INFLAMMATION-DERIVED GROWTH FACTORS

Optic nerve regeneration is becoming a reality.


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Optic nerve trauma, ischemia, and certain degenerative eye diseases can lead to permanent vision loss due to the inability of retinal ganglion cells (RGCs) to regenerate the axons that convey visual information from the eye to the brain and the subsequent death of RGCs. Over the past 20 years, considerable progress has been made in defining factors that promote or suppress axon regeneration and RGC survival after optic nerve injury.

This article describes research from our lab that has identified trophic factors derived from inflammatory cells that promote appreciable levels of optic nerve regeneration. These findings provide the basis for a potentially viable gene therapy–based approach that might in the future help enable damaged retinal axons to grow back to the brain and restore vision to patients with optic nerve damage.

INTRAOCULAR INFLAMMATION INDUCES OPTIC NERVE REGENERATION

We discovered that an unintentional injury to the lens induces considerable axon regeneration and that this effect could be mimicked by inducing intraocular inflammation with zymosan, a fragment of the yeast cell wall.[1,2] This regeneration was found to be associated with a change in RGCs' intrinsic growth state, as evidenced by a massive upregulation of *GAP-43*, *SPRR1A*, and other growth-associated proteins (GAPs; also called regeneration-associated gene products, or RAGs) in a pattern similar to that seen during peripheral nerve regeneration.[3]

Genetic deletion of two receptors that are expressed by inflammatory cells, Toll-like receptor 2 (*TLR2*) and dectin-1, eliminates the proregenerative effects of zymosan, despite not altering the general profile of infiltrative cells.[4] β-glucan is a component of zymosan that stimulates cells of the innate immune system via dectin-1, and curdlan, a particulate form of β-glucan, mimics the effects of zymosan on regeneration.[4]

Combining intraocular inflammation with elevation of cAMP and *PTEN* deletion in RGCs and other cells infected with an adeno-associated virus serotype 2 (AAV2) expressing an anti-*PTEN* short hairpin RNA (shRNA) has strongly synergistic effects. These include increasing axon regeneration 10-fold compared with any of the treatments alone and enabling some axons to reach the optic chiasm by 6 weeks[5] and to reinnervate subcortical visual nuclei by 10 to 12 weeks.[6] These regenerating axons become myelinated, although the process proceeds slowly.[7] The brain target reinnervation leads to a limited recovery of simple visual reflexes such as the optomotor response.[6]

These findings raise the question of whether the positive factors associated with inflammation can be identified to promote regeneration in a clinically useful way.

Oncomodulin. Our earlier work showed that the carbohydrate mannose, which is abundant in the vitreous and cerebrospinal fluid, stimulates appreciable axon growth from goldfish RGCs and moderate outgrowth from rat RGCs. These effects require elevation of cAMP and are strongly augmented by a protein secreted by activated macrophages.[2,8] Using column chromatography, mass spectrometry, and bioassays, we identified the 11 kDa Ca^{2+}-binding protein oncomodulin (Ocm) as a major growth-promoting factor associated with inflammation.[9]

Ocm is secreted by infiltrative neutrophils and macrophages, and it accumulates in the neural retina 12 to 24 hours after induction of intraocular inflammation, binding to a high-affinity receptor on RGCs (K_d ~ 28 nM).[5,9,10] Elevation of cAMP alone induces only modest axon regeneration[11] but is required for Ocm and other trophic factors to bind to their cognate receptors on RGCs.[5,9,12,13] Delivery of Ocm and a cAMP analog via slow-release polymer beads mimics the proregenerative effects of zymosan; conversely, blocking the effects of Ocm with either a neutralizing antibody or a blocking peptide strongly suppresses the effects of zymosan.[9,10,14] Regeneration is also diminished by immune-depletion of neutrophils, implying that these first responders of the innate immune system mediate most of the effects of inflammation on optic nerve regeneration.[10]

Ocm has also been reported to synergistically promote axon outgrowth

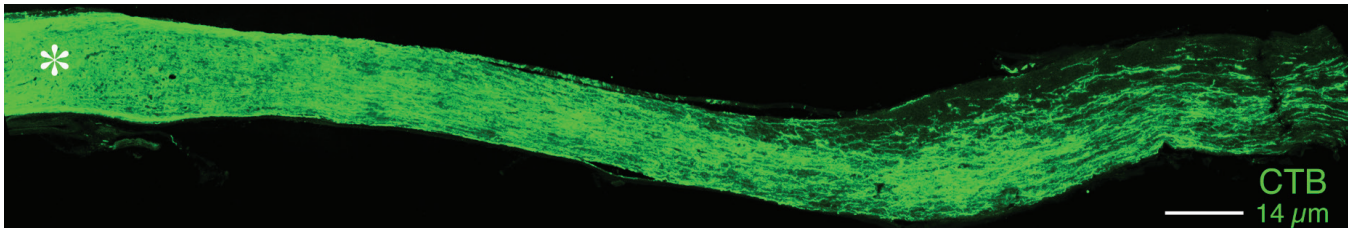

Figure. An example of a combinatorial gene therapy treatment leading to full-length optic nerve regeneration. CTB-positive axons are visualized in a longitudinal section through the optic nerve (14 µm thick) 6 to 8 weeks after nerve crush followed by intraocular injection of an adeno-associated virus expressing an shRNA to knock down expression of the *PTEN* gene (AAV2-sh*PTEN*), a shH10-oncomodulin virus, AAV2-c/a-adenylate cyclase, and the chemokine stromal cell-derived factor 1 (SDF1). Asterisk: nerve injury site; scale bar: 150 µm.

in RGCs when combined with a small interfering RNA against the Nogo-66 receptor[15] and to contribute to the *conditioning lesion effect* in the peripheral nervous system. This, in turn, enables injured dorsal root ganglion (DRG) neurons to extend axons following the accumulation of infiltrative cells into peripheral nerves and DRGs.[16]

SDF1. A second growth factor associated with intraocular inflammation is the chemokine stromal cell-derived factor 1 (SDF1; also called CXCL12). SDF1 acts through the receptor CXCR4, which is expressed in neurons, inflammatory cells, and other cell types[17-19] as well as through CXCR7.[20] SDF1 has a wide range of effects on central nervous system development and hematopoiesis.[21,22] It is highly expressed in infiltrative macrophages and acts synergistically with Ocm to induce optic nerve regeneration.[23] Deletion of SDF1 in myeloid cells, using CXCL12$^{fl/fl}$-LysM$^{Cre-/+}$ mice, or deletion of its receptor CXCR4 in RGCs, using CXCR4$^{fl/fl}$ mice injected intraocularly with AAV2-Cre virus, diminished inflammation-induced optic nerve regeneration by approximately one-third and fully eliminated inflammation on RGC survival.[23]

Blockade of both Ocm and SDF1 decreased inflammation-induced regeneration by 70% to 80%.[23] In gain-of-function experiments, although SDF1 alone has only modest effects on regeneration,[24,25] SDF1 combined with Ocm and cAMP mimics most of the proregenerative effects of intraocular inflammation.[23] The level of SDF1 associated with intraocular inflammation appears to be below optimal levels, as adding exogenous SDF1 to intraocular inflammation doubles the number of axons that regenerate the full length of the optic nerve and increases the number of axons that extend through the optic chiasm and the optic tract and into the dorsal lateral geniculate nucleus.[23]

SDF1 exerts its effects by activating phosphatidylinositol 3-kinase (PI3K) signaling, elevating intracellular cAMP, and antagonizing the axon repellant effects of slit/robo.[23,25,26] Thus, Ocm and SDF1 are two of the major proregenerative constituents of intraocular inflammation, and together they may be useful in promoting optic nerve repair clinically.

CNTF. Ciliary neurotrophic factor (CNTF) has also been proposed to mediate the effects of intraocular inflammation on axon regeneration.[27] Although CNTF and other cardiotrophin family chemokines become elevated in the eye after intraocular inflammation,[10,28] at physiologic concentrations recombinant CNTF (rCNTF) alone has little axon-promoting effect on RGCs in cell culture[9,14,29] and weak or no effects on optic nerve regeneration in vivo.[1,30-34] In the paradigm in which a segment of peripheral nerve is grafted onto the cut end of the optic nerve, high concentrations of rCNTF augmented axon regeneration,[35] but these effects were due to the chemotactic effects of CNTF on macrophages.[36,37]

In contrast, unlike rCNTF, AAV2-mediated CNTF delivery induces considerable axon regeneration through the optic nerve. However, we recently showed that this effect is due to the infiltration of inflammatory cells into the eye that express Ocm, SDF1, and other trophic factors.[38] One reason for the low efficacy of rCNTF is that *SOCS3*, a repressor of the Jak-STAT signaling pathway, increases postnatally and increases even further after optic nerve injury.[3,32,39] Accordingly, deletion of *SOCS3* amplifies the effects of rCNTF.[33] In addition, mature RGCs do not express appreciable CNTFRa, the specific receptor subunit for CNTF (unpublished data). Elsewhere in the nervous system, this subunit can be released from one type of cell and become anchored to another cell type via a glycosylphosphatidylinositol linkage to form part of a tripartite receptor complex with LIFRβ and glycoprotein 130 (gp130).[40]

CNTFRa is heavily expressed on astrocytes and inflammatory cells, and, as noted above, the effects of CNTF gene therapy on optic nerve regeneration are mediated by factors secreted by these cells. Nonetheless, CNTF and related trophic factors appear to play an important role in the visual system, as double deletion of CNTF and leukemia inhibitory factor (LIF) accelerates RGC death after optic nerve injury and prevents regeneration.[28]

Other growth factors. Several other growth factors have been reported to stimulate RGC survival but limited optic nerve regeneration; whether these factors act directly on RGCs is generally not known. These factors include, but are not limited to, fibroblast growth factor-2 (FGF2),[41] BDNF,[31,42-49] GDNF,[50,51] and insulin-like growth factor 1 (IGF1)[52-55] combined with osteopontin.[56]

Gene therapy and full-length optic nerve regeneration. Combinatorial treatment of inflammation-derived growth factors via gene therapy

has potential clinical significance. By administering AAV2-SDF1, shH10-Ocm, and other cofactors after optic nerve injury, we have been able to get hundreds of mouse RGCs to extend axons the entire length of the optic nerve and into the optic chiasm after 8 weeks (Figure).[57]

FUTURE DIRECTIONS

Over the past 2 to 3 decades, optic nerve regeneration has gone from being considered impossible to becoming a reality. Clearly, much more needs to be done to increase the number of axons that reach their appropriate destinations, evaluate whether regenerating axons form a topographically organized map of visual space in the lateral geniculate nucleus and superior colliculus, and assess visual acuity.

Perhaps these advances will take the form of manipulating transcriptional and epigenetic regulators of the regenerative program (material submitted for publication), counteracting cell-extrinsic suppressors of regeneration associated with myelin and the fibrotic scar that forms at the injury site,[58,59] or altering the intraretinal signaling pathway that leads to a toxic elevation of free zinc in the retina.[60] Alternatively, they may take the form of identifying additional potent trophic agents for RGCs, discovering ways to alter the immune and glial response to injury, reprogramming the entire retina, or perhaps something that is currently outside our conceptual models. In any event, the strides that have been made to date are considerable and point to the possibility that one day we may be able to restore vision after optic nerve injury in a clinically meaningful way. ■

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