to be most relevant for vision, they must satisfy several distinct criteria. One is **target specificity**. The optic projection innervates several subcortical nuclei that serve different aspects of visual processing (Fig. 2). Whereas most RGC axons in the human brain project to the lateral geniculate nucleus (LGN) of the thalamus, other RGCs project to alternative sites, such as the suprachiasmatic nucleus (SNC), olivary pretectal complex, several nuclei concerned with image stabilization, and superior colliculus (SC), the most distal subcortical projection. For now, the goal of regeneration ought to be to restore vision – any vision – where it has been lost, period. However, our expectation is that regenerative strategies eventually will evolve that ensure the right RGCs project to the right neurons in the right target area.

The second issue we must consider is **retinotopic specificity**. During development of the visual system, RGCs of a specific functional type mature to cover the retina such that cells representing adjacent patches of the photoreceptor array maintain the same