Encyclopedia of the Eye - CONTRIBUTORS' INSTRUCTIONS

PROOFREADING

The text content for your contribution is in final form when you receive proofs. Read proofs for accuracy and clarity, as well as for typographical errors, but please DO NOT REWRITE.

Titles and headings should be checked carefully for spelling and capitalization. Please be sure that the correct typeface and size have been used to indicate the proper level of heading. Review numbered items for proper order – e.g., tables, figures, footnotes, and lists. Proofread the captions and credit lines of illustrations and tables. Ensure that any material requiring permissions has the required credit line and that we have the relevant permission letters.

Your name and affiliation will appear at the beginning of the article and also in a List of Contributors. Your full postal address appears on the non-print items page and will be used to keep our records up-to-date (it will not appear in the published work. Please check that they are both correct.

Keywords are shown for indexing purposes ONLY and will not appear in the published work.

Any copy-editor questions are presented in an accompanying Author Query list at the beginning of the proof document. Please address these questions as necessary. While it is appreciated that some articles will require updating/revising, please try to keep any alterations to a minimum. Excessive alterations may be charged to the contributors.

Note that these proofs may not resemble the image quality of the final printed version of the work, and are for content checking only. Artwork will have been redrawn/relabelled as necessary, and is represented at the final size.

DESPATCH OF CORRECTIONS

PLEASE KEEP A COPY OF ANY CORRECTIONS YOU MAKE.

Proof corrections should be returned in one communication to Mike Nicholls (visiproofs@elsevier.com), by 05-Jun-2009 using one of the following methods:

1. **PREFERRED**: Corrections should be listed in an e-mail and sent to Mike Nicholls in the Elsevier MRW Production Department at **visiproofs@elsevier.com**.

The e-mail should state the article code number in the subject line. Corrections should be consecutively numbered and should state the paragraph number, line number within that paragraph, and the correction to be made.

2. If corrections are substantial, send the amended hardcopy by courier to **Mike Nicholls, Elsevier MRW Production Department, The Boulevard, Langford Lane, Kidlington, Oxford, OX5 1GB, UK.** If it is not possible to courier your corrections, please fax the relevant marked pages to the Elsevier MRW Production Department **(fax number: +44 (0)1865 843974)** with a covering note clearly stating the article code number and title.

Note that a delay in the return of proofs could mean a delay in publication. Should we not receive corrected proofs within 7 days, Elsevier may proceed without your corrections.

CHECKLIST

Author queries addressed/answered?	❑
Affiliations, names and addresses checked and verified?	❑
Permissions details checked and completed?	❑
Outstanding permissions letters attached/enclosed?	❑
Figures and tables checked?	❑

If you have any questions regarding these proofs please contact the Elsevier MRW Production Department at: **visiproofs@elsevier.com**

VISI: 00219

Non-Print Items

Abstract:

Injury to the mammalian retina produces a number of cellular wound-healing types of responses, with similarities and differences to injury responses in the brain and spinal cord. Retinal detachment is one such serious, sight-threatening injury that produces responses from almost all cell types including cellular degeneration and death, neuronal remodeling, some poorly understood immune responses, and glial cell reactivity. Glial cell reactivity is particularly important in glial scar formation which may, in turn, have future downstream effects on visual recovery. The glial cell response is prominent in all injuries to the central nervous system and, yet, its exact purpose and regulatory mechanisms are poorly understood.

Keywords: Glia; Glial scar; Müller cell; Outer segment; Photoreceptor; Regeneration; Retina; Retinal detachment; Retinal pigmented epithelium; Retinal reattachment; Rhegmatogenous retinal detachment

Au1 Author and Co-author Contact Information:

Steven K Fisher
Department of Molecular Cellular and Developmental Biology
Neuroscience Research Institute
University of California, Santa Barbara
Santa Barbara
CA 93106-5060
USA
fisher@lifesci.ucsb.edu

Geoffery P Lewis
Neuroscience Research Institute
University of California, Santa Barbara
Santa Barbara
CA 93106-5060
USA
g_lewis@lifesci.ucsb.edu

VISI: 00219

Author Query Form

Book: Encyclopedia of the Eye (VISI)
Article No.: 00219

Dear Author,

During the preparation of your manuscript for typesetting some questions have arisen. These are listed below. Please check your typeset proof carefully and mark any corrections in the margin of the proof or compile them as a separate list. Your responses to these questions should be returned within seven days, by email, to MRW Production, email: VISIproofs@elsevier.com

Query	Details Required	Author's response
AU1	Please check the long affiliations for accuracy. These are for Elsevier's records and will not appear in the printed work.	
AU2	Please check whether the changes made to the construct ''An important physiological . . . . . behind the RPE'' are OK.	
AU3	Do Figures 1–9 require permission? If yes, please provide the relevant correspondence granting permission and the source of the figures.	
AU4	As part label (d) in Figure 7 has been mentioned twice, it has been changed (caption, image and text citation) to (e) and, subsequently, (e) has been changed to (f). Please check.	
AU5	Please provide better quality artwork for Figures 1 and 2.	

a0005

Injury and Repair Responses: Retinal Detachment

Au3, 5

S K Fisher and G P Lewis, University of California, Santa Barbara, Santa Barbara, CA, USA

Glossary

g0005 **Glial scar** – The proliferation and growth of glial cells (astrocytes and oligodendrocytes in the brain and spinal cord) in response to a physical wound to the central nervous system.

g0010 **Immunocytochemistry** – A laboratory technique that uses antibodies (the primary antibody) targeted to specific peptides or proteins (antigens) in the cell. The primary antibody is made to recognize its specific protein by the immune system of a specific species (i.e., it may be a rabbit immunoglobulin G (IgG) antibody). In addition, it can be bound to an antibody made in a second species (i.e., goat anti-IgG) that is bound with some detectable marker, often a fluorescent dye. Using appropriate imaging techniques, the localization can be subcellular.

g0015 **Laser scanning confocal microscope** – An optical imaging technique used to increase contrast and/or resolution by eliminating out-of-focus planes within a tissue section by using a spatial pinhole. Optical image plane thickness can be selected (usually 0.5–1.0 μm) and images from several optical sections collapsed together, played as a movie loop, or used to reconstruct objects in three dimensions. In biological research, this technique is most commonly used with immunocytochemistry with fluorescent dyes.

g0020 **Pneumatic retinopexy** – A method for repairing a retinal detachment by the injection of expanding gasses (usually sulfur hexafluoride (SF_6) or perfluoropropane (C_3F_8)) into the vitreous cavity.

g0025 **Retinal detachment** – A site-threatening condition that results from the physical separation of two cellular layers of the retina, the retinal pigmented epithelium and the neural retina.

g0030 **Rhegmatogenous retinal detachment** – A specific type of retinal detachment in which the neural retina is torn, allowing fluid from the vitreous to separate the two layers. This type is the most common form of retinal detachment.

g0035 **Scleral buckle** – A surgical method for repairing a retinal detachment by placing a band or bands of material (now usually silicone rubber and/or silicone sponges in a variety of configurations) to encircle the globe and to indent the wall of the eye in the region of the detachment. A scleral buckle is used in conjunction with cyrotherapy or laser treatment to seal the retinal break.

g0040 **Serous retinal detachment** – A retinal detachment that occurs when fluid accumulates between the neural retina and retinal pigmented epithelium, but without a tear in the retina.

Injury to the Retina

s0005

Introduction

s0010

Retinal glial cells

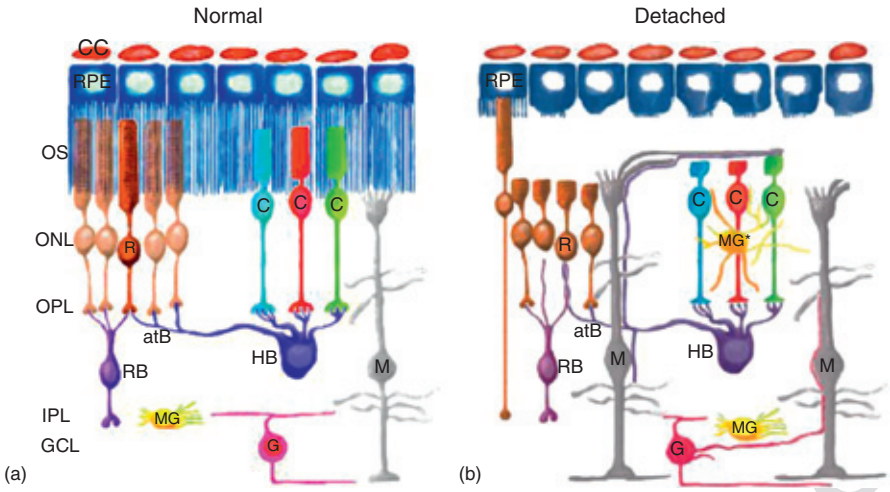
s0015

p0005 Damage to the retina, whether a result of physical injury, disease, or a genetic condition, produces similar cellular responses on the part of glial cells. Glial cells are non-neuronal, supportive cells found in all parts of the central nervous system (CNS). In the brain and spinal cord, where they also play an important role in injury, there are two types of true glia: oligodendrocytes and astrocytes. Oligodendrocytes are found in the optic nerve, but not in the retina itself. Astrocytes, similar to those in the brain and spinal cord, are star-shaped cells limited to the bundles of axons that run across the surface of the retina and exit the eye as the optic nerve. The glial cell with the most prominent role in the retina's response to injury is a highly modified, structurally complex radial astrocyte referred to as the Müller cell (first described in the mid-nineteenth century by the German anatomist Heinrich Müller). This cell spans the entire width of the neural retina (**Figure 1**), and, in many ways, its reaction to injury mirrors that of astrocytes. Microglia comprise another cell type important in the retina's response to injury. However, these are not true glia since they are derived from blood-borne marcrophages and have a different developmental lineage than that of astrocytes and Müller cells.

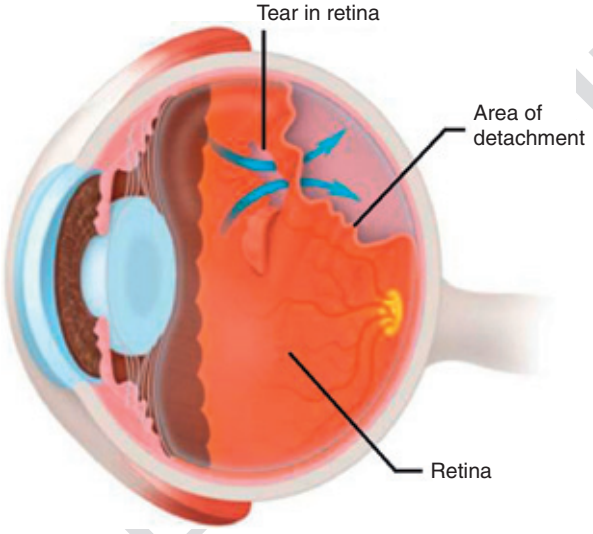
Retinal injury and retinal detachment: A close relationship

s0020

p0010 Physical injury to the retina can occur in many ways, from a penetrating wound to a spontaneous tearing of the retina in highly myopic individuals. Anytime the retina is physically damaged, there is great risk that the neural retina will separate from the underlying retinal pigmented epithelium (RPE in **Figure 1**) in a condition known as a rhegmatogenous retinal detachment (RD; **Figure 2**; the prefix rhegma is derived from the Greek word meaning a



f0005 **Figure 1** Diagrammatic representation of major cell types in which important reactions to injury of the retina by retinal detachment have been identified. (a) Normal retina and (b) representative changes after detachment. The rod photoreceptor on the left in (b) (gold cell) represents a characteristic response that occurs in some rods after reattachment; that is, they regenerate an axon that grows beyond its normal target, the OPL. CC, choriocapillaris, the main vasculature supply for the RPE and photoreceptor cells; RPE, retinal pigmented epithelium; R and C, rod and cone photoreceptors; OS, outer segments; ONL, outer nuclear layer (cell bodies of rods and cones); OPL, outer plexiform layer (region where photoreceptors synapse with second-order neurons); HB, a subclass of horizontal cells; atB, the axon of the B type horizontal cell; RB, rod bipolar cell; M, Müller's cell (the radial glial cell of the retina); IPL, inner plexiform layer (region where second-order neurons synapse with third-order neurons); GCL, ganglion cell layer, the third-order and output neurons of the retina; MG, microglia; MG*, activated microglia.



f0010 **Figure 2** A rhegmatogenous retinal detachment forms when a hole or tear occurs across the neural retina, allowing fluid to flow from the vitreous and separate the neural retina from the retinal pigmented epithelium.

break in continuity). This condition occurs with a prevalence estimated at 1:10 000 in the United States and is usually treated by a retinal surgeon who repairs the retinal tear and reapposes the two layers of tissue. Thus, studying RD is, in essence, a study of retinal injury, and used to demonstrate the variety of cellular responses that can occur in many different insults or injuries to the retina.

What is an RD?

s0025

p0015 An RD refers to a separation between the neural retina and the RPE. As discussed above, rhegmatogenous detachment (hereafter, simply detachment or RD) involves a specific injury to the retinal tissue. An important physiologicalramification of such a detachment is an increase in the physical distance between the photoreceptor cells and their blood supply, a highly branched capillary network (the choroicapillaris, which lies behind the RPE; see **Figure 1**). The choriocapillaris provides nearly all of the oxygen and nutrients to photoreceptors and also carries away metabolic waste products from these extraordinarily active cells. A decrease in oxygen availability across the expanded extracellular space may be a critical step in the rapid degeneration of photoreceptor outer segments that occurs after detachment (**Figure 1**). A certain number of these photoreceptors are destined to die by apoptosis, in most species reaching a peak about 3 days after detachment and declining thereafter to a low, but steady level. Indeed, some studies in animal models have demonstrated that placing animals in an environment of 70% oxygen has the capacity to stop many of the degenerative effects of detachment including photoreceptor cell death. Detachment also creates a foreign environment for the photoreceptor outer segments as components from the vitreous wash through the tear and into that space. Indeed , it is this movement of fluid through the tear and under the retina that is generally assumed to create and maintain a detachment. Another type of RD is known as a serous

Au2

detachment and involves the accumulation of fluid within the same space, but without a retinal tear. Its symptoms and treatment are very different.

s0030 ### *Symptoms and signs of RD in people*

p0020 All detachments are accompanied by some loss of visual function, but this will vary depending upon the size and retinal location, making it difficult to ascribe one set of symptoms to the condition. Diagnosing RD is complex, with many qualifications. Abnormal vision is the only reliable symptom of RD; however, the types of abnormal vision are large and varied: light flashes, floaters, changes in the peripheral visual field, decreased acuity, defective color vision, distorted vision (metamorphopsia), or even unilateral double vision (diplopia). The detachment of the fovea always involves a loss of central visual acuity.

s0035 ### *Brief history*

p0025 Greg Joseph Beer provided the earliest systematic description of RD in the early eighteenth century. After Hermann von Helmholtz recognized the clinical significance of the ophthalmoscope in *c.* 1850, detailed descriptions of detachments and accompanying retinal breaks or tears proliferated rapidly. The first treatment of rhegmatogenous detachment by sealing the retinal break with a red-hot probe occurred in 1889, and was revived as a standard treatment by Jules Gonin in the early 1920s. Gonin devoted much of his research career to studying the relationship between retinal tears and detachment and also was the first to suggest a relationship between detachment duration and successful visual recovery. His technique is credited with moving an inevitably blinding retinal injury into a treatable one. The next major advance occurred 70 years later when Ernst Custodis described the scleral buckle, a band or bands of material (now usually silicone rubber and/or silicone sponges) surgically placed to encircle and indent the wall of the eye in the region of the detachment, thus reapposing the RPE and neural retina. A scleral buckle is used in conjunction with cyrotherapy or laser treatment to seal the retinal break, probably by creating a localized glial scar. In the early 1970s, pneumatic retinopexy, or injection of an expanding gas bubble (sulfur hexafluoride or perfluoropropane), into the vitreous cavity to reappose the retina and RPE became widely used. There is still much ongoing discussion on the use of scleral buckling, primary vitrectomy, and pneumatic retinopexy to treat RD.

s0040 ## Cellular Responses of the Retina to Injury

s0045 ### General Events

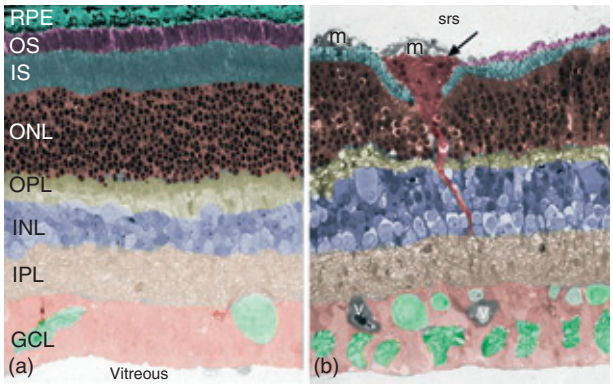
p0030 Overall, the retina may be described as including two major cellular layers, the RPE and the intimately apposed neural retina. The neural retina is highly organized, consisting of three cellular layers and two synaptic layers (**Figure 1**). Since it is so highly structured, changes in its normal architecture are readily noted by conventional histology and light microscopy as shown in **Figure 3**.

p0035 In the 1960s through the 1980s, investigators used combinations of light and electron microscopy to reveal cellular and subcellular structural changes induced by detachment in photoreceptors including outer segment degeneration, loss of mitochondria, and the general disorganization of organelles involved in protein synthesis. It was also recognized in these early structural studies that Müller's cells underwent vast changes in structure (hypertrophy) with growth out of the retina and into the subretinal space (**Figure 3(b)**). In recent years, it has been recognized that cellular effects of detachment occur well beyond the site of separation of the RPE and neural retina. Almost all of the cellular changes have been validated in tissue samples taken from human detachments including the capacity of photoreceptors to regenerate outer segments after surgical reattachment. When immunocytochemistry became a routine laboratory procedure in the late 1980s, and laser scanning confocal imaging allowed the efficient collection of high-resolution immunofluorescence images by light microscopy, it became apparent that cellular responses to detachment were far more complex than the degeneration of photoreceptor outer segments and the hypertrophy of Müller's cells. It first became clear in these animal studies that a number of photoreceptors actually die (see **Figure 3**).

p0040 As Müller's cells are the primary players in the retina's gliotic response to injury, they are discussed first and in most detail. In animal models, these range from molecular changes that occur within minutes to hours (e.g., phosphorylation of the fibroblast growth factor receptor 1, and the extracellular signal-regulated kinase, with later synthesis of the AP1 family transcriptional activators cFos and cJun in Müller's cells), cellular changes recognizable within hours (photoreceptor outer segment degeneration) to cellular events that begin in days and can extend for weeks or months (e.g., Müller cell reactivity, rod cell death, glial scar formation, and neuronal remodeling). The cellular level events are summarized in **Figure 4**.

s0050 ### *Müller's cells*

p0045 Except for the death of photoreceptors, the reactivity of Müller's cells may be one of the most important consequences of injury to the retina. The cell body of the Müller cell lies among the interneurons of the inner nuclear layer and extends main stalks in both directions (M in **Figure 1**). The inner stalk expands along the vitreal border of the retina to form a so-called endfoot. Müller's cells are arrayed across the retina to assure that adjacent endfeet form a continuous layer on the vitreal surface (the green cells in **Figure 5(c)** are good examples). They are not, however, linked by tight junctions and, hence,



f0015 **Figure 3** (a) Light micrographs of a normal mammalian retina and (b) micrograph showing the overall disorganization of the retina with a characteristic glial scar in the subretinal space (arrow in (b), dark red) after a retinal detachment. The retinal layers were colored to allow for easier comparison between the two images. The retinal pigmented epithelium (RPE, light green in A) that is anatomically tightly apposed to the photoreceptor outer segments (OS, light purple) in normal retina becomes several hundred micrometers removed from the neural retina after detachment and does not appear in (b). There is a dramatic degeneration of light-sensitive rod and cone outer segments (OS, light purple) and inner segments (IS, light gray) in the detached retina. The latter are responsible for most of the energy production and protein synthesis in normal photoreceptors. The outer nuclear layer (ONL, dark brown) which contains the cell bodies and nuclei of the photoreceptors is greatly thinned in the detached retina as a result of the loss of cells by apoptotic cell death. Notice that many of the photoreceptor cell bodies in (b) appear swollen with clear cytoplasm surrounding highly condensed nuclei, indicative the fact that they are in the process of undergoing apoptosis. The outer plexiform layer (OPL, light green) is greatly thinned in the detached retina by the loss of rod photoreceptor terminals and probably dendrites of second-order neurons whose cell bodies reside in the inner nuclear layer (INL, blue). The INL appears thickened in the detached retina due to the significant swelling of the cells and also due to the hypertrophy and migration of Müller's cells within the layer (see text). Although we know there are subtle changes in the inner plexiform layer (IPL, light brown) after detachment, these are difficult to discern by light microscopy. The endfeet of Müller's cells (pink) form a continuous layer along the vitreous cavity of the retina (vitreous) and assume a somewhat thickened and contorted appearance as they expand after detachment (this effect is more obvious when the Müller cells are specifically stained by immunolabeling as in **Figures 5** and **6**). The cells of the ganglion cell layer (GCL, green) and their axons (green) appear little affected in this thin histological section, but immunolabeling studies show that they undergo significant remodeling through the sprouting of neurites (see **Figure 8(b)**). Müller's cells expanding beyond the boundaries of the retina form a layer of scar tissue on the photoreceptor surface (arrow, dark red), in the subretinal space (srs). The dark round structures embedded in the subretinal scar represent nuclei of Müller's cells that migrate through their processes into the scar as well as some photoreceptor cell bodies that migrate from the ONL to appear among the scar tissue. These subretinal scars can block the regeneration of outer segments after surgical reattachment, and they can also contract, wrinkling and redetaching the retina after reattachment. Two macrophage cells (m) appear in the subretinal space in (b). These cells engulf debris from degenerating outer segments. Two retinal blood vessels (v) appear among the Müller cell endfeet in (b).

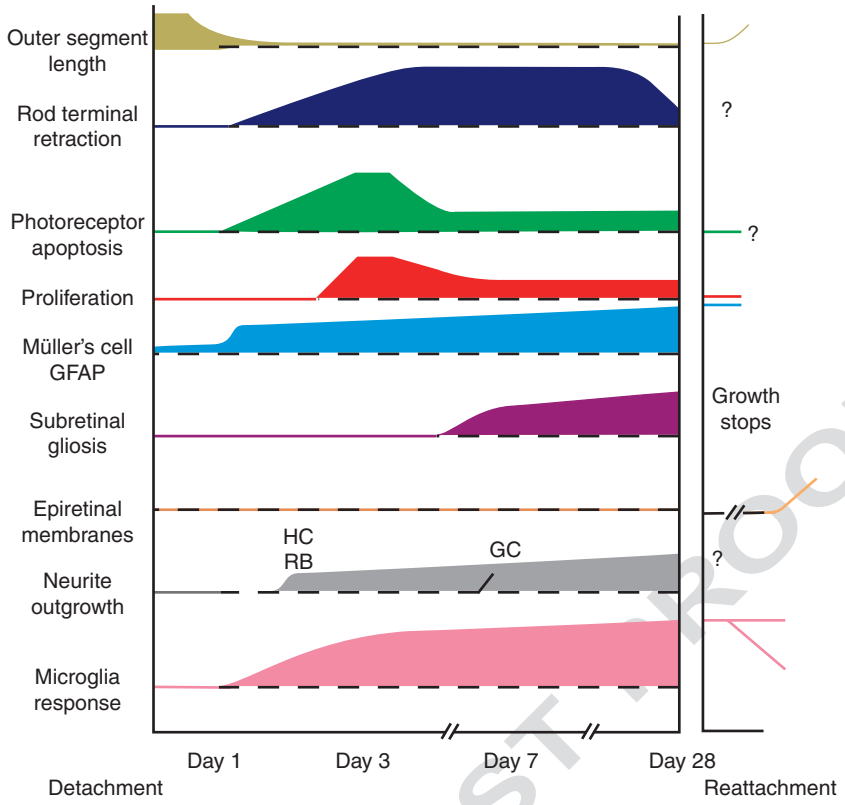
extracellular molecules can flow freely between them. Some branching of the main stalk occurs in all retinal layers, but reaches its greatest complexity as the individual cells interweave between all photoreceptor cell bodies so that each is separated from the next by a thin layer of Müller cell cytoplasm. The Müller cell ends as a tuft of microvilli at the level of the photoreceptor inner segments. Thus, this cell is ideally situated to sample the internal environment of the retina, the vitreous, and the space between photoreceptors and RPE. Adhering junctions between adjacent Müller's cells and photoreceptors form a continuous, recognizable layer known as the outer limiting membrane.

Functionally, Müller's cells are responsible for much of p0050 the homeostasis of the neural retina. They are known to actively recycle glutamate released as a neurotransmitter by retinal neurons through uptake and conversion to glutamine. Potassium balance, water regulation, and pH balance are all thought to depend strongly on Müller's cells. There is even some evidence that they may act as light pipes – optically funneling light through the retinal layers to the outer segments. While much of the focus of this article is on the formation of glial scars by Müller's cells as part of the injury response, there is also significant experimental evidence that activated Müller's cells can play a neuroprotective role in retinal injuries resulting from excitotoxicity generated by the excess release of the neurotransmitter glutamate.

Müller's cells and RD: Glial scarring s0055

Following RD, there are rapid changes in molecular p0055 events in Müller's cells as discussed above. This fairly quickly translates into two major events: proliferation of the cells and hypertrophy or excessive growth. Both are poorly understood, but the latter is known to have important ramifications. If a retina is labeled with markers for DNA synthesis (tritiated-thymidine, bromodeoxyuridine, or antibodies to cell-cycle-specific proteins such as Ki-67), many cell types can be shown to divide, with Müller's cells and microglia being among the most prominent (**Figure 5**).

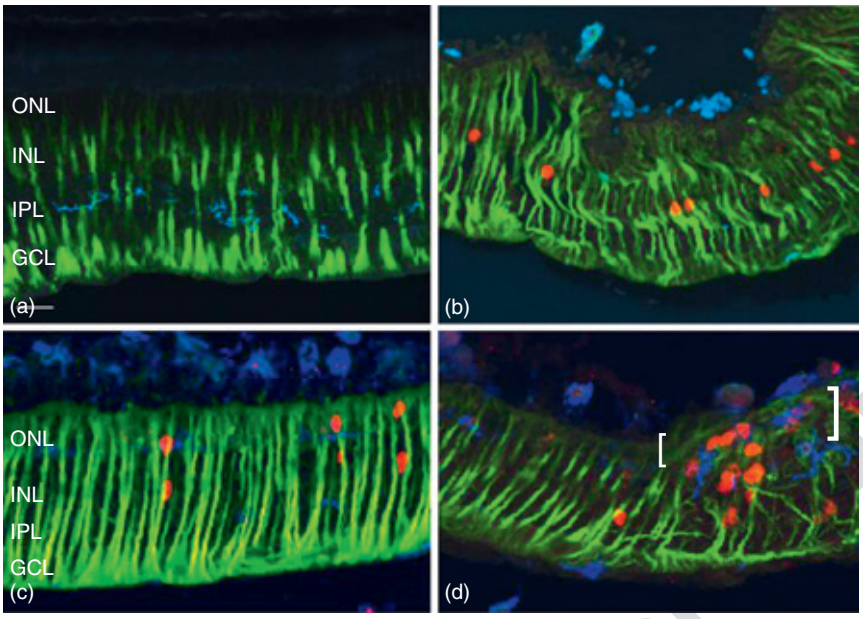
This newly activated cell division appears to peak p0060 3–4 days after detachment in the feline and rabbit retina, but continues at low levels thereafter as long as the retina is detached. The fate of the daughter cells produced by dividing Müller's cells is still controversial, although, in species such a chick or teleost fish, they appear to regress to an earlier pluripotent stage where they can regenerate retinal neurons. There is scant evidence that this occurs in mammals. Details of how these morphologically complex cells divide or exactly what cell types they produce in the mammalian retina are unknown. One intriguing scenario is that after nuclear division, a daughter nucleus migrates into the outer retina and then buds off a daughter cell. If this is true, the proliferating Müller's cells may indeed



f0020 **Figure 4** A diagram representing cellular events that occur after retinal detachment. The purpose is to show how the various events are temporally related. For example, outer segments degenerate very quickly with rod terminal retraction and photoreceptor apoptosis starting around the end of the first day of detachment. Epiretinal membranes do not form in the detached retina, but begin forming when the retina is reattached. Their time of formation is highly variable, but can range from weeks to months. Rod terminal retraction and apoptosis are stopped by reattachment, but the timing is unknown. Indeed, following reattachment, rod axons regrow into the outer plexiform layer. Müller cell proliferation is stopped by reattachment, but glial fibrillary acidic protein (GFAP) expression can remain elevated for an unknown period of time. Neurite sprouting occurs from horizontal and rod bipolar cells (HC, RB) before it does from ganglion cells (GC). The microglial response is variable after reattachment, remaining high in areas of poor regeneration, but returning to normal in areas of good regeneration.

be producing a small population of pluripotent cells that have remained unidentified in mammals. The proliferation of glial cells is common to virtually all types of CNS injuries.

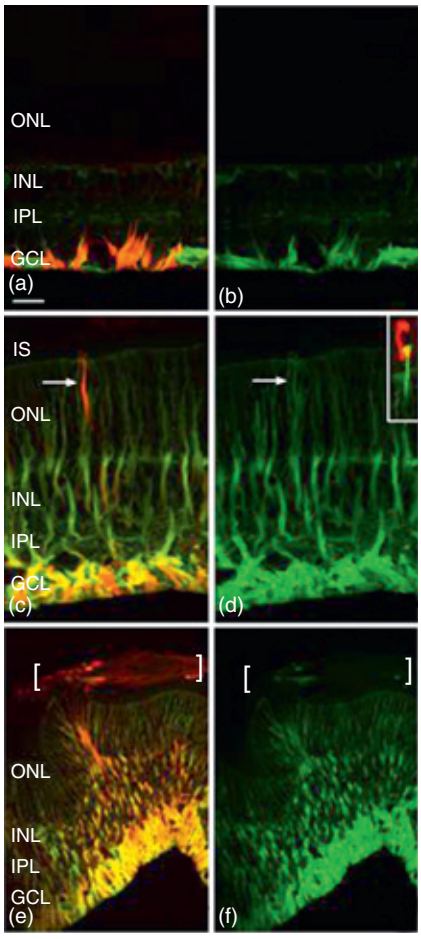
p0065 Structural remodeling of Müller's cells can be observed as early as 1 day after detachment by the hypertrophy of their main trunk and lateral branches within the retina. At this time, they also decrease their expression of the proteins glutamine synthetase, carbonic anhydrase II, and cellular retinaldehyde-binding protein, and dramatically increase their expression of two intermediate filament proteins, glial fibrillary acidic protein (GFAP) and vimentin (**Figures 6(a)–6(d)**). The increased expression of these two proteins is especially useful as hallmarks of the retina's reaction to injury. Indeed, their tissue expression profile varies by species, but an increased expression of GFAP is widely used as an indicator of retinal stress or injury. In the normal feline retina, both of these proteins are mainly restricted to the endfoot of the Müller cell (**Figures 6(a)** and **6(b)**). There are interesting species differences in the expression of these molecules. In rabbit, ground squirrel, rat, and mouse retinas, vimentin is expressed from the endfoot into the layer of photoreceptors, whereas GFAP is limited to the endfoot or virtually undetectable by immunocytochemistry. GFAP does, in all of these species, occur abundantly in astrocytes while vimentin does not. Following detachment or injury, the proteins rapidly increase their expression so that within a few days, they fill the entire cytoplasm of the cell (**Figures 6(c)** and **6(d)**; see also **Figure 5**). The interesting exception occurs in the cone-dominant ground squirrel retina which increases its expression of vimentin, but not GFAP, and does not show the Müller cell hypertrophy characteristic of the all other species studied. In feline retina, the balance of expression of GFAP and vimentin within individual Müller's cells shifts fairly rapidly over the first few days of detachment. Within a day or so, there is a shift so that GFAP expression in the endfoot increases rapidly as it acquires a more elongated and branched appearance. This is one of the earliest recognizable stages of the hypertrophic growth of these cells after



f0025 **Figure 5** A series of immunofluoresence images acquired by laser scanning confocal microscopy, illustrating the proliferative response of Müller's cells in reaction to retinal detachment and their participation in glial scar formation. The red-colored portions indicate the signal from immunolabeled bromodeoxyuridine, a nucleotide that can become incorporated into DNA as cells undergo DNA synthesis in preparation for division. Green labeling is a marker for Müller's cells and blue a marker for microglia and macrophages. Panel (a) is an image from normal retina. Notice that there are no red cells because neurons and Müller's cells of the adult mammalian retina are postmitotic. In (b)–(d), the bromodexoyuridine-labeled nuclei are associated with Müller's cells. In (b), these occur at the normal location of Müller cell nuclei in the inner nuclear layer (INL), while 24 h later (c), many of them have migrated into the outer nuclear layer (ONL). At a later time point, Müller's cells begin to to form a characteristic glial scar (brackets in (d)) on the photoreceptor layer and many of the bromodeoxyuridine-labeled Müller's cells reside within the glial scar, suggesting a link between Müller cell proliferation and glial scar formation. Notice that many of the microglial cells (blue) occur within the glial scar as well. IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bar = 20 μm.

injury of the retina (**Figures 6(c)** and **6(d)**). By 3 days, GFAP expression increases throughout the cell and the cell shows expansive growth of its processes within the retina, in later stages forming large columns of cytoplasm across the retina and filling space left by dying photoreceptors. An important subpopulation of Müller's cells will eventually grow into the space between the retina and RPE to form glial scars over the photoreceptor surface (**Figures 6(e)** and **6(f)**). Interestingly, these cells will quickly begin heavy expression of vimentin in their outer half. The extension of Müller's cells into the subretinal space is part of a reaction comparable to glial scarring in the brain and spinal cord where the scars block axon growth. Here, they effectively block outer segment regeneration after reattachment. The growth of Müller's cells into this space does not appear to be random, but, in feline retina, spatially associated with the presence of cone photoreceptors (inset to **Figure 6(d)**), suggesting some attraction between cones and the vimentin-rich Müller's cells. There is no molecular mechanism known to underlie this process. Once growth into the subretinal space is initiated, it continues at a rapid pace so that a Müller cell scar can rapidly expand broadly over the photoreceptor surface of the retina (**Figures 6(e)** and **6(f)**). Besides inhibiting outer segment regeneration, these scars also appear permissive for the growth of neurites out of the retina (see below).

p0070 Retinal reattachment appears to greatly slow much of the intraretinal hypertrophy of the Müller cells as well as their growth into the subretinal space. It does not, however, completely halt their reactivity since cell division can still occur at low levels. In addition, reattachment appears to stimulate a redirection in the growth of Müller's cells from the subretinal space toward the vitreal surface of the retina where they break through the vitreoretinal interface to form glial scars on this surface of the retina. Curiously, the Müller cell processes that expand into the vitreous predominately express GFAP instead of vimentin. Their growth into the vitreous may mark the beginning for a devastating disease called proliferative vitreoretinopathy (PVR). The Müller cells can form long fibers on the retinal surface and become populated with a variety of other cell types, including RPE and immune cells. There are no pharmacological treatments to date that will inhibit this disease from occurring or reverse its progression. It continues to occur in a small percentage of successful reattachment surgeries. The cellular strands can become contractile, causing a secondary tractional detachment (essentially a rhegmatogenous



f0030 **Figure 6** A series of immunofluoresence images acquired by laser scanning confocal microscopy, illustrating the upregulation of the intermediate filament proteins vimentin (red) and GFAP (green) that occurs as Müller's cells react to injury. Panels (a), (c), and (e) show the fluorescent signals from both (when the signals overlap, yellow results). Panels (b)–(e) show only the signal from GFAP. Panels (a) and (b) are images from a normal retina. The retinal astrocytes reside among the ganglion cells with their axons containing GFAP, while the endfeet of the Müller's cells (where these cells terminate on the vitreal border of the retina as shown in **Figure 1**) have a mixture of the two proteins (with vimentin being at a higher level of expression than GFAP). Panels (c)–(f) show the very large uprergulation of both of these molecules as the Müller's cells react to injury. Panels (c) and (d) show that although both increase their expression in Müller's cells after 3 days of detachment, the largest overall increase is associated with GFAP. However, one Müller's cell predominately shows vimentin expressed at its apex (arrow), and this cell is just beginning to grow out of the retina to form a glial scar in the subretinal space (the space created between the neural retina and RPE by the detachment). Panels (e) and (f) show further upregulation of both at 7 days of detachment and also a large subretinal glial scar (brackets) in which the Müller's cell processes predominately express vimentin. The inset to panel (d) shows a characteristic phenomenon where Müller's cell processes (yellow/green) grow out of the retina at the location of cone photoreceptors (red). ONL, outer nuclear layer; IS, photoreceptor inner segment layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bar = 20 μm.

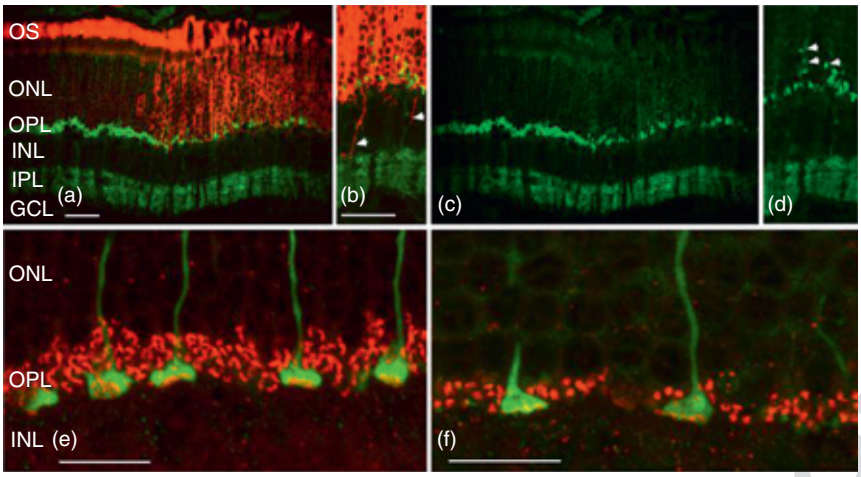
detachment caused by the contractional force generated by this strand of fibrous-like scar tissue). Similar to their counterparts in the subretinal space, these epiretinal membranes can act as substrates for the growth of ganglion cell neurites (discussed below) out of the retina. From these examples, it is clear that Müller's cells are highly involved in the reaction of the retina to detachment. There is evidence in mice which are genetically modified so that they do not express vimentin and GFAP that subretinal scars do not form. There is also some experimental evidence that certain inhibitors of cellular proliferation may help prevent the formation of glial scars. There are currently no drugs that target the former, although inhibiting proliferation of cells with antiproliferative drugs after retinal injury is an area of active investigation.

Injury and Other Cell Types s0060

Photoreceptors s0065

Besides undergoing degeneration of their light-sensitive outer segment, photoreceptors undergo a number of other changes when the retina is detached, or when they lie in the immediate vicinity of retinal injury. Within a day after detachment, they undergo changes in the way they express proteins; the light-sensitive protein, opsin, normally localized in the membranes of the outer segment soon becomes distributed throughout the plasma membrane of the cell (red staining, right side of **Figure 7**(**a**)), essentially outlining the whole cell. This may occur because the cell cannot construct an outer segment as part of its ongoing membrane renewal process; however, since the cell does continue to synthesize opsin, the protein is then aberrantly inserted into the plasma membrane. Some proteins (e.g., peripherin/rds) remain inside transport vesicles that accumulate within the cytoplasm. Rods and cones appear to differ in this respect inasmuch as cones may actually downregulate much of their protein synthetic machinery, perhaps giving these critical cells a better chance of survival in a stressful environment. p0075

Rod photoreceptors have short axons that retract after detachment, pulling their synaptic terminal back toward the cell body. This can be seen as a loss of synaptic terminals in the outer plexiform layer and the appearance of these terminals among the cell bodies of photoreceptors (**Figures 7**(**c**) and **7**(**d**)). Cone photoreceptor axons do not appear to retract, but there are numerous structural modifications in their synapses (**Figures 7**(**e**) and **7**(**f**)). Given the complexity of photoreceptor synapses, it seems reasonable to expect that these structural changes effect communication to second-order neurons. Importantly, rod axons appear to regrow back toward their normal location after reattachment; some even overshoot their target and grow into the inner retina (**Figure 7**(**b**)), p0080



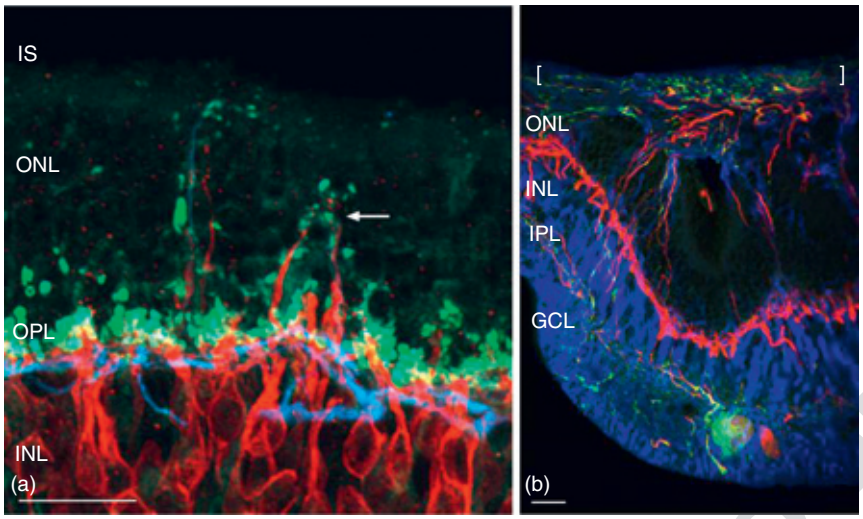
f0035 **Figure 7** A series of immunofluoresence images acquired by laser scanning confocal microscopy, illustrating various responses of photoreceptor cells caused by detachment. In panels (a)–(d), the retina has actually been surgically reattached, but shows characteristics of both normal morphology and that after detachment. In panels (a) and (b), the retinal sections were labeled with antibodies to the rod photopigment, rhodopsin (red), and a synaptic vesicle protein, synaptophysin (green). Panels (c) and (d) show labeling with synaptophysin alone. In panel (a), the layer of rod outer segments (OS) is uniform in the nondegenerate area to the left, but highly disrupted in the degenerate area to the right. Characteristically, as the OS structure is disrupted by injury, the rhodopsin molecules become distributed throughout the plasma membrane of the rod photoreceptors instead of being restricted to the outer segment, hence the heavy red labeling in the ONL in panels (a) and (b). Also shown in panel (b) is the characteristic overgrowth of some axons (arrowheads) that occur in some rods after reattachment (also see **Figure 1**). The fate of these overgrown axons is unknown. The green labeling compares the organization of the photoreceptor synaptic terminals in areas that are normal (to the left in (a) and (c)) to the organization in areas of photoreceptor degeneration (to the right in (a) and (c)). Notice the sparseness of terminals in areas of degeneration. This is caused largely by the withdrawal of rod axons and the retraction of the terminals into the layer of photoreceptor nuclei (ONL, arrowheads in (d)). Axonal retraction appears to be a common response of rod photoreceptors to retinal injury but the same Au4 is not true of cones. Figures (e) and (f)show the responses of cone photoreceptors (green) as well as an organelle characteristic of all photoreceptor synapses, the synaptic ribbon (red). In the normal retina, the cone synaptic terminals are very large by comparison to those of rods and contain many of the synaptic ribbons (red/yellow areas within the green terminals). Rod terminals, on the other hand (unstained in these images), contain only one or two of the crescent-shaped synaptic ribbons. As rod and cone photoreceptors degenerate, cone axons (green) do not withdraw as do those of rods, but the cone synaptic terminals assume a flattened appearance and lose the characteristic synaptic morphology of normal terminals. Notice that the rod synaptic ribbons are transformed from a crescent shape in (e) to a small clumped appearance in (f). There are also many fewer of the rod terminal ribbons in (f) due to the loss of rod synaptic terminals from their normal location in the OPL. OS, layer of photoreceptor outer segments; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bar = 20 μm.

a phenomenon that also occurs in retinal development. There is almost nothing known about the recovery of connections to second-order neurons, but the repopulation of the outer plexiform layer with rod terminals appears imperfect after a month of reattachment. It may be the reformation of synaptic connections that partially accounts for the prolonged period of visual recovery that can occur after reattachment, rather than simply a defect in the recovery of outer segments, since the latter are known to have a remarkable capacity to regenerate and recovery quickly, usually within a few weeks of reattachment.

s0070 ## Second- and Third-Order Neurons

p0085 The interneurons of the inner retina and the ganglion cells undergo remarkable structural remodeling as the photoreceptors undergo degenerative changes. Rod bipolar cells and horizontal cells show rapid neurite sprouting with fine thread-like processes extending deep into the outer nuclear layer, usually terminating near withdrawn rod terminals (**Figure 8(a)**). However, many neurites from horizontal cells grow across the retina, following intermediate filament-filled Müller cell processes into the subretinal space (**Figure 8(b)**). Postsynaptic processes are probably pruned from both of these cell types as well. A subpopulation of ganglion cells, those with the largest cell bodies, undergoes changes that mirror the responses of horizontal cells. They upregulate the expression of at least two proteins, GAP 43 and neurofilament protein, both of which are expressed at low levels in adult ganglion cell bodies and dendrites (**Figure 8(b)**). The same cells also sprout neurites that can become extensive and grow into the vitreous or across the retina and into the subretinal space, always following gliotic Müller cell scars.

p0090 Thus, the detachment of the neural retina from the RPE initiates a series of events in neurons not just among photoreceptors, but also throughout the retina. Ensuing



f0040 **Figure 8** A series of immunofluoresence images acquired by laser scanning confocal microscopy, illustrating the remodeling response of second- and third-order neurons to retinal injury and the relationship of that reaction to glial scars formed by Müller's cells in the subretinal space. (a) All rod bipolar cells (red) label with an antibody to protein kinase C. Synaptic terminals of the photoreceptors (green) are labeled by an antibody to the synaptic vesicle protein synaptophysin. Notice that many of the synaptic terminals have withdrawn into the outer nuclear layer (ONL) as part of the injury response (white arrow). The dendrites of rod bipolar cells normally terminate only among the layer of rod terminals in the outer plexiform layer (OPL), but, in the detached retina, these cells extend neurites deep into the ONL with many of them terminating close to the withdrawn rod terminals. Electron microscopic studies show that these usually do not terminate close enough to the rod terminal to participate in the normal transmission of signals from the rod cells. (b) Horizontal cells and ganglion cells can show extreme remodeling in response to many retinal degenerations including retinal detachment. This image shows processes from horizontal cells (red) and ganglion cells (green) extending across the retina and into a glial scar formed by Müller cell processes in the subretinal space (white brackets). In a normal retina, the horizontal cell postsynaptic processes would terminate among the layer of photoreceptor synaptic terminal in the OPL, and ganglion cell processes would terminate in the IPL where they are postsynaptic to bipolar and amacrine cells. Müller's cells and their processes forming the subretinal scar are labeled with an antibody to GFAP (blue). Scale bar = 20 μm.

changes in synaptic circuitry could have a profound effect on retinal function and there is evidence that the activity of ganglion cells is abnormal in the detached feline retina. The reorganization of synaptic circuitry after reattachment may underlie the long-term changes in vision that are known to occur in many reattachment patients.

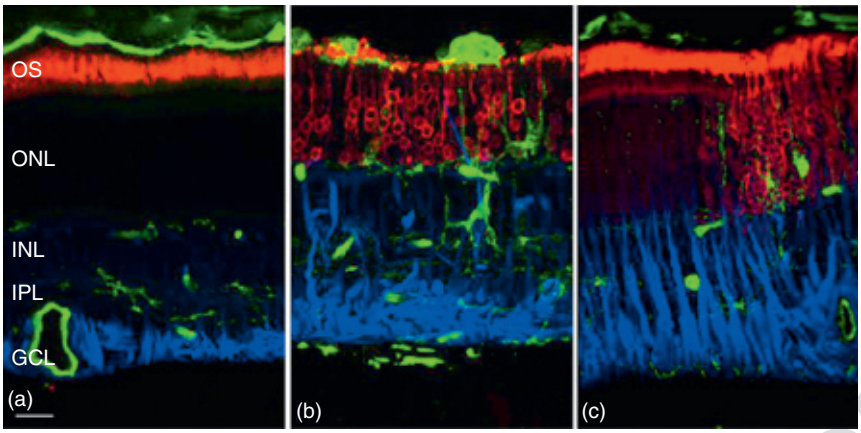
s0075 ## Microglia and the Immune Response

p0095 Microglial cells are immune cells that normally reside in the inner retina. Almost any injury to the retina will activate these cells, meaning that they begin to proliferate and migrate from their normal location in the inner retina into the photoreceptor layer where they scavenge dead or dying cells (**Figure 9**).

p0100 Microglia may cause or prevent photoreceptor cell death by modulating the release of trophic factors from Müller's cells. Macrophages from the circulation enter the subretinal space where they also scavenge debris from degenerated outer segments. Microarray analysis of messenger ribonucleic acid (mRNA) in a number of species has now identified significant changes in the expression levels of many genes involved in the immune and inflammatory responses, with most of these genes being upregulated. In the reattached retina, the presence of microglia correlates strongly with the degree of photoreceptor recovery; areas showing less outer segment recovery have a greater population of microglia distributed among the photoreceptor cells (**Figure 9(c)**). The role of the immune system and the inflammatory response following detachment and retinal injury is only beginning to be appreciated. It is quite clear from cellular localization studies that both macrophages and microglia can populate glial scars formed on either surface of the retina. What role they play there is unknown.

Retinal Pigmented Epithelial cells s0080

p0105 Retinal pigmented epithelial cells react to detachment by retracting their highly specialized apical microvillii – actin-filled processes that normally drape the outer segments (**Figure 1**). The cells do, however, retain a fringe of conventional microvilli much like those that appear on cultured RPE cells and these microvilli then have the capability of regenerating after reattachment. RPE cells can also proliferate after injury to the retina and then migrate from their monolayer to form complex assemblies of cells in the expanded subretinal space. This is an important response to injury because these cells are structurally and physiologically polarized so that their apical



f0045 **Figure 9** A series of immunofluoresence images acquired by laser scanning confocal microscopy, illustrating the microglial and macrophage component of the response to retinal detachment. Microglia and macrophages (green) are labeled by a lectin molecule that binds to sugar groups on protein molecules. Notice that this lectin also binds to blood vessels that are represented by the large choroidal vessels above the OS and a large inner retinal vessel bridging the GCL/IPL layers in panel (a). Rod photoreceptors (red) are labeled with an antibody to the protein rod opsin, and Müller's cells by an antibody to GFAP (blue). Rod opsin is restricted to the layer of outer segments (OS) and GFAP to the Müller cell endfeet and astrocytes, both in the ganglion cell layer (GCL). The microglia are the green spindly cells within the INL. (b) In a detached retina, the OS are highly degenerated; rod opsin labeling now extends throughout the rod cell plasma membrane and the Müller cells have upregulated the expression of GFAP. Lectin-labeled microglia are now found in the outer retina, and macrophages are present on top of the degenerated outer segments. (c) An example of a retina that has been reattached but shows patchy areas of good and poor outer segment regeneration. In the area of good outer segment regeneration (to the right), opsin is once again restricted to the layer of outer segments and microglia are no longer found in the outer retina. In the area of poor outer segment regeneration, opsin is found distributed throughout the plasma membrane of the rod cells and microglia still reside in the outer retina. Notice that even though this retina was reattached for 28 days after a 3-day period of detachment, there is still a large amount of GFAP in the Müller cells. Scale bar = 20 μm.

and basal surfaces physiologically differ. If a new layer of RPE cells is generated in response to detachment and their basal surface faces the retina, no outer segment regeneration will occur. These cells also have the remarkable ability to migrate across the neural retina and integrate into epiretinal membranes (referred to above as glial scars) that contribute to the disease PVR. Indeed, because of the presence of melanin pigment granules, RPE cells within these membranes are highly visible and, for many years, PVR was thought to be a disease of RPE cell origin. This is still a point of controversy, but it seems clear that RPE cells can be involved along with glia and immune response cells.

Summary

s0085

p0110 Rhegmatogenous RD is a form of retinal injury that produces cellular responses common to many other injuries. Detachment as well as other physical injuries to the retina can result in long-lasting visual deficits. New information at the cellular and molecular level may lead to an understanding of why successful anatomical reattachment can still leave a patient with imperfect vision or the mechanisms by which some repairs fail. Müller's cells of the retina respond similarly to astrocytes in the brain and spinal cord and there is still much to learn about the role of these enigmatic cells in the retina's responses to injury and its ability to recover.

See also: Rhegmatogenous retinal detachment (00265).

Further Reading

Anderson, D. H., Guérin, C. J., Erickson, P. A., Stern, W. H., and Fisher, S. K. (1986). Morphological recovery in the reattached retina. *Investigative Ophthalmology and Visual Science* 27: 168–183.

Bringmann, A., Pannicke, T., Rosche, J., et al. (2006). Müller cells in the healthy and diseased retina. *Progress in Retinal and Eye Research* 25: 397–424.

Cook, B., Lewis, G. P., Fisher, S. K., and Adler, R. (1995). Apoptotic photoreceptor degeneration in experimental retinal detachment. *Investigative Ophthalmology and Visual Science* 36: 990–996.

Fisher, S. K., Lewis, G. P., Linberg, K. A., and Verardo, M. R. (2005). Cellular remodeling in mammalian retina: Results from studies of experimental retinal detachment. *Progress in Retinal and Eye Research* 24: 395–431.

Harada, T., Harada, C., Kohsaka, S., et al. (2002). Microglia–Müller glia cell interactions control neurotrophic factor production during light-induced retinal degeneration. *Journal of Neuroscience* 22: 9228–9236.

Jacobs, G. H., Calderone, J. B., Sakai, T., Lewis, G. P., and Fisher, S. K. (2003). Effects of retinal detachment on S and M cone function in an animal model. In: Mollon, J. D., Pokorny, J., and Knoblauch, K. (eds.) *Normal and Defective Colour Vision*, pp. 381–388. Oxford: Oxford University Press.

Lewis, G. P. and Fisher, S. K. (2003). Upregulation of GFAP in response to retinal injury: Its potential role in glial remodeling and a comparison to vimentin expression. In: Jeon, K. W. (ed.) *International Review of*

Cytology. A Survey of Cell Biology, vol. 230, pp. 263–290. San Diego, CA: Academic Press.
Lewis, G. P. and Fisher, S. K. (2005). Retinal plasticity and interactive cellular remodeling in retinal detachment and reattachment. In: Pinaud, R., Tremere, L., and De Weerd, P. (eds.) *Plasticity in the Visual System: From Genes to Circuits*, pp. 55–78. Dordrecht: Springer.
Lewis, G. P., Sethi, C. S., Carter, K. M., Charteris, D. G., and Fisher, S. K. (2005). Microglial cell activation following retinal detachment: A comparison between species. *Molecular Vision* 11: 491–500.
Lewis, G. P., Sethi, C. S., Charteris, D. G., et al. (2002). The ability of rapid retinal reattachment to stop or reverse the cellular and molecular events initiated by detachment. *Investigative Ophthalmology and Visual Science* 43: 2412–2420.
Ryan, S. (ed.) (2006). *Retina,* 4th edn., vol. I–III. Philadelphia, PA: Mosby.
Sarthy, V. and Ripps, H. (2001). *The Retinal Müller Cell. Structure and Function.* New York: Kluwer/Plenum.
Zacks, D. N., Hanninen, V., Pantcheva, M., et al. (2003). Caspase activation in an experimental model of retinal detachment. *Investigative Ophthalmology and Visual Science* 44: 1262–1267.

Relevant Websites

http://biology.about.com – About.com: Biology. The starting place for exploring biology.
http://www.sciencedirect.com – Current Opinion in Neurobiology.
http://webvision.med.utah.edu – Introduction.
http://www.nei.nih.gov – National Eye Institute, Retinal Detachment.
http://www.sfn.org – SfN Brain Briefings.