Chapter 5

Restoring Vision to the Blind: Endogenous Regeneration

The Lasker/IRRF Initiative for Innovation in Vision Science

Discussion Leaders: Daniel Goldman and David Hyde

Scribe: Curtis Powell

Session Participants: John Ash, David Beebe, John Dowling, David Gamm, Peter Hitchcock, Amy Laster, Matthew LaVail, Pamela Raymond, Tonia Rex, Josh Sanes, Jane Sowden, Jeffrey Stern, Sally Temple, and Donald Zack

Introduction

The human eye is made up of many tissues, each of which directly or indirectly influences our visual perception of the world. Tissues that directly impact vision include the cornea, lens, retina, and optic nerve. The cornea and lens serve to focus light as it enters into the eye; this light is detected and analyzed by the retina and the visual signal communicated to the rest of the brain via retinal ganglion cell (RGC) axons that form the optic nerve. Because they directly influence our vision, damage to any one of these tissues, due to disease or injury, can dramatically impact one's quality of life. Many approaches are being developed to aid those who suffer from vision loss due to damage of these tissues; some of which have been quite successful, like corneal transplants, corneal epithelial regeneration, and artificial intraocular lenses. However, approaches to treat damage of the retina and optic nerve have proven more challenging.

Unlike mammals, some vertebrate species are able to repair damage to the retina and optic nerve via endogenous regenerative events. Two fundamentally different categories of neuronal regeneration are important in the eye: neurogenesis (cell proliferation leading to replacement of neurons that have been lost) and axonal regeneration (regrowth of retinal ganglion cell axons following damage to the optic nerve). Depending on which cells are damaged, and the vertebrate species being studied, regeneration of retinal neurons through neurogenesis is accomplished by mitotic activation of retinal pigment epithelial (RPE) cells, retinal progenitor cells in the ciliary marginal zone (CMZ), or Müller glia. For optic nerve regeneration, RGCs whose axons are severed exhibit an axonal growth-permissive state. In both types of regeneration, immune cells and injury-related changes likely play a critical but poorly understood role. Importantly, these regenerative events ultimately can lead to the restoration of visually mediated behaviors.

While mammals do not actively demonstrate these modes of regeneration in response to retinal disease or injury, current research supports the possibility that at least some of these regenerative cell types maintain an intrinsic regenerative potential in mammals and that this potential could be harnessed for retinal repair if the proper stimuli were provided. One can easily imagine how advantageous it would be to treat human retinal disease using endogenous reparative strategies.

The purpose of this targeted session was to review the current state of research aimed at stimulating endogenous regeneration of retinal neurons and axons in the optic nerve and to provide guidance for future research. To this end, we provide a brief background describing the various modes of retinal and optic nerve regeneration employed by species that exhibit a robust regenerative response (amphibians and teleost fish) and those that demonstrate a limited regenerative response (birds). A discussion of progress made in initiating endogenous regenerative events in nonregenerative species (mammals) is then presented. Finally, recommendations are provided to stimulate and direct future research in this field.

Figure 5.1. Injury-dependent Müller glia proliferation in zebrafish retina. Zebrafish retinas were mechanically injured and 4 days later retinal sections prepared and immunofluorescence used to identify glutamine synthetase-positive Müller glia (*red*) and BrdU-positive proliferating Müller glia-derived progenitors (*green*). (Image courtesy of Jin Wan, MBNI, University of Michigan).

Amphibians

Amphibians are the only adult animals known to possess the ability to regenerate the entire retina after its removal. In urodele amphibians, such as newts, the new retina arises predominantly from the RPE (Okada, 1980); however, a small domain of the new retina is derived from the circumferential germinal zone (CGZ): a ring of retinal progenitor cells at the periphery of the neural retina (Fischer, Bosse, & El-Hodiri, 2013). Amazingly, regeneration of the neural retina occurs without the addition of exogenous factors and without preserving the vascular membrane of the eye. In anuran amphibians, the mode of regeneration varies by species, but the integrity of the vascular membrane is universally crucial (Reh & Nagy, 1987). Similar to urodele amphibians, regenerated retinal neurons in *Xenopus laevis* are derived from the RPE (Yoshii et al., 2007). In contrast, retinal regeneration in *Xenopus tropicalis* is carried out exclusively by the CGZ (Miyake & Araki, 2014).

Because the studies mentioned above involve the removal of the entire neural retina, these models by necessity also require growth of a new optic nerve. Optic nerve regeneration in amphibians can also be stimulated without such a drastic injury (e.g., following optic nerve crush or cut). This damage leads to the activation of RGCs, which establish a developmental-like growth permissive state, ultimately restoring vision (Stelzner, Bohn, & Strauss, 1986).

Teleost Fish

Although teleost fish are unable to replace an entire retina, they do mount a robust regenerative response following damage to the retina or optic nerve. Similar to the amphibian retina, the teleost retina possesses a CGZ to facilitate persistent neurogenesis and retinal growth throughout the life of the fish (Johns, 1977). The Müller glia in the inner nuclear layer of the fish retina also retain a radial glial, stem-cell-like neurogenic ability. Under normal circumstances, they undergo sporadic, self-renewing divisions in the inner nuclear layer to produce retinal progenitors that migrate to the outer nuclear layer (Bernardos et al., 2007), where they proliferate as rod precursors and are committed to differentiate as rod photoreceptors (Johns & Fernald, 1981). When the retina is injured and neurons are lost, Müller glial proliferation is enhanced and the resulting retinal progenitors (Fig. 5.1) differentiate into various types of retinal neurons (Bernardos et al., 2007; Fausett & Goldman, 2006; Fimbel et al., 2007; Thummel et al., 2008). Interestingly, intraocular injection of agents that impact a variety of signaling pathways can also stimulate Müller glia proliferation, even in the absence of overt

Figure 5.2. Enhanced optic nerve regeneration following Sfpq morpholino knockdown. Regenerating zebrafish optic axons were lesioned and back-labeled with fluorescein dextran. Whole mount retinas were examined under a fluorescent microscope to visualize RGCs regenerating their optic axon as indicated by fluorescein in RGC bodies (*green*). Morpholino-modified antisense oligonucleotides (MOs) were used to knockdown the expression of specific proteins in RGCs. Control MO shows the number of RGCs whose axons have regenerated past the lesion site without protein knockdown. Note that Sfpq (splicing factor proline glutamine rich) knockdown resulted in increased numbers of back-labeled RGCs indicating improved axonal regeneration. (Photo courtesy of Rose Elsaedi, MBNI, University of Michigan).

loss of retinal neurons, and a small number of the resulting cells can express neuronal markers (Ramachandran, Zhao, & Goldman, 2011; Wan, Ramachandran, & Goldman, 2012). Thus, Müller glia are primarily responsible for the ability of teleost fish to regenerate any of the lost neuronal cell types following retinal damage (Bernardos et al., 2007; Fausett & Goldman, 2006; Fimbel et al., 2007; Thummel et al., 2008). This Müller glia-derived regenerative response represents a fundamental difference between amphibians and teleost fish in their modes of retinal regeneration.

Teleost fish also exhibit a robust ability to regenerate their optic nerve that leads to restoration of lost sight (Bernhardt, 1999; McDowell et al., 2004). This regenerative response is regulated by gene expression programs and signaling cascades in RGCs after optic nerve injury (Fig. 5.2; Elsaedi et al., 2014; Kato et al., 2013; Veldman et al., 2007). Unlike mammals, in fish most RGCs do not die following optic nerve lesion.

Birds

In contrast to amphibians and teleost fish, adult birds do not regenerate a damaged retina; however, the embryonic and post-hatch chick retina does possess a limited regenerative potential. If a region of the neural retina is removed from the chick embryo within the first four days of incubation, an RPE-dependent regenerative event ensues, generating a new retina (Coulombre & Coulombre, 1965). Like teleost fish, Müller glia also can serve as a source of retinal progenitors in the chick retina following excitotoxic injury, but unlike teleosts, they do so only through the first few days post-hatch (Fischer & Reh, 2001). Intraocular injection of exogenous growth factors can enhance this response and even initiate Müller glia proliferation in the absence of injury (Fischer et al., 2002; Fischer & Reh, 2002). In contrast to Müller glia-dependent regeneration in teleost fish, the majority of Müller glia-derived cells in the chick retina either do not survive or fail to differentiate (Fischer & Bongini, 2010; Fischer & Reh, 2001).

While the majority of work using the chick model system has focused on regeneration of retinal neurons, there is some evidence of a limited potential for RGC-dependent optic nerve regeneration; however, both regenerative abilities decline rapidly post-hatch (Halfter & Deiss, 1984).

Mammals

While RPE, CMZ, and Müller glia can generate neuronal progenitors following retina damage in some vertebrate species, these cell types do not do so in mammals. In contrast to amphibians, teleost fish, and birds, the mammalian retina does not add retinal neurons after birth. However, pigmented cells in the mammalian CMZ can proliferate and express neuronal markers under certain circumstances (Cicero et al., 2009). Interestingly, lid fusion stimulated proliferation of progenitor-like cells in the retinal periphery in juvenile macaques (Tkatchenko et al., 2006), perhaps suggesting a source of cells for regeneration in primates. Furthermore, the human RPE harbors multipotent cells that, under certain conditions, can be activated and may serve as a potential source of progenitors for repair (Salero et al., 2012). Activation of RPE proliferation and regeneration contributes to RPE layer wound repair that supports the neural retina (Lopez et al., 1995). After activation, RPE stem cell progeny can be directed to differentiate into RPE or cells that express neuronal markers in cell culture. The fact that the (presumptive) RPE domain can transdifferentiate into neural retina in

mammalian embryos lacking MITF, Pax6, or Pax2 (Baumer et al., 2003; Bharti et al., 2012) indicates that specific stages of RPE could exhibit neurogenic potential, with appropriate manipulation. While strategies to direct RPE differentiation into neurons in vivo may result in a potential therapy for retinal diseases, these approaches will need to be carefully modulated to avoid fibrosis and epiretinal membrane formation.

Although mammalian Müller glia do not normally generate neurons in vivo, they do express low levels of markers associated with a retinal progenitor state (Jadhav, Roesch, & Cepko, 2009), and they can generate neurons and glia when placed in cell culture (Giannelli et al., 2011; Singhal et al., 2012). A number of studies suggest that mammalian Müller glia can be coaxed to proliferate and generate a small number of cells with neuronal markers in vivo by combining retinal damage with growth factor stimulation (Karl et al., 2008; Osakada et al., 2007; Wan et al., 2008). These studies also suggest that this Müller glia response may decline with age.

Similarly, in mammals, RGCs lose their intrinsic capacity for axonal growth soon after birth (Moore et al., 2009). In adult mammals, there is no appreciable regenerative response following damage to the optic nerve in the absence of exogenously introduced stimuli. Some success has been achieved with enhancing the intrinsic potential of RGCs or modulating inhibitory extrinsic factors, but even with these changes, a large percentage of the RGCs do not survive and only a small percentage of RGC axons regrow past the optic chiasm (de Lima, Habboub, & Benowitz, 2012; Fischer et al., 2012; Sun et al., 2011).

Recommendations for Future Work

While these results delineate a stark difference between the regenerative response of mammals following damage to the retina or optic nerve relative to amphibians or teleost fish, studies do suggest some level of endogenous regeneration can be achieved under certain circumstances. Because research suggests that mammalian Müller glia, RPE cells, and RGCs possess an intrinsic regenerative potential, the majority of our discussion in this targeted session focused on a better understanding of these cell types so that their regenerative potential could be realized. What follows are questions, the answers to which would greatly benefit the field:

1. What are the mechanisms that drive regeneration in species where these processes are robust?
2. What are the mechanisms that limit avian and mammalian Müller glia, RPE cells, and RGCs from mounting a more robust regenerative response either in vivo or when placed in cell culture?
3. Are there molecular differences between the regeneration-permissive retinas and the regeneration-limited retinas? Are these differences intrinsic to the Müller glia, RPE cells, or RGCs?
4. What early developmental programs enhance the regenerative capacity of Müller glia, RPE cells, and RGCs?
5. What role does the retinal/optic nerve environment have in the regenerative capacity of Müller glia, RPE cells, and RGCs? Do inhibitory environments need to be neutralized or are stimulatory signals sufficient for a regenerative response?
6. What molecular mechanisms underlie and distinguish the gliotic/fibrotic response versus the regenerative responses resulting from Müller glia and RPE cell activation. Can the gliotic/fibrotic responses to retinal injury be shifted to regenerative?
7. How are the proliferative/regenerative responses of Müller glia, RPE, and RGCs terminated to prevent tumor formation and exuberant axonal growth in the regeneration-permissive retinas?
8. What pathfinding and synaptogenic mechanisms are used by RGC axons in the adult brain so they reform functional connections with appropriate brain targets?
9. What role does the immune system and inflammation play in stimulating and repressing retinal and optic nerve regeneration?
10. Can more realistic disease models be developed in model organisms to help guide regenerative strategies for vision restoration in a disease context?
11. Can Müller glia, RPE cells, and RGC cell cultures and retinal explants be used to identify small molecules that stimulate regeneration?

Answering these questions will require investigation of a variety of biological systems, including animal models that have already proven their value in driving discovery. In addition, the advent of human stem cell technologies and the development of protocols to efficiently grow retina and RPE cells in 2D and 3D configurations, now

provide powerful tools to investigate and test hypotheses on human cells, with the ultimate goal of translating discoveries into therapies.

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Correspondence: See Appendix 2 in the Supplementary Material.

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