Molecular Vision 2005; 11:491-500 <http://www.molvis.org/molvis/v11/a57/>
Received 27 January 2005 | Accepted 28 June 2005 | Published 13 July 2005

Microglial cell activation following retinal detachment: a comparison between species

Geoffrey P. Lewis,[1] Charanjit S. Sethi,[2] Katrina M. Carter,[1] David G. Charteris,[2] Steven K. Fisher[1,3]

[1]Neuroscience Research Institute and [3]Department of Molecular, Cellular, and Developmental Biology, University of California Santa Barbara, Santa Barbara, CA; [2]Moorfields Eye Hospital, London, UK

Purpose: To compare the activation of microglia in response to retinal detachment in four species.
Methods: Experimental detachments were created in cats, rabbits, and ground squirrels and the retinas harvested 1, 3, 7, or 28 days later. Retinal reattachments of 28 days in duration were also performed in cats following a 3-day detachment. Human tissue was obtained during reattachment surgery. Microglia and macrophages were labeled with the lectins *Griffonia simplicifolia* and *Ricinus communis* and the antibody CD11b. Müller cell and photoreceptor responses were followed immunocytochemically on the same tissue sections labeled with microglial markers. Images were collected by laser scanning confocal microscopy.
Results: Lightly labeled microglia were observed primarily in the inner retina of control tissue. In the cat and rabbit, a progressive increase in the number of labeled cells occurred in the outer retina beginning at 1 day of detachment. In both long term human and cat detachments numbers of microglia were elevated throughout the retina. This is in contrast to the rabbit and ground squirrel retinas where microglial activation was dramatically diminished in longer term detachments. Presumptive macrophages (anti-CD11b labeled cells) occurred only in the subretinal space. Retinal reattachment in cats significantly attenuated the response except in areas of poor outer segment regeneration.
Conclusions: The robust microglial response to retinal detachment is an indicator of the importance of this cell type in the overall response of the retina. Our data suggest that the feline retina is a particularly appropriate model system for understanding this response in humans. Inhibiting the microglial response in that species should help us understand more precisely its potential role in photoreceptor survival in human pathology.

Microglial cells are the resident macrophages of the central nervous system (CNS) although they are neither a true glial cell nor a macrophage. They are derived from bone marrow precursor cells that enter the CNS early in development and generally lie "dormant" in adult tissue unless activated in response to various pathological changes including infectious diseases, trauma, inflammation, ischemia, and neurodegeneration [1]. Activated microglia become scavenger cells, phagocytosing cellular debris and killing invading microorganisms. The rapidity of their activation may be in part due to their responsiveness to subtle changes in the microenvironment such as imbalances in ion homeostasis [2] and signaling molecules such as ATP [3,4], acetylcholine and noradrenaline [5], all of which precede structural changes in surrounding cells. Thus, in their "resting" state, microglia seem to monitor their surroundings and remain poised to react quickly to physiological insult.

In the retina, microglia become activated in various forms of retinal injury or disease including light damage [6,7], glaucoma [8,9], laser photocoagulation [10], intraocular surgery [11], and the inherited degeneration of the RCS rat [12,13], human retinitis pigmentosa, and age related macular degeneration [14,15]. It is generally presumed that activation of microglia benefits surviving cells by removing debris that may be toxic. However, recent evidence suggests that activated microglia may be detrimental to CNS neuron survival. Indeed, it has been shown that inhibiting microglia activation slows photoreceptor degeneration in the RCS rat [16] and media taken from microglial cell cultures kills photoreceptors [17]. Microglia are known to secrete molecules such as pro-inflammatory cytokines, proteases, tumor necrosis factor, and nitric oxide, all of which can kill neurons [18-20]. For this reason, microglia inhibiting factors are being explored in the treatment of various diseases in the CNS including multiple sclerosis, stroke, Alzheimer's, and Parkinson's diseases.

The lectin *Griffonia simplicifolia* labels both microglia and macrophages in the retina of several different species [21], but it does not label cells in human retinal tissue. The lectin *Ricinus communis* agglutinin I has been used to label microglia and macrophages in human retinal samples [15]. The antibody to CD11b labels a cell surface antigen expressed by granulocytes, monocytes, and macrophages. In some species this antibody also labels microglia within the neural retina [22-24], but this is not the case in our preparations of feline retina.

Retinal detachment, or the separation of the neural retina from the retinal pigment epithelium (RPE), can occur as a result of varied conditions including trauma, inflammatory eye disease, or the presence of retinal holes or tears. In all cases, detachment initiates a cascade of events beginning in photoreceptors with outer segment degeneration [25-27] and fol-

Correspondence to: Geoffrey P. Lewis, PhD, Neuroscience Research Institute, University of California Santa Barbara, Santa Barbara, CA, 93106; Phone: (805) 893-3611; FAX: (805) 893-2005; email: g_lewis@lifesci.ucsb.edu

lowed quickly by overall photoreceptor cell deconstruction and the apoptotic death of some photoreceptors [28]. These events lead to extensive structural remodeling of bipolar, horizontal [29], and ganglion cells [30]. In addition, Müller cells hypertrophy within the area of detachment [31], and all non-neuronal cell types, including microglia, are stimulated to divide [32,33]. This response begins within a day of detachment, peaks between days 3 and 4, but continues at reduced levels for as long as the retina remains detached. Photoreceptor apoptosis peaks between days 3 and 4 and continues at low levels in detached regions [34]. Interestingly, no other retinal cell type appears to undergo cell death in response to detachment, at least in the feline retina. It is generally assumed that a major cause of photoreceptor cell deconstruction and death is a result of distancing the retina from its source of oxygen and nutrients in the choriocapillaris. However, given the evidence in other model systems of CNS degeneration indicating that microglia may accentuate neuronal cell death we have begun to determine what role microglia may play in the retina's response to detachment and reattachment. As a first step, we have compared the microglial response in the retinas of four different species: the rod dominated cat, rabbit, and human and the cone dominated California ground squirrel. In all cases, microglia become activated and migrate into the photoreceptor layer, but not all species are equally reactive. Activated microglia remain only in localized regions of poor photoreceptor recovery in reattached retinas. In areas that appear to have recovered more completely, microglia occur in similar locations as in the non-detached retina. These data suggest a significant role for microglia in modulating the retina's response to detachment and reattachment.

METHODS

Tissue preparation: Retinal detachments were created in cats, rabbits, and California ground squirrels. Since it is difficult to create large stable detachments in cats, the lens and vitreous were first removed. A solution of sodium hyaluronate (Healon; Pharmacia, Piscataway, NJ) was then infused via a glass pipette (with an inner diameter of approximately 100 μm) between the neural retina and RPE [35]. The Healon is necessary to prevent spontaneous reattachment of the retina. All surgery was done via an entry site at the level of the pars plana. Approximately half the retina was detached in each eye, consisting primarily of superior retina, leaving attached regions as internal controls. For the rabbits and ground squirrels it was not necessary to remove the lens and vitreous since these retinas detached more easily and remained detached for the duration of the experiment. Following 1, 3, 7, or 28 days of detachment, the animals were euthanized (sodium pentobarbital, 120 mg/ml, IV) and the tissue placed in fixative (4% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4; Electron Microscopy Sciences, Fort Washington, PA).

Small 1-2 mm human retinectomy samples were obtained at the time of reattachment surgery and immediately placed in fixative. One human eye with an exudative retinal detachment secondary to malignant melanoma was also analyzed. This situation is different from the experimental detachments in animals and the human retinectomy samples where there is a break in the retina either from the pipette (animals) or some underlying pathology (human) allowing for the flow of fluid under the retina thereby creating a "rhegmatogenous" detachment. Following enucleation of the melanoma eye the entire globe was placed in fixative.

Four animals were analyzed at each time point. Multiple regions were sampled from the detached nasal, temporal, and superior retina and from the attached naso-temporal and inferior areas of retina within each eye. Six retinectomy samples were used. Only one exudative detachment was obtained but multiple regions from both the detached and attached regions were examined. All experimental procedures conformed to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research and the guidelines of the Animal Resource Center of the University of California Santa Barbara (Santa Barbara, CA). All human surgery was conducted at Moorfields Eye Hospital (London, UK) by Consultant vitreoretinal surgeons or senior Fellows, and adhered to the tenets of the Declaration of Helsinki for research involving human subjects and human tissue. All patients provided informed consent for the use of retinal tissue excised during surgery.

Retinal reattachments were performed in four cats. Following a 3-day detachment where the detachments were made using a balanced salt solution (BSS; Alcon, Ft. Worth, TX) rather than Healon, a fluid-gas exchange was performed, which caused the fluid from under the retina to flow through the hole thereby allowing the retina to become reapposed to the RPE. The eye was then flushed with 20% sulfur hexafluoride (SF-6; Alcon, Ft. Worth, TX) in filtered room air to act as a retinal tamponade. The animals were euthanized using sodium pentobarbital (120 mg/ml, IV) 28 days after the reattachment procedure and the eyes immediately placed in fixative and stored until used. Similar retinal regions to those sampled in the detached retina (listed above) were sampled in the reattached retinas.

Immunocytochemistry: For the experimental animals and the eye with the exudative detachment, retinal pieces approximately 3 mm square were excised from the globe following at least 24 h of fixation, rinsed in phosphate buffered saline (PBS), embedded in low-melt agarose (5%; Sigma, St. Louis, MO) and sectioned at 100 μm using a Vibratome (Technical Products International, Polysciences, Warrington, PA). The 1-2 mm human retinectomy samples were embedded in agarose in their entirety and sectioned at 100 μm. Sections were incubated in normal donkey serum (1:20) in PBS, 0.5% BSA, 0.1% Triton X-100, and 0.1% azide (PBTA) overnight at 4 °C on a rotator. The following day the primary antibodies or lectins were added and incubated overnight at 4 °C on a rotator in PBTA. The isolectin B4, *Griffonia simplicifolia* (1:50; Vector Labs, Burlingame, CA) was used as a label for microglia and macrophages in cat, rabbit and squirrel retinas. The lectin *Ricinus Communis* agglutinin I (1:1000; Vector Labs) was used for the human samples since the isolectin B4 does not label human retinal tissue. Both lectins were purchased from the company as a biotinylated product whereas CD11b was purchased as a mouse monoclonal antibody (1:50; Serotec, Raleigh, NC).

The mouse monoclonal antibody to rod opsin (1:50; kind gift of Dr. Robert Molday, University of British Columbia, Vancouver, Canada) and the rabbit polyclonal antibody to GFAP (1:400; Dako, Carpinteria, CA) were used on the same sections to follow the response of rod photoreceptors and Müller cells, respectively. Following rinsing of the primary antibodies in PBTA, for most experiments, the secondary antibodies (streptavidin CY2, donkey anti-mouse CY3, and donkey anti-rabbit CY5; Jackson ImmunoResearch, West Grove, PA) were added together, each at 1:200 in PBTA, overnight at 4 °C on a rotator. Donkey anti-rabbit CY3 and donkey anti-mouse CY5 were used for Figure 1. On the final day, the sections were rinsed in PBTA, mounted on glass slides using 5% n-propyl gallate in glycerol and viewed on a BioRad 1024 laser scanning confocal microscope (Hercules, CA).

RESULTS

Feline: In the normal feline retina, cells labeled with the lectin *Griffonia simplicifolia* (but not the anti-CD11b), were found exclusively in the inner retina and most often in the inner plexiform layer (Figure 2A). These cells had a small cell body and a stellate appearance with distinctive wavy processes consistent with the morphology of microglia. At one day of detachment, as photoreceptor outer segments began to degenerate and opsin became redistributed to the rod cell bodies, labeled microglia were observed in the outer nuclear layer (Figure 2B). At 3 days of detachment numerous microglia were observed, with varying morphologies, throughout the retina (Figure 2C). Some were elongated and extended the entire width of the outer nuclear layer with both thick and thin processes while others were more rounded. Labeled cells were also seen

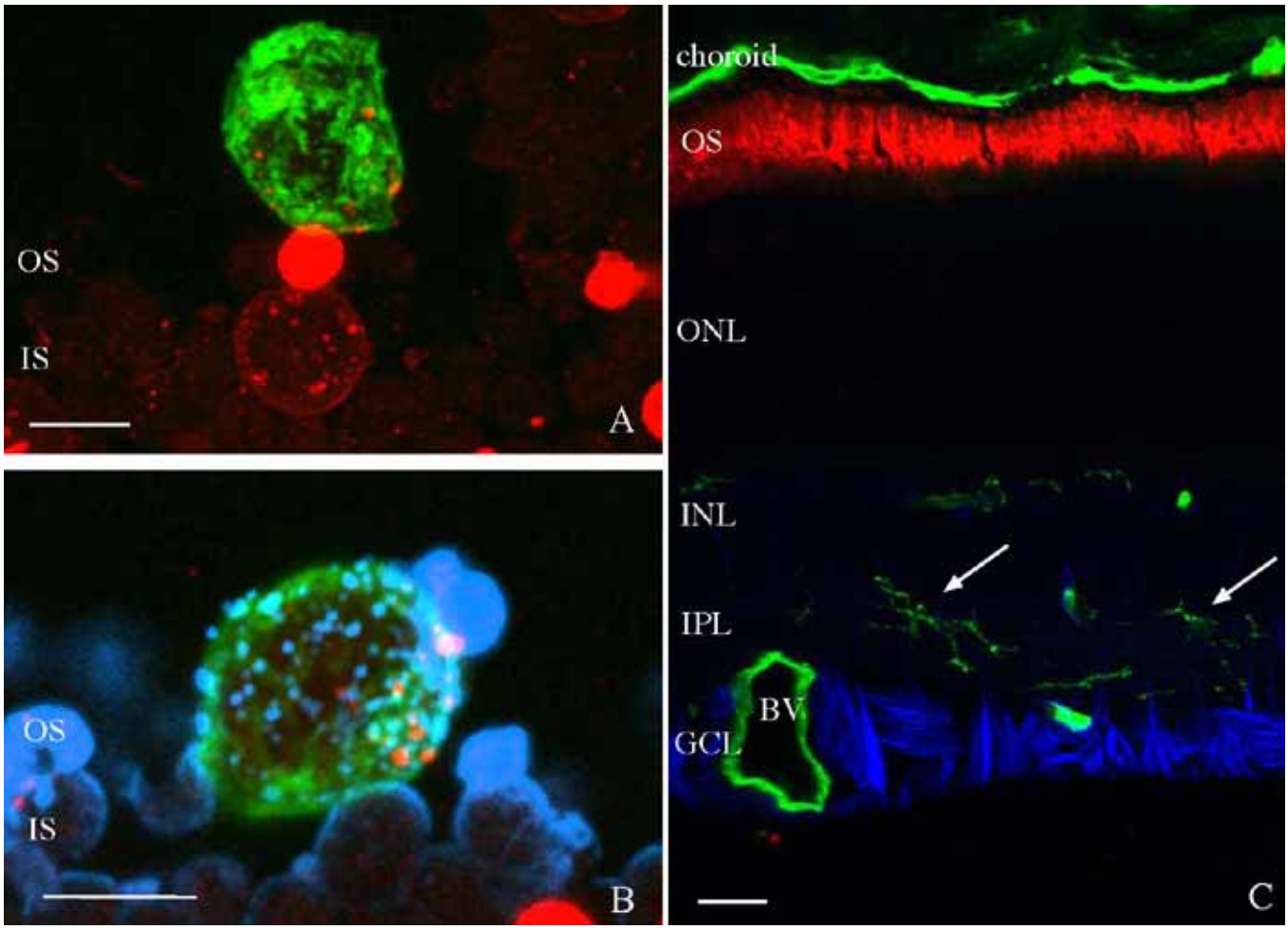

Figure 1. Lectin labeling of detached and attached feline retina. **A**: A laser scanning confocal image is shown of a cell in the subretinal space from 3 day detached feline retina labeled with the lectin *Griffonia simplicifolia* (green) and antibodies to M cone opsin (red). The cone outer segment (OS) is truncated and the opsin is redistributed to the inner segment (IS). Cone opsin material is present within the cell (small dots). **B**: A laser scanning confocal image is shown of a cell in the subretinal space from 3 day detached feline retina labeled with the lectin *Griffonia simplicifolia* (green) and antibodies to both rod opsin (blue) and M cone opsin (red). The cell (green) appears in association with the degenerate rods but contains both rod and cone opsin labeled material (small dots). **C**: A laser scanning confocal image is shown of attached feline retina from an eye with a 28 day detachment, labeled with the lectin *Griffonia simplicifolia* (green) and antibodies to rod opsin (red) and glial fibrillary acidic protein (GFAP; blue). No significant activation of microglia is apparent as microglia continue to reside in the inner plexiform layer (IPL; arrows). The outer segments (OS), outer nuclear layer (ONL), inner nuclear layer (INL), ganglion cell layer (GCL), and blood vessels (BV) are also identified. The scale bars represent 5 μm in **A**,**B** and 20 μm in **C**.

interspersed among photoreceptor outer segments. At 28 days of detachment the labeled microglia continued to be present in high numbers with cells distributed throughout the retina and extending into the subretinal space where they associated with subretinal glial scars (Figure 2D). At this time point many of these cells had long, fine processes that course through the retina although some of these cells had a rounded morphology. Lectin labeled cells in the subretinal space were found adjacent to both cones (Figure 1A) and rods (Figure 1B). In addition, these cells appeared to phagocytose cone and rod outer segment debris and often contained phagosomes with an origin from both (Figure 1B). No activation of microglia was observed in the attached retina from the eye with the detachment at any time point (Figure 1C; day 28).

Double labeling experiments with the *Griffonia simplicifolia* lectin and the antibody to CD11b identified two populations of cells in the feline retina. Figure 3 shows two images separated from one double-labeled section using the lectin and anti-CD11b on a 3 day detached retina. The lectin labeled many cells (and blood vessels) within the retina and

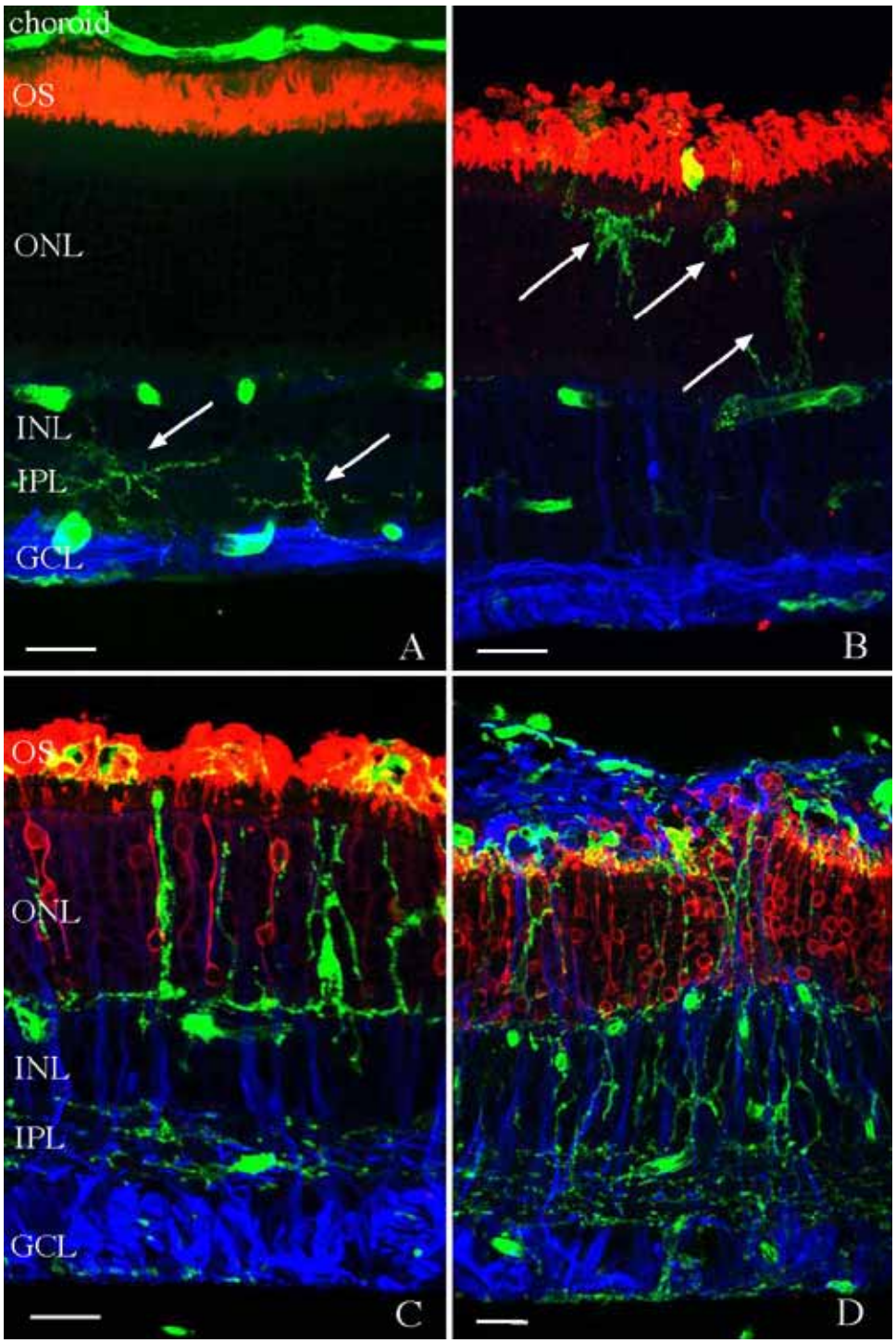

Figure 2. Localization of microglia in the normal and detached feline retina. Laser scanning confocal images of feline retina are shown labeled with the lectin *Griffonia simplicifolia* (green) and antibodies to rod opsin (red) and glial fibrillary acidic protein (GFAP; blue). **A**: In normal retina, the lectin labels microglia in the inner plexiform layer (IPL; arrows) and blood vessels in the retina and choroid. Anti-rod opsin labels rod outer segments (OS). Anti-GFAP labels Müller cell endfeet and astrocyte processes. **B**: One day after retinal detachment, lectin labeled cells are observed in the outer nuclear layer (ONL) as rod OS begin to degenerate and Müller cells upregulate GFAP expression. **C**: Three days after retinal detachment lectin-labeled cells with varied morphologies are distributed throughout the inner and outer retina and in the OS layer. Anti-rod opsin is present in the degenerating OS and in the plasma membrane of some rod photoreceptors. In the ONL and anti-GFAP, labeling extends throughout the retina. **D**: Twenty-eight days after retinal detachment, numerous lectin-labeled cells are present throughout the retina and in a subretinal glial scar labeled with anti-GFAP. Anti-rod opsin labeling illustrates the extensive OS degeneration and redistribution of the protein to the entire photoreceptor plasma membrane at this time. The inner nuclear layer (INL) and ganglion cell layer (GCL) are also identified. The scale bars represent 20 μm.

cells in the subretinal space (Figure 3B). The CD11b antibody labeled only the surface of cells in the subretinal space and no cells within the retina (Figure 3A, arrows). Thus, in terms of these two markers, we observed two populations of cells responding to detachment.

In retinas that were reattached for 28 days following a 3-day detachment, the microglia response, for the most part, appeared diminished; that is, rarely were labeled cells observed in the outer retina (Figure 4A). Also at this time, most photoreceptors appeared structurally normal; photoreceptor outer segments had regenerated and opsin was once again restricted to the outer segment (Figure 4A). However, small, focal regions of poor photoreceptor recovery ded occur, and in these areas outer segments showed incomplete regeneration, opsin was still elevated in the plasma membrane of the cell bodies, and microglia were still prevalent in the outer retina (Figure 4B). The transition between these regions was always quite abrupt and similarly the transition to areas rich in microglial cells also occurred abruptly (Figure 4C). In addition, as was reported previously [36], some rods extended neurites deep into the inner retina in these focal regions (Figure 4B and inset) and microglial cells often appeared in direct association with these neurites. No specific association of microglial cells with Müller cells was observed.

Human: In normal human retinal tissue, the lectin *Ricinus communis* labeled cells that were restricted to the inner retina, predominantly the inner plexiform layer (Figure 5A, arrow). These cells had the same morphology and location as the *Griffonia simplicifolia* labeled cells in the feline retina. Following detachment, the labeled cells become dispersed throughout the retina (Figure 5B). These detachments are termed "complex" detachments, that is, the retinas underwent multiple reattachment surgeries before the tissue was excised from the eye, so the exact duration of detachment is not known. In all cases, however, these can be considered longstanding detachments of a month or more; anti-opsin labeling was elevated in the ONL and anti-GFAP labeling was increased in the Müller cells. Labeling with the lectin also occurred in epiretinal membranes (cells that have grown on the vitreal surface of the retina; Figure 5B, brackets).

In the human eye with a malignant melanoma that induced an "exudative" detachment, labeled cells occurred in all retinal layers but were restricted to the detached zone (Figure 5D). The exact duration of the detachment was unknown but most likely was long standing based on the extent of the outer segment degeneration and rod opsin redistribution throughout the entire rod plasma membrane. Attached retinal regions from this same eye showed normal appearing photoreceptors and a normal distribution of lectin-labeled microglia (Figure 5C) demonstrating once again the localized nature of the activation.

Rabbit: The rabbit retina initially responded similarly to the feline and human retina. By one day of detachment (Figure 6B), *Griffonia Simplicifolia* labeling showed an increase in the number of microglia in the outer retina compared to normal (Figure 6A). At one week of detachment, however, few lectin labeled cells were observed (Figure 6C), a trend that continueed at the 28-day time point (data not shown). Anti-rod opsin labeling indicateed that there was significant photoreceptor degeneration; anti-GFAP labeling indicated a robust glial response.

California ground squirrel: The photoreceptors in the California ground squirrel retina degenerated even more quickly than those in the rabbit retina in response to detachment [37]. In this species, labeled cells were rare at any detachment time point (Figure 6D, arrow, day 7). The few la-

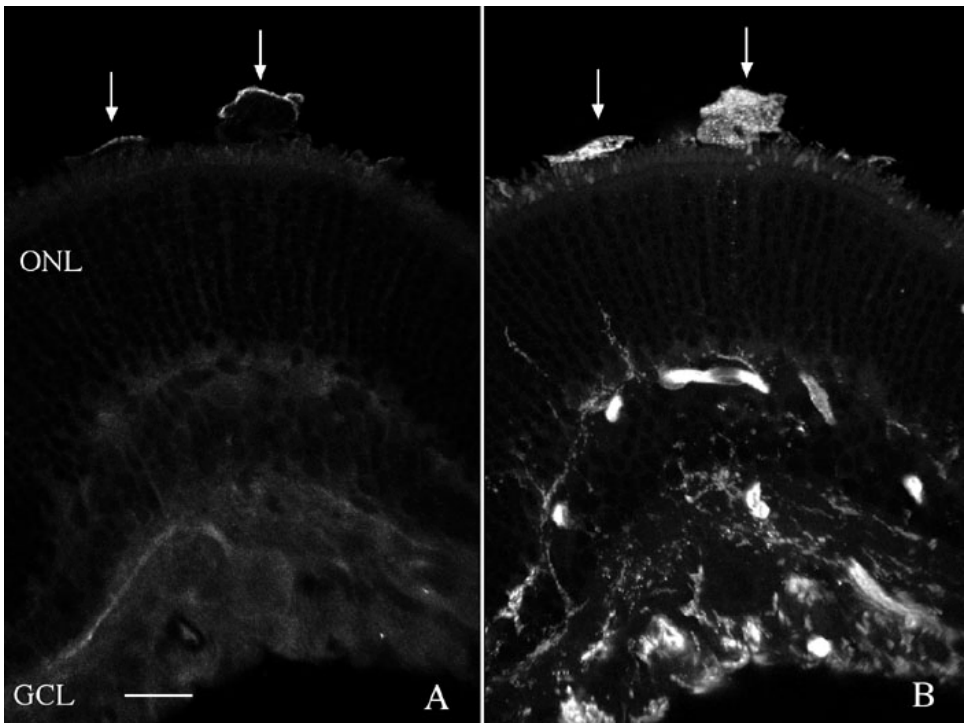

Figure 3. Localization of *Griffonia simplicifolia* and CD11b in the feline retina. Laser scanning confocal images are shown of a 3-day detached feline retina labeled with anti-CD11b (**A**) and the lectin *Griffonia simplicifolia* (**B**). A single section was originally double labeled but the two channels are separated here to more easily visualize the labeling patterns. **A**: Anti-CD11b labeling is present only in the presumptive macrophages in the subretinal space (arrows). **B**: The same section shown in **A** demonstrating that the lectin labels cells throughout the retina, in the subretinal space (arrows), and blood vessels. The outer nuclear layer (ONL) and ganglion cell layer (GCL) are also identified. The scale bar represents 20 μm.

beled cells that were observed had the same morphology of labeled cells in the other species. Occasionally labeled cells were also observed in the subretinal space (Figure 6E, arrows, day 7). There was no increase in anti-GFAP labeling in Müller cells at this time even though there was considerable photoreceptor degeneration, as illustrated by the truncated outer segments and redistribution of the opsin protein throughout the rod cell plasma membrane.

DISCUSSION

Following retinal detachment, microglia become activated in all species examined and migrate towards the outer retina. This is in accordance with results in other forms of retinal degeneration where lectin labeled cells, present in the inner retina are stimulated to migrate towards degenerating photoreceptors (see references in Introduction). In addition to their migration, however, there almost certainly was an increase in

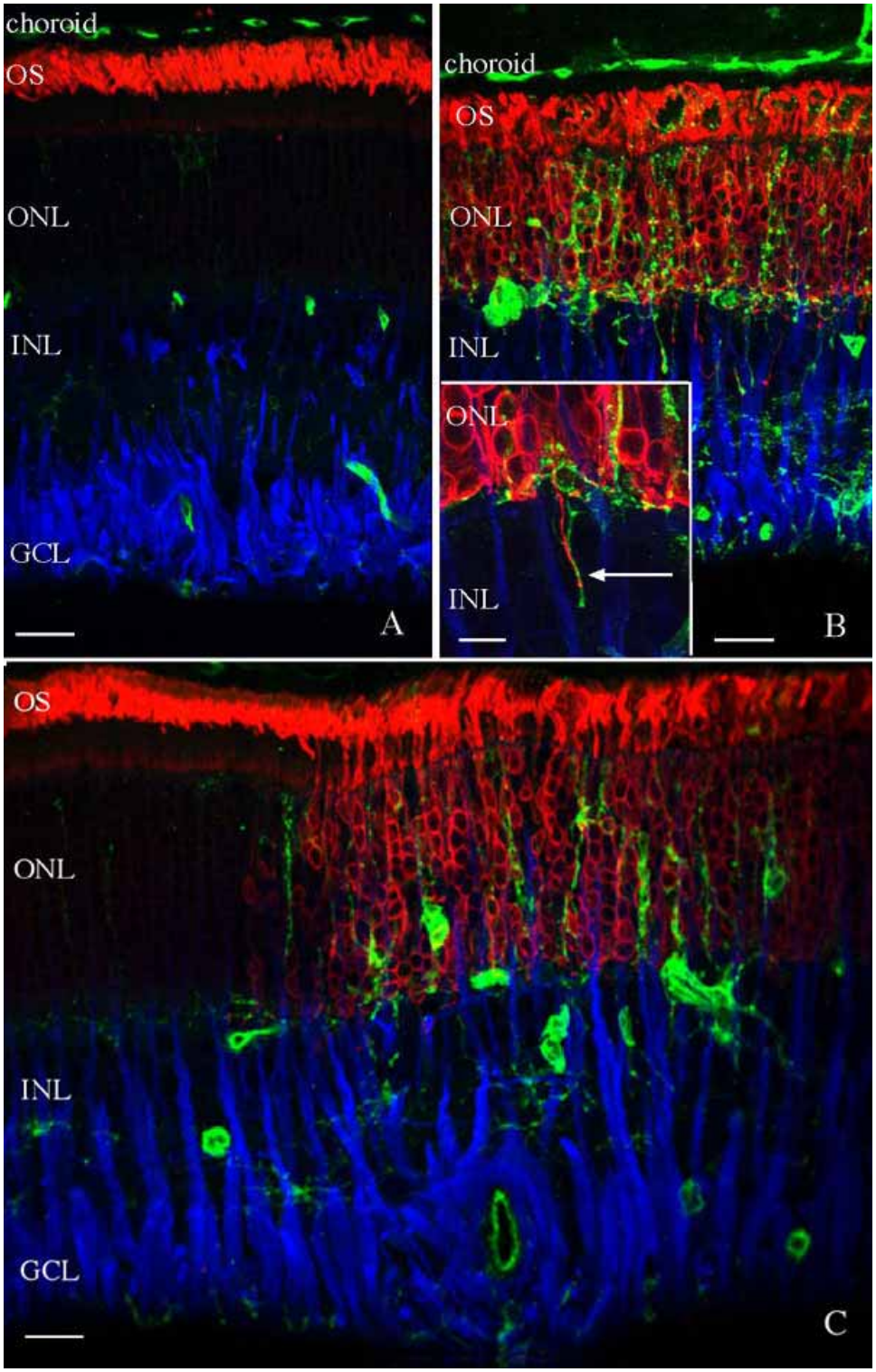

Figure 4. Localization of microglia in the reattached feline retina. Laser scanning confocal images are shown of feline retina, detached for 3 days and reattached for 28 days, labeled with the lectin *Griffonia Simplicifolia* (green) and antibodies to rod opsin (red) and glial fibrillary acidic protein (GFAP; blue). **A**: A typical region of reattached retina demonstrates that few lectin labeled cells are present in the outer nuclear layer (ONL) at this time. Anti-rod opsin labeling is present in the now organized outer segments (OS) and anti-GFAP labeling extends into the inner nuclear layer (INL). **B**: In focal "patchy" regions with poor photoreceptor recovery, as illustrated by the continued anti-opsin labeling of the ONL and disorganized OS, many lectin labeled cells are present throughout the retina. Lectin-labeled processes are often observed in close association with rod axons that extend into the inner retina following reattachment (arrow). **C**: A reattached retina is shown illustrating the abrupt transition from retinal areas with few lectin labeled cells in the ONL (left half of image) to regions with many lectin-labeled cells in the ONL (right half of image). The lectin-labeled cells present in the ONL occur predominantly in regions of poor photoreceptor recovery as illustrated by the increased anti-opsin labeling. The lectin also labeled blood vessels in the retina and choroid. The ganglion cell layer (GCL) is also identified. Scale bars represent 20 μm in **A**,**B**,**C** and 10 μm in the inset to **B**.

the number of microglia following retinal detachment; the immunocytochemistry showed large numbers of labeled cells in the longer term detachments, particularly in the human and feline retina. Indeed, cell proliferation studies have shown that these cells are stimulated to divide following detachment [32,33]. Both migration and proliferation are indicative of microglia undergoing a transition from a "resting" to an "activated" state.

Based on their morphology, location in normal retina, response to injury and the observation that only cells in the subretinal space label with anti-CD11b, we conclude that the lectin labeled cells in the retina were microglial cells and the majority of those in the subretinal space were most likely macrophages that entered the eye in response to the detachment. We cannot, however, rule out the possibility that the cells in the subretinal space represent a mixture of macrophages and microglia that migrated into the subretinal space and began to express the CD11b antigen. Indeed it has been shown in rats following optic nerve transection that as microglia become reactive they can transform into cells that express macrophage/monocyte markers [38]. In the feline retina both cell types were present as long as the retina remained detached although the CD11b-positive cells appeared to remain associated with photoreceptor outer segments in the subretinal space

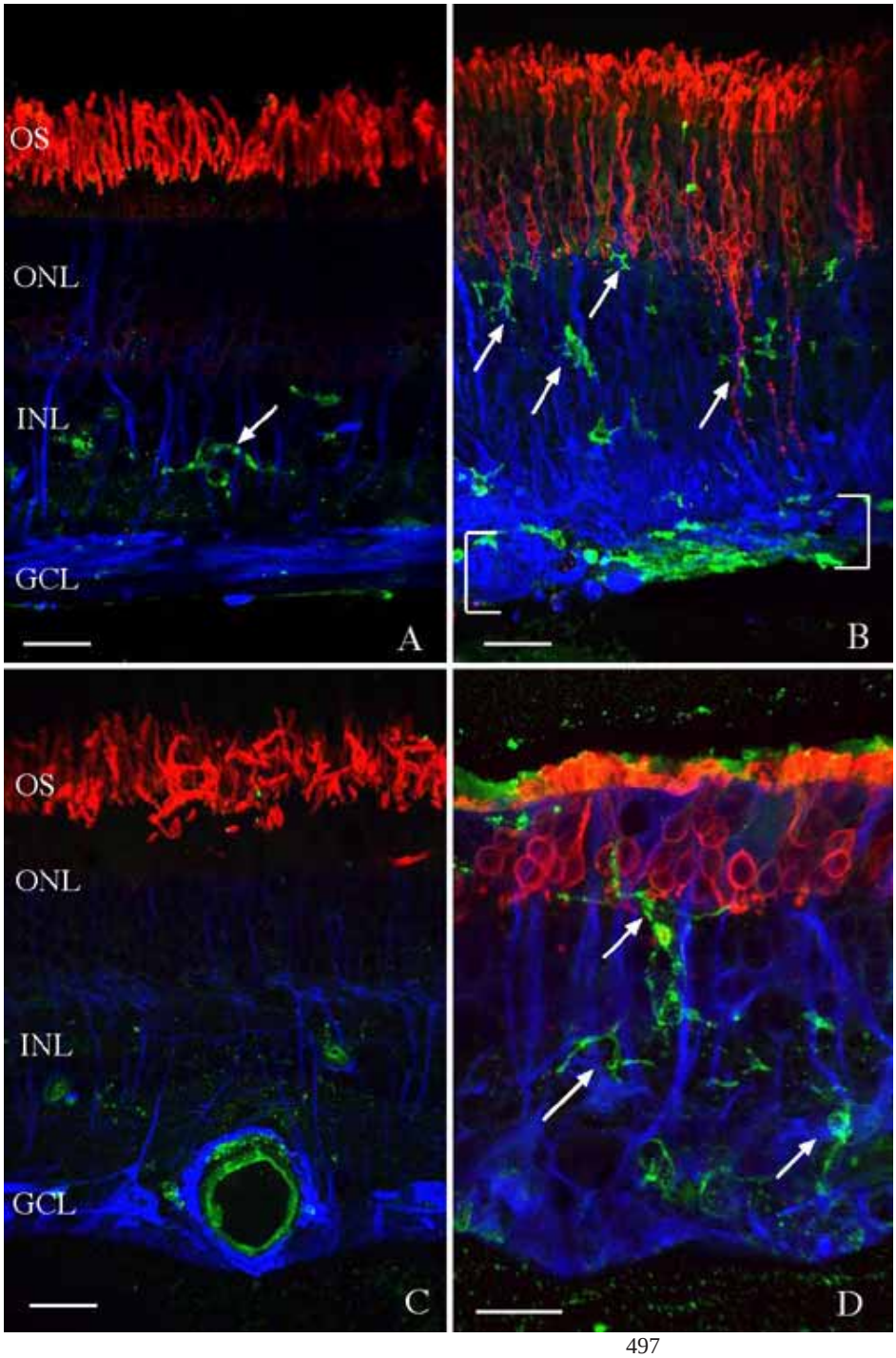

Figure 5. Localization of microglia in normal and detached human retina. Laser scanning confocal images are shown of human retina labeled with the lectin *Ricinus communis* (green) and antibodies to rod opsin (red) and glial fibrillary acidic protein (GFAP; blue). **A**: In normal retina the lectin labels cells in the inner plexiform layer; anti-rod opsin is present in the rod outer segments (OS) and anti-GFAP is present in Müller cells and astrocytes. **B**: In longstanding detachments (exact duration unknown but at least 1 month), lectin-labeled cells (arrows) are dispersed throughout the retina and within an epiretinal membrane (brackets). Anti-opsin labeling is elevated in the outer nuclear layer (ONL) and rod axons are observed extending into the inner retina. Anti-GFAP labeling of Müller cells is elevated. **C**: An attached retina from an eye with an exudative detachment as a result of a malignant melanoma is shown illustrating the normal distribution of lectin, anti-opsin, and anti-GFAP labeling. The large lectin-labeled structure in the ganglion cell layer (GCL) is a blood vessel. **D**: A detached retina is shown from the same eye as shown in **C** illustrating the numerous lectin-labeled cells dispersed throughout the retina (arrows), elevated anti-opsin labeling in the ONL, and anti-GFAP labeling of Müller cells. The inner nuclear layer (INL) is also identified. Scale bars represent 20 μm.

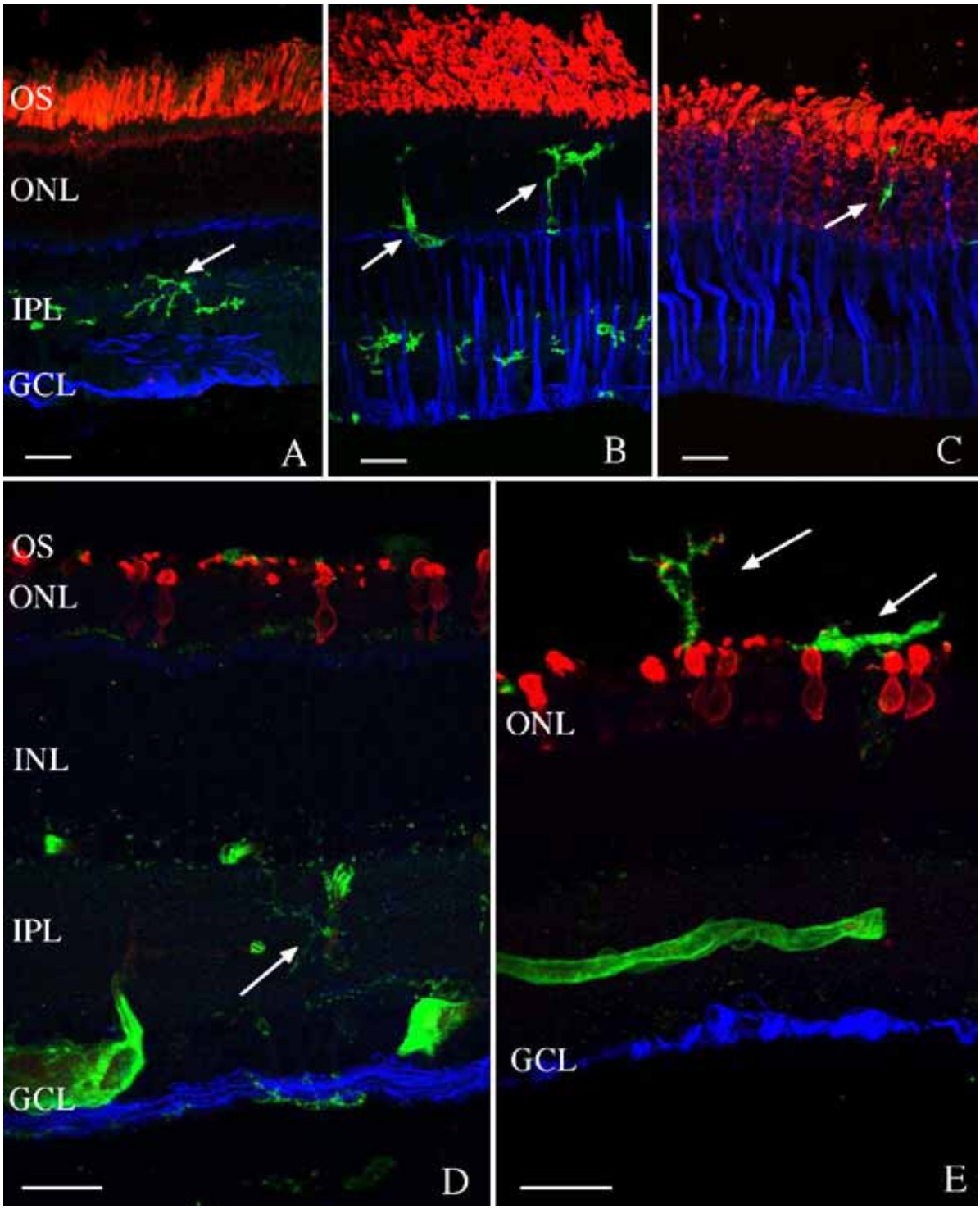

Figure 6. Localization of microglia in normal and detached rabbit and ground squirrel retina. Laser scanning confocal images of rabbit (**A**,**B**,**C**) and California ground squirrel (**D**,**E**) retina are shown labeled with the lectin *Griffonia simplicifolia* (green) and antibodies to rod opsin (red) and glial fibrillary acidic protein (GFAP; blue). **A**: In normal rabbit retina the lectin labels cells in the inner plexiform layer (IPL), anti-rod opsin labels outer segments, and anti-GFAP labels astrocytes and Müller cells in the inner retina. **B**: At one day of detachment, lectin-labeled cells are observed in the outer nuclear layer (ONL) as the outer segments (OS) begin to degenerate. **C**: At 7 days of detachment, few lectin-labeled cells are observed, rod photoreceptors are highly degenerate and are labeled with anti-opsin throughout their plasma membrane, and anti-GFAP is elevated in Müller cells. **D**,**E**: Two examples of ground squirrel retina detached for 7 days. Few lectin-labeled cells (arrows) are observed in either the retina or the subretinal space. Anti-opsin labeling illustrates the highly degenerate morphology of the photoreceptors, labeling the truncated OS and the entire plasma membranes of the cells. No increase in anti-GFAP is observed in Müller cells. The large lectin-labeled structures in the inner retina are blood vessels. The inner nuclear layer (INL) and ganglion cell layer (GCL) are also identified. The scale bars represent 20 μm.

and the lectin-positive cells (microglia) were confined to the retina and Müller cell scars. Following one month of reattachment the number in both populations was greatly reduced, appearing about the same as in the control retina. These observations suggest that many of the cells died and those microglia that remained, returned to their original location in the inner retina. A similar phenomenon is observed following optic nerve transection in rats; microglia are initially activated in the region of ganglion cell death, undergo a change in morphology and increase in numbers, but following a 6-month recovery period, return to their original number and normal morphology [38].

Microglial activation occurred to differing degrees in different species. In the cat and rabbit retina the response began quickly, within a day of detachment. This activation can also represent the beginning of a long term response since increased numbers of activated cells were still observed in the outer retina of feline retinas reattached for a month and in longstanding complex human detachments. The short term nature of the microglial response in the rabbit and the minimal response in the ground squirrel may be related to the fact that photoreceptor degeneration was extremely rapid and complete in these species. It is not known if microglia play a role in the death of photoreceptors in the other species, where it occurs more slowly, but this would not seem to be the case in the ground squirrel where we observed essentially no activation. There is also evidence that photoreceptor death following injury may be due to interactions between microglia and Müller cells. Harada et al. [7] demonstrated that microglia modulate the production of a variety of trophic factors from Müller cells including some that may promote photoreceptor survival and some that may promote cell death. It is interesting in this context that ground squirrels mounted neither a robust microglial nor a Müller cell response and photoreceptor degeneration was rapid. In the cat and human retina, where there was continued microglial cell reactivity there was also continued robust Müller cell reactivity which may lead to an increased production of photoreceptor survival factors by these cells.

The microglial cell response was highly localized to the injured retinal regions. Rarely were these cells observed in the outer nuclear layer of reattached regions that showed good recovery from the detachment, even though these areas were adjacent to regions with incomplete photoreceptor recovery and extensive microglial activation. In addition, microglia did not become reactive in non-detached regions from eyes with detachments nor did they appear within the attached portions of the human eye with an exudative detachment. While another study demonstrated that vitreal manipulation during surgery (with or without a detachment) induced an activation of microglia within the nerve fiber layer of the rabbit retina two days later [11] we found no evidence for this in the attached retina from the eyes with detachments. The data from this study suggests that photoreceptor degeneration was the primary stimulus for activating microglia in responsive species; in regions with less photoreceptor degeneration, there was less microglia activation.

In all four species studied, events consistent with microglial cell activation occurred in response to detachment, albeit to different degrees. Questions remain, however, about the functional roles of these cells. As elsewhere in the CNS they are almost certainly involved in scavenging cellular debris, in this case, from dead photoreceptors [15]. Based on evidence from other studies, they may also be involved in either causing or preventing photoreceptor cell death, depending on the factors they release and their effects on Müller cells [7]. Indeed, our data also suggests a correlation between the amount of microglial cell activation and Müller cell reactivity. Functional links between these events may be revealed through the use of agents that inhibit the microglial cell response after detachment [16]. Given the prevalence of this response, it seems significant to determine more precisely its role in the retina's response to injury.

ACKNOWLEDGEMENTS

The authors thank Ken Linberg and Kellen Betts for their discussions and technical assistance. The work was supported by NIH grant EY00088 (SKF), the Medical Research Council, UK (grant G84/5452), the Special Trustees of Moorfields Eye Hopsital, The Royal College of Surgeons of Edinburgh and the Royal Blind Asylum/Scottish National Institution for the War Blinded (DGC).

REFERENCES

1. Thanos S, Moore S, Hong YM. Retinal Microglia. Prog Retin Eye Res 1996; 15:331-61.
2. Gehrmann J, Mies G, Bonnekoh P, Banati R, Iijima T, Kreutzberg GW, Hossmann KA. Microglial reaction in the rat cerebral cortex induced by cortical spreading depression. Brain Pathol 1993; 3:11-7.
3. Walz W, Ilschner S, Ohlemeyer C, Banati R, Kettenmann H. Extracellular ATP activates a cation conductance and a K+ conductance in cultured microglial cells from mouse brain. J Neurosci 1993; 13:4403-11.
4. Langosch JM, Gebicke-Haerter PJ, Norenberg W, Illes P. Characterization and transduction mechanisms of purinoceptors in activated rat microglia. Br J Pharmacol 1994; 113:29-34.
5. Whittemore ER, Korotzer AR, Etebari A, Cotman CW. Carbachol increases intracellular free calcium in cultured rat microglia. Brain Res 1993; 621:59-64.
6. Ng TF, Streilein JW. Light-induced migration of retinal microglia into the subretinal space. Invest Ophthalmol Vis Sci 2001; 42:3301-10.
7. Harada T, Harada C, Kohsaka S, Wada E, Yoshida K, Ohno S, Mamada H, Tanaka K, Parada LF, Wada K. Microglia-Muller glia cell interactions control neurotrophic factor production during light-induced retinal degeneration. J Neurosci 2002; 22:9228-36.
8. Naskar R, Wissing M, Thanos S. Detection of early neuron degeneration and accompanying microglial responses in the retina of a rat model of glaucoma. Invest Ophthalmol Vis Sci 2002; 43:2962-8.
9. Lam TT, Kwong JM, Tso MO. Early glial responses after acute elevated intraocular pressure in rats. Invest Ophthalmol Vis Sci 2003; 44:638-45.
10. Humphrey MF, Moore SR. Microglial responses to focal lesions of the rabbit retina: correlation with neural and macroglial reactions. Glia 1996; 16:325-41.

11. Uhlmann S, Bringmann A, Uckermann O, Pannicke T, Weick M, Ulbricht E, Goczalik I, Reichenbach A, Wiedemann P, Francke M. Early glial cell reactivity in experimental retinal detachment: effect of suramin. Invest Ophthalmol Vis Sci 2003; 44:4114-22.
12. Thanos S. Sick photoreceptors attract activated microglia from the ganglion cell layer: a model to study the inflammatory cascades in rats with inherited retinal dystrophy. Brain Res 1992; 588:21-8.
13. Roque RS, Imperial CJ, Caldwell RB. Microglial cells invade the outer retina as photoreceptors degenerate in Royal College of Surgeons rats. Invest Ophthalmol Vis Sci 1996; 37:196-203.
14. Penfold PL, Madigan MC, Gillies MC, Provis JM. Immunological and aetiological aspects of macular degeneration. Prog Retin Eye Res 2001; 20:385-414.
15. Gupta N, Brown KE, Milam AH. Activated microglia in human retinitis pigmentosa, late-onset retinal degeneration, and age-related macular degeneration. Exp Eye Res 2003; 76:463-71.
16. Thanos S, Richter W, Thiel HJ. The protective role of microglia-inhibiting factor in the retina suffering of hereditary photoreceptor degeneration. In: Pleyer U, Schmidt K, Thiel HJ, editors. Cell and Tissue Protection in Ophthalmology. Stuttgart: Hippokrates Verlag; 1995. p. 195-203.
17. Roque RS, Rosales AA, Jingjing L, Agarwal N, Al-Ubaidi MR. Retina-derived microglial cells induce photoreceptor cell death in vitro. Brain Res 1999; 836:110-9.
18. Colton CA, Gilbert DL. Production of superoxide anions by a CNS macrophage, the microglia. FEBS Lett 1987; 223:284-8.
19. Banati RB, Gehrmann J, Schubert P, Kreutzberg GW. Cytotoxicity of microglia. Glia 1993; 7:111-8.
20. Lee YB, Nagai A, Kim SU. Cytokines, chemokines, and cytokine receptors in human microglia. J Neurosci Res 2002; 69:94-103.
21. Cho EY, Choi HL, Chan FL. Expression pattern of glycoconjugates in rat retina as analysed by lectin histochemistry. Histochem J 2002; 34:589-600.
22. Broderick C, Duncan L, Taylor N, Dick AD. IFN-gamma and LPS-mediated IL-10-dependent suppression of retinal microglial activation. Invest Ophthalmol Vis Sci 2000; 41:2613-22.
23. Rhodes KE, Moon LD, Fawcett JW. Inhibiting cell proliferation during formation of the glial scar: effects on axon regeneration in the CNS. Neuroscience 2003; 120:41-56.
24. Marella M, Chabry J. Neurons and astrocytes respond to prion infection by inducing microglia recruitment. J Neurosci 2004; 24:620-7.
25. Machemer R. Experimental retinal detachment in the owl monkey. IV. The reattached retina. Am J Ophthalmol 1968; 66:1075-91.
26. Erickson PA, Fisher SK, Anderson DH, Stern WH, Borgula GA. Retinal detachment in the cat: the outer nuclear and outer plexiform layers. Invest Ophthalmol Vis Sci 1983; 24:927-42.
27. Anderson DH, Stern WH, Fisher SK, Erickson PA, Borgula GA. Retinal detachment in the cat: the pigment epithelial-photoreceptor interface. Invest Ophthalmol Vis Sci 1983; 24:906-26.
28. Fisher SK, Lewis GP. Cellular effects of detachment on the neural retina and the retinal pigment epithelium. In: Ryan SJ, Hinton DR, Schachat AP, Wilkinson CP, editors. Retina. 4th ed. St. Louis, MO: Mosby. In press 2005.
29. Lewis GP, Linberg KA, Fisher SK. Neurite outgrowth from bipolar and horizontal cells after experimental retinal detachment. Invest Ophthalmol Vis Sci 1998; 39:424-34.
30. Coblentz FE, Radeke MJ, Lewis GP, Fisher SK. Evidence that ganglion cells react to retinal detachment. Exp Eye Res 2003; 76:333-42.
31. Lewis GP, Erickson PA, Guerin CJ, Anderson DH, Fisher SK. Changes in the expression of specific Muller cell proteins during long-term retinal detachment. Exp Eye Res 1989; 49:93-111.
32. Fisher SK, Erickson PA, Lewis GP, Anderson DH. Intraretinal proliferation induced by retinal detachment. Invest Ophthalmol Vis Sci 1991; 32:1739-48.
33. Geller SF, Lewis GP, Anderson DH, Fisher SK. Use of the MIB-1 antibody for detecting proliferating cells in the retina. Invest Ophthalmol Vis Sci 1995; 36:737-44.
34. Cook B, Lewis GP, Fisher SK, Adler R. Apoptotic photoreceptor degeneration in experimental retinal detachment. Invest Ophthalmol Vis Sci 1995; 36:990-6.
35. Lewis GP, Linberg KA, Geller SF, Guerin CJ, Fisher SK. Effects of the neurotrophin brain-derived neurotrophic factor in an experimental model of retinal detachment. Invest Ophthalmol Vis Sci 1999; 40:1530-44.
36. Lewis GP, Sethi CS, Linberg KA, Charteris DG, Fisher SK. Experimental retinal reattachment: a new perspective. Mol Neurobiol 2003; 28:159-75.
37. Linberg KA, Sakai T, Lewis GP, Fisher SK. Experimental retinal detachment in the cone-dominant ground squirrel retina: morphology and basic immunocytochemistry. Vis Neurosci 2002; 19:603-19.
38. Garcia-Valenzuela E, Sharma SC, Pina AL. Multilayered retinal microglial response to optic nerve transection in rats. Mol Vis 2005; 11:225-31.

The print version of this article was created on 28 Jul 2005. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.