Changes in the Organization and Expression of Cytoskeletal Proteins During Retinal Degeneration Induced by Retinal Detachment

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Purpose. The goal of this study was to determine the changes in the organization of the retinal cytoskeleton after experimental retinal detachment.

Methods. Cat retinas were detached from the retinal pigment epithelium and then processed for Western blot analysis and fluorescence microscopy. Proteins examined included glial fibrillary acidic protein (GFAP), vimentin, tubulin, and actin. Sections were viewed using a laser scanning confocal microscope.

Results. GFAP and vimentin: At 1 day after detachment, there was an aggregation of intermediate filaments in the endfoot of Müller cells. At 3 days, intermediate filament containing Müller cell processes could be detected within the subretinal space, and, at 28 days, these processes formed large glial scars in the subretinal space. β-tubulin: At 3 days after detachment, an increase in immunolabeling could be detected within the Müller cell endfoot and in Müller cell processes within the subretinal space. Actin: At 3 days after detachment, rhodamine–phalloidin staining decreased in the inner segments, the photoreceptor synaptic terminals, and the outer limiting membrane.

Conclusions. The decrease in labeling of the photoreceptor inner segment and synaptic terminal cytoskeleton may be a key indicator of early changes in photoreceptors after detachment. The increase in cytoskeletal proteins GFAP, vimentin, and tubulin within the retinal Müller cells after detachment may help to stabilize this cell type as it hypertrophies during glial scar formation. Inhibition of this response may aid in the treatment of diseases in which Müller cell hypertrophy plays a role. Invest Ophthalmol Vis Sci. 1995;36:2404–2416.

Separation of the neural retina from the retinal pigment epithelium (RPE) initiates a number of cellular and biochemical changes within the retina.[1,2] These events can be placed in four main categories: photoreceptor cell degeneration, nonneuronal cell proliferation, Müller cell hypertrophy, and changes in the levels of expression of various proteins. Within 1 day of retinal detachment, the photoreceptor outer segments begin to degenerate. Inner segments also shorten and show disruption of their characteristic compartmentalization of organelles. By day 3, photoreceptor synaptic terminals retract into the outer nuclear layer (ONL),[3] and some of the photoreceptor cell bodies become apoptotic.[4] Although the photoreceptor appears to be able to synthesize at least two of its major proteins used to construct outer segment discs, opsin, and peripherin–rds, even after long-term detachments,[5,6] the actual ability to build and organize an outer segment is never regained unless the retina is surgically reattached.

Concomitant with the structural disruption and, in some cases, the death of photoreceptors is the induction of proliferation of all nonneuronal retinal cell types. Within 2 days of detachment, Müller cells, astrocytes, vascular endothelial cells, RPE cells, and the microglial cells all begin to divide.[7,8] Müller cell nuclei frequently are translocated to the ONL or subretinal space, a process that also begins within the

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Supported by U.S. Public Health Service research grants EY-00888 (SKF) and EY-07191 (BM).
Submitted for publication June 2, 1995; revised August 7, 1995; accepted August 8, 1995.
Proprietary interest category: N.
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Investigative Ophthalmology & Visual Science, November 1995, Vol. 36, No. 12

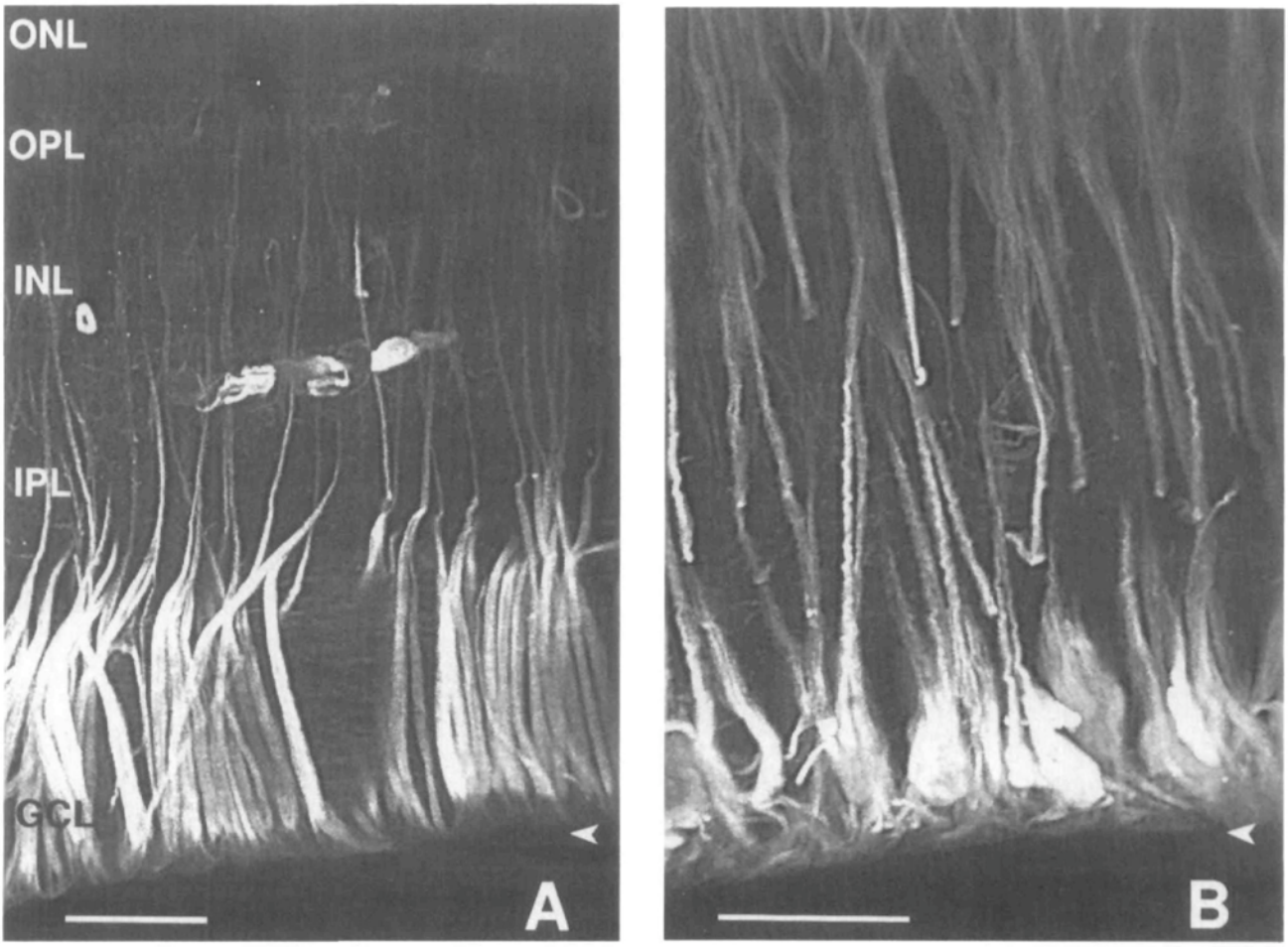

FIGURE 1. Anti-vimentin labeling. Normal retina and 1-day detached retina. (**A**) Normal retina. The heaviest labeling occurs in the Müller cell endfoot region and extends from the inner limiting membrane (ILM; *arrowhead*), through the ganglion cell layer (GCL), just reaching the outer plexiform layer (OPL). (**B**) One-day retinal detachment. The intermediate filament proteins appear to aggregate within the endfoot region, and finer filaments radiate toward the ILM (*arrowhead*) from this aggregate. Bundles of filaments within the Müller cell cytoplasm appear thickened within the inner nuclear layer (INL) and inner plexiform layer (IPL) and now extend into the outer nuclear layer (ONL). Anti-glial fibrillary acidic protein labeling appears identical to anti-vimentin labeling (not shown). **A** is a projection of 21 images; **B** is a projection of 20 images. Bar = 25 μm (**A**,**B**).

first 2 or 3 days of detachment. Although the proliferative response peaks between 3 and 4 days of detachment and subsequently declines to baseline levels after a few weeks, there is also the hypertrophy of Müller cells that continues for months after detachment. As a result, Müller cell processes expand within the retinal layers and often extend into the subretinal space, where they can interfere with the regeneration of the outer segments after successful anatomic reattachment of the retina.[3,9,10]

Because many of the changes that occur after detachment involve changes in cell shape, size, position, or intracellular organization, it is likely that the cytoskeleton of the affected retinal cell types plays a key role in their responses to retinal detachment. It has been demonstrated that the intermediate filament proteins, glial fibrillary acidic acid protein (GFAP) and vimentin, are concentrated in the Müller cell endfoot region in normal feline retinas. However, after detachment or other injury to the retina, upregulation of these proteins occurs and intermediate filaments eventually fill the cell's entire cytoplasm.[11-17] Microfilaments and microtubules, on the other hand, are known to be present in all retinal cell types. The precise cellular location of these proteins suggests that they may be involved in the response of the retina to injury. For example, filamentous (F)-actin has been localized to the adherens junctions between the

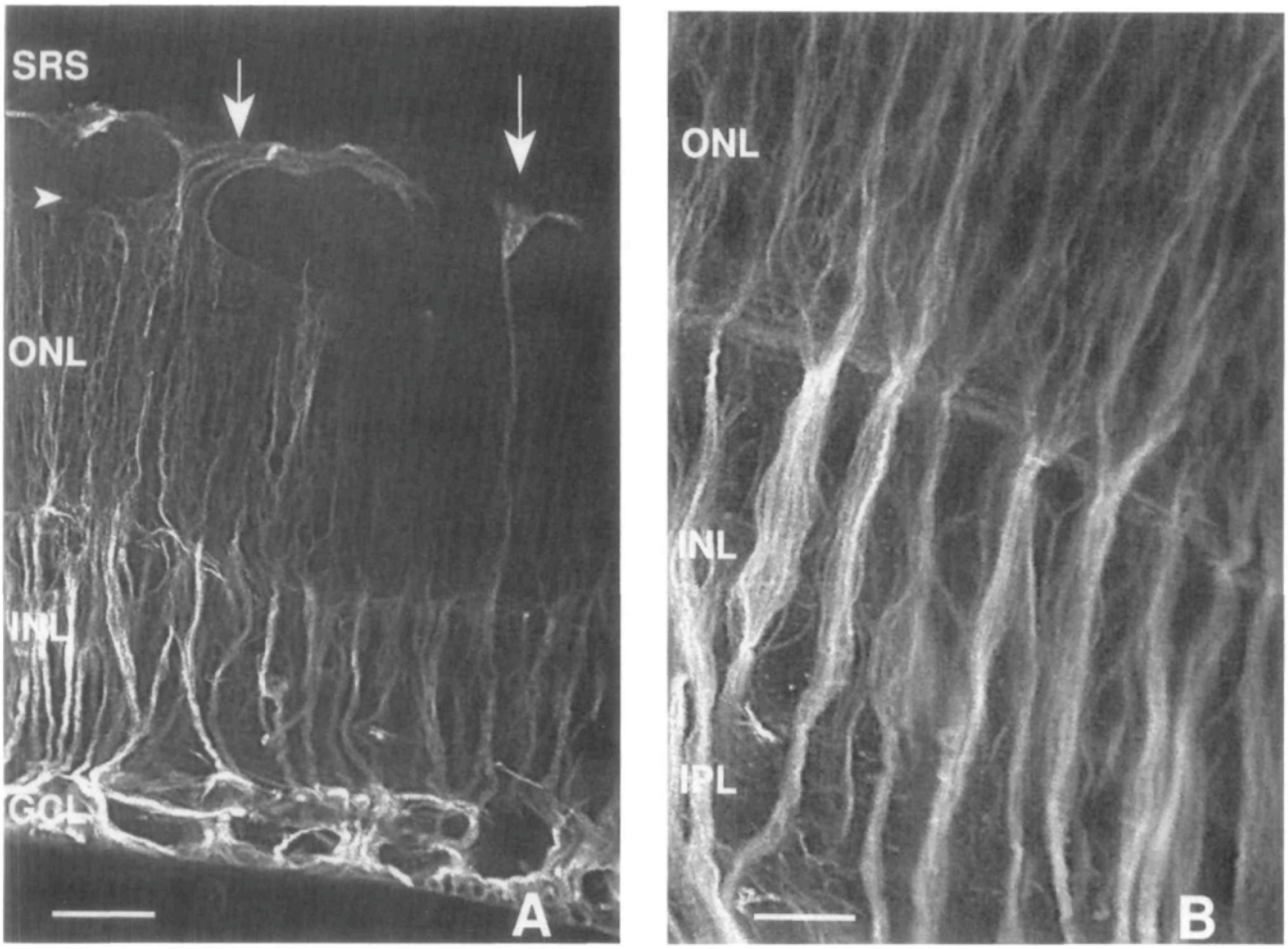

FIGURE 2. Anti-vimentin labeling, 3-day detached retina. (**A**) Labeled intermediate filaments are present within the Müller cell cytoplasm across the full width of the retina, and they extend into the subretinal space (SRS; *arrows*) onto the surface of the photoreceptors. (**B**) At higher magnification, the labeling within the Müller cells appears as numerous cables that run through the cytoplasm. This is especially apparent within the expanded cytoplasm of the cell body in the inner nuclear layer (INL). IPL = inner plexiform layer; outer limiting membrane, *arrowhead*. **A** is a projection of 26 images; **B** is a projection of 14 images. Bar = 25 μm (**A**); bar = 10 μm (**B**).

Müller cells and photoreceptors (i.e., at the outer limiting membrane [OLM]), to the site of disc morphogenesis in photoreceptor outer segments, to precisely organized cables in the photoreceptor inner segments, to RPE apical processes, and to synaptic terminals in the plexiform layers.[18–26] After retinal detachment, all these structures undergo changes. The outer limiting membrane becomes disrupted; the photoreceptor outer segments degenerate; the subcellular compartmentalization within the inner segments becomes disorganized; the photoreceptor synaptic terminals retract; the RPE cells lose their specialized apical processes, proliferate, and migrate from the monolayer; and the Müller cells can proliferate, migrate, hypertrophy, and shift the location of their nuclei.[2]

Determining the fine cytoskeletal changes that occur in the retina after detachment may lead to an understanding, and possible prevention, of the steps leading to photoreceptor degeneration and cellular proliferation, as well as lead to a better understanding of the function of these prominent proteins in retinal pathology. Because the structural changes observed in the retina after detachment resemble changes documented in other retinal disorders,[27–32] the changes described here are likely to be characteristic of retinal degenerations in general. In this study, we have taken advantage of the capabilities of the laser scanning confocal microscope to study at high-resolution changes in the cytoskeleton. Because we also are able to collect high-resolution information from thick sections and from various depths in the section, this technique provides superior information to that collected from ei-

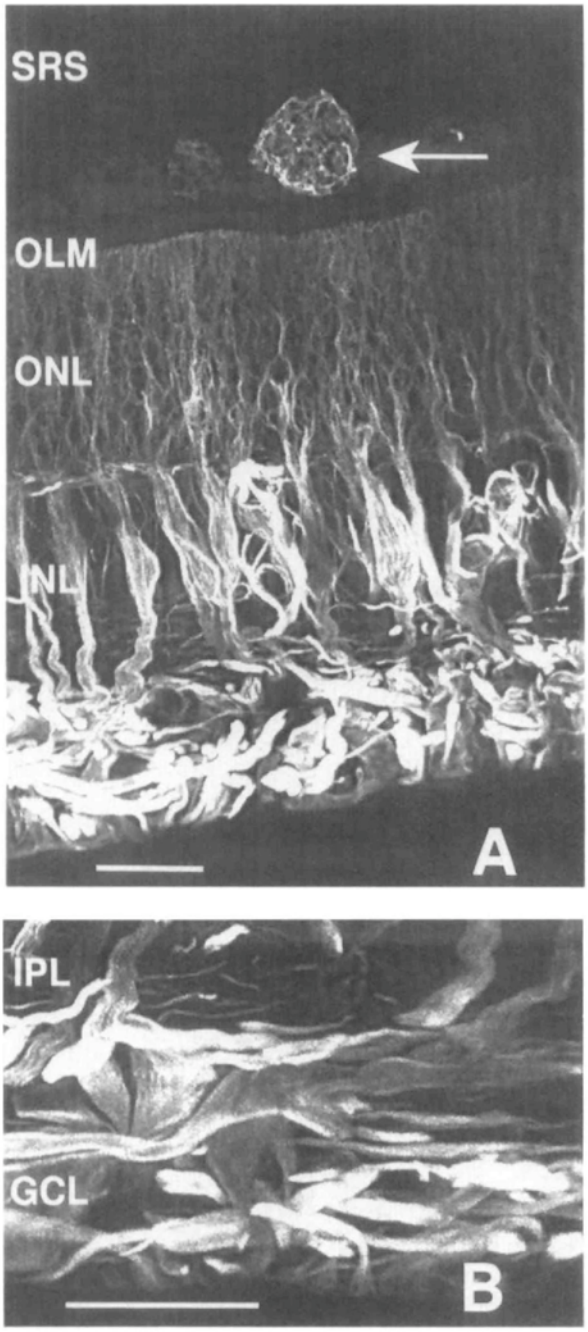

FIGURE 3. Anti-vimentin labeling, 28-day detached retina. (**A**) Large bundles of labeled intermediate filaments are present within the cytoplasm of the greatly hypertrophied Müller cells extending throughout the retina and into the subretinal space (SRS; *arrow*). (**B**) Higher magnification of the ganglion cell layer (GCL) and the inner plexiform layer (IPL) showing bundles of labeled filaments running laterally within the retinal layers. (INL = inner nuclear layer; ONL = outer nuclear layer; OLM = outer limiting membrane.) **A** is a projection of 16 images; **B** is a projection of 18 images. Bar = 25 μm (**A**,**B**).

ther traditional immunofluorescence or electron microscopy.

METHODS

Retinal Detachments

Cat retinas (n = 2 for each time point) were detached experimentally from the retinal pigment epithelium for 1, 3, 7, 14, or 28 days as described previously.[10] Briefly, the lens was removed and the eye was allowed to heal for at least 2 weeks, at which time the vitreous was removed and a solution of 0.25% Healon (sodium hyluronate; Pharmacia, Piscataway, NJ) in a balanced salt solution (Alcon, Fort Worth, TX) was infused between the neural retina and the RPE using a micropipette. All procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Confocal Microscopy

The technique of pre-embedding immunocytochemistry was used in conjunction with imaging by the laser scanning confocal microscope, as described previously.[33,34] Briefly, retinas were cut into quadrants and fixed in 4% paraformaldehyde in sodium cacodylate buffer (0.1 N; pH 7.4). Tissue approximately 2 mm square was excised and embedded in 5% agarose (Sigma, St Louis, MO) in phosphate-buffered saline (PBS). Sections 100 μm thick were cut on a vibratome (Technical Products International, Polysciences, Warrington, PA) and incubated in normal donkey serum (1:20) overnight at 4°C on a rotator. The next day, the blocking serum was removed and the primary antibodies were added: those against GFAP and vimentin (DAKO, Carpenteria, CA) were used at 1:500, and that against β-tubulin (gift from Dr. Michael Klymkowsky, University of Colorado) was used at 1:200. All primary antibodies were immunoglobulin G (IgG) fractions. Some sections were treated with either IgG or whole serum (both 20 μg/ml) in place of the primary antibody as negative control. After rotating overnight at 4°C, the sections were rinsed in PBS–bovine serum albumin (BSA) and incubated in either donkey anti-rabbit (GFAP) or donkey anti-mouse IgG (vimentin and tubulin) conjugated to the fluorochrome Cy3 (1:200; Jackson ImmunoResearch Laboratories, West Grove, PA) overnight at 4°C on a rotator. The sections were rinsed, mounted in 5% n-propyl gallate in glycerol, and viewed on the laser scanning confocal microscope (BioRad 500 MRC; BioRad, Hercules, CA). All the antibodies were diluted in PBS containing 0.5% BSA, 0.1% triton X-100, and 0.1% sodium azide. For the actin localization study, rhodamine–phalloidin (Molecular Probes, Eugene, OR), diluted 1:10 in PBS

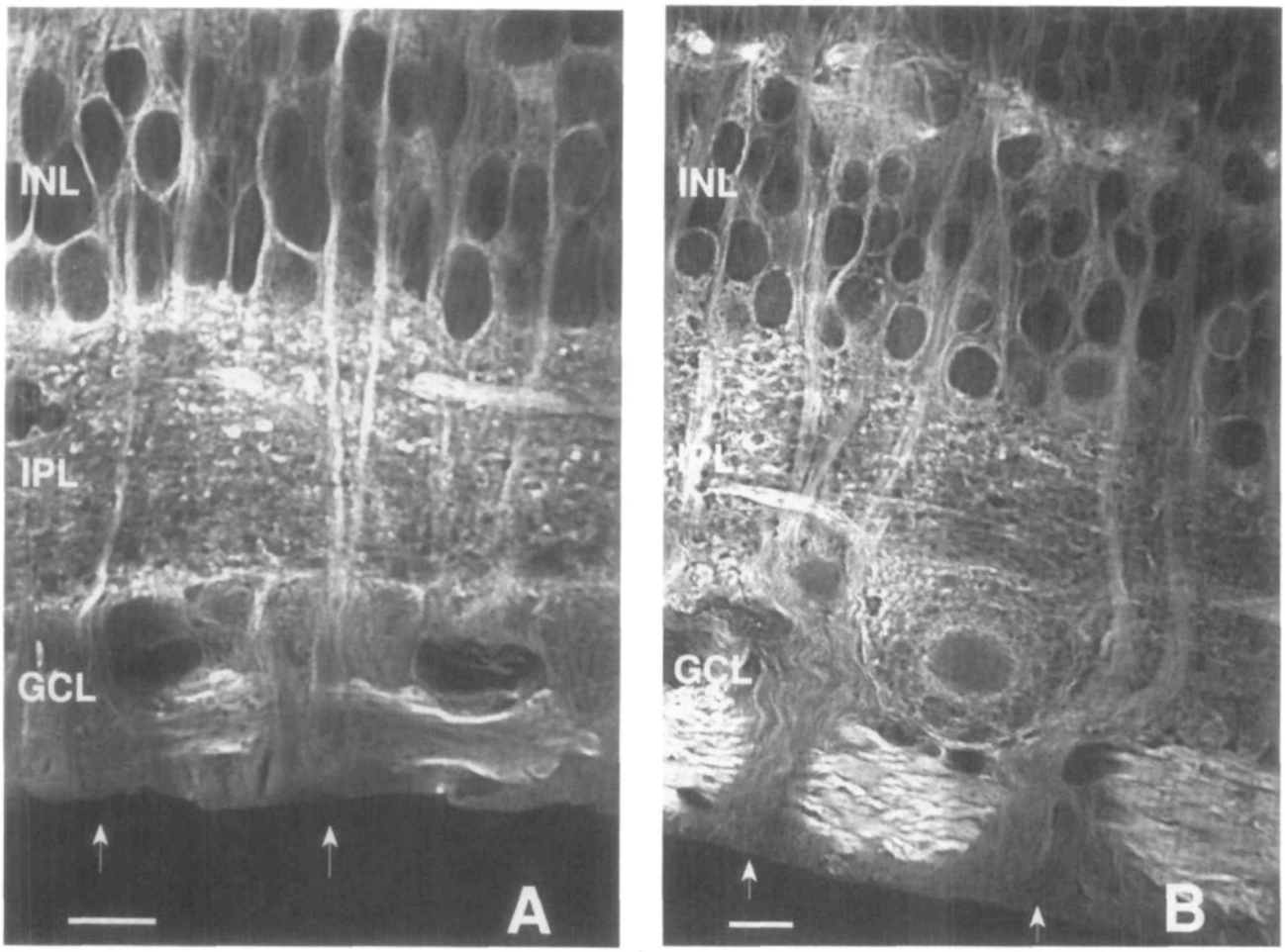

FIGURE 4. Anti-β-tubulin labeling. Inner retina, normal retina, and 3- and 28-day detached retinas. (**A**) Normal retina. Labeling is present within the cell bodies, neuronal processes within the inner plexiform layer (IPL), and ganglion cell axons. The Müller cell labeling is heaviest within the IPL and inner nuclear layer (INL), with only fine labeled filamentous structures present in the endfoot region (*arrows*). (**B**) 3-day retinal detachment. Müller cell processes appear thickened, and there is more labeling extending into the endfoot (*arrow*).

containing 0.1% Triton X-100 and 0.1% sodium azide, was added overnight at 4°C. The sections were viewed on a BioRad 500 MRC. In some cases, multiple planes of focus were collected (i.e. a z-series) and then projected as a two-dimensional image. Each image plane represents 1 μm. The number of images projected is included in the figure captions.

Western Blot Analysis of β-Tubulin

Retinas for sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western blot analysis were homogenized at 4°C in 0.02M PBS containing 0.1 mM phenylmethylsulfonylfluoride, 10 mM EDTA, 0.5% Triton X-100, and 2.55% SDS. Protein concentrations were determined by the BCA Protein Assay (Pierce Chemical, Rockford, IL). Fifty micrograms of protein were loaded in each lane of a 11% to 23% gradient gel (Phorcast; Amersham, Arlington Heights, IL). The separated retinal proteins were transferred to nitrocellulose paper, blocked in 3% BSA in Tris-buffered saline (TBS, pH 7.5) and incubated overnight in primary antibody. The same antibody to β-tubulin referred to above was used at a 1:400 dilution in 1% BSA in TBS. Blots were washed in TBS containing 0.5% Tween (TTBS) and incubated with goat anti-rabbit or goat anti-mouse IgG conjugated to HRP (1:1000; BioRad) for 1 hour. After washing in TTBS and TBS, the blots were placed in HRP Color Development Reagent (BioRad) for 15 minutes, rinsed in distilled water, and air dried.

RESULTS

Intermediate Filaments

In normal retinas, immunocytochemical labeling with anti-GFAP and anti-vimentin occurs predominantly in

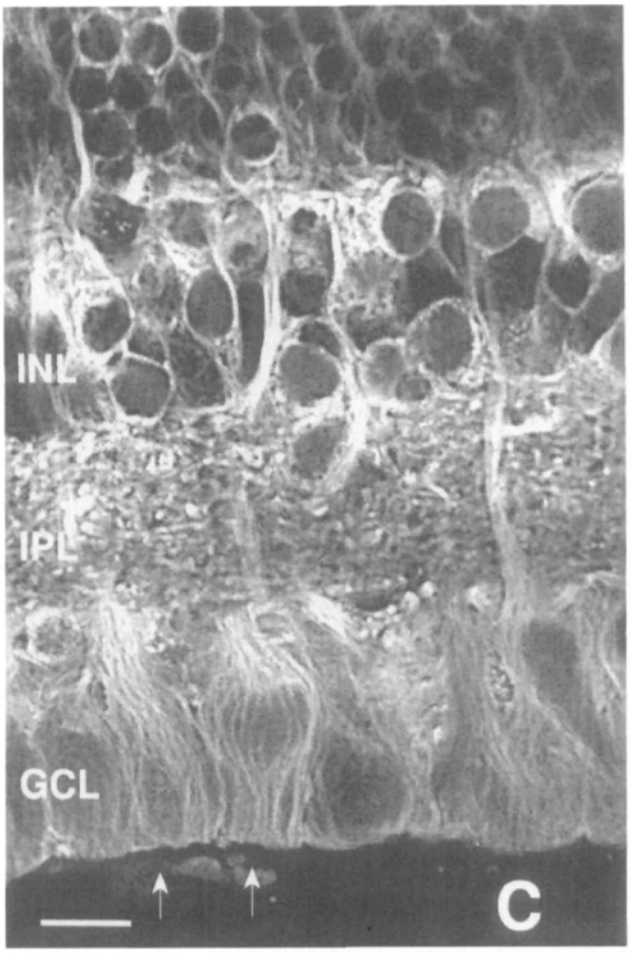

FIGURE 4. (*Continued*) (**C**) 28-day retinal detachment. Labeled microtubules fill the Müller cell endfoot. (Note: The region of the retina as well as the plane of section accounts for the differences in labeling of ganglion cell axons.) GCL = ganglion cell layer. Figures **A**, **B**, and **C** are single images. Bar = 10 μm (**A**,**B**,**C**).

the Müller cell endfoot region (Fig. 1A; vimentin). By confocal microscopy, this heavy labeling within the endfoot appears as bands of filaments that extend from the inner limiting membrane, through the ganglion cell layer, and taper into fine filaments that often reach as high as the outer plexiform layer (OPL). Because the labeling patterns of anti-GFAP and anti-vimentin are identical in the normal and detached retinas, their distribution within Müller cells will be referred to collectively. No labeling was observed in either the IgG or whole serum controls (data not shown).

One day after retinal detachment, the intermediate filaments within the endfoot cytoplasm formed a tight aggregate of filaments from which finer filamentous structures radiated toward the inner limiting membrane or into the inner retina (Fig. 1B). In addition, the bundles of labeled filaments within the inner plexiform layer (IPL) already appeared thickened at this time. By 3 days after detachment, labeled intermediate filaments were present within the Müller cell cytoplasm across the full width of the retina, often extending past the OLM into the subretinal space where they sometimes extended laterally on the surface of the photoreceptors for several hundred microns (Fig. 2A). Not only did the filaments extend the length of the entire Müller cell, the bundles of filaments within the Müller cell processes in the inner retina appeared greatly thickened. These bundles appeared as multiple arrays that ran through the Müller cell body in the inner nuclear layer, then converged at the level of the OPL only to spread out as fine bundles that extend through Müller cell processes in the ONL (Fig. 2B). As can be seen in Figure 2A, labeled filaments now often extended laterally, especially at the IPL–ganglion cell interface. This is in striking contrast to the completely "vertical" arrays that occur in normal retina labeled with the antibody to vimentin (Fig. 1A). This "vertical" and "lateral" increase in labeling of the intermediate filaments within the Müller cell's cytoplasm continued at 7 days, and by 28 days the labeling often appeared as masses of thick bundles of filaments projecting across the subretinal surface of the retina and always laterally within the retinal layers (Figs. 3A, 3B).

Microtubules

Anti-β-tubulin labeling in the normal retina was present in all retinal layers with distinctive labeling of cell bodies, the photoreceptor inner segments, the rod and cone synaptic terminals in the OPL, the neuronal processes within IPL, and the ganglion cell axons (Figs. 4A, 5A, 6A). The heaviest labeling within the Müller cell cytoplasm was located at the level of the IPL and INL with only very fine labeled filamentous structures present in the endfoot region. The filamentous labeling pattern produced by this antibody undoubtedly represented labeling of groups of microtubules, whereas the more diffuse labeling most likely represents monomers of tubulin. By 3 days after detachment, there was an overall increase in labeling of the Müller cells with greater numbers of filamentous structures extending sclerad across the inner retina and into the endfoot region (Fig. 4B). In addition, heavily labeled Müller cell processes were observed extending through the ONL and projecting into the subretinal space (Fig. 5B). At 28 days after detachment, while most of the cell types show little if any change from the labeling pattern exhibited at 3 days, the Müller cell labeling continued to increase both in the endfoot region and in processes extending across the retina (Figs. 4C, 5C). The inner segments continued to be labeled in long-term detachments though

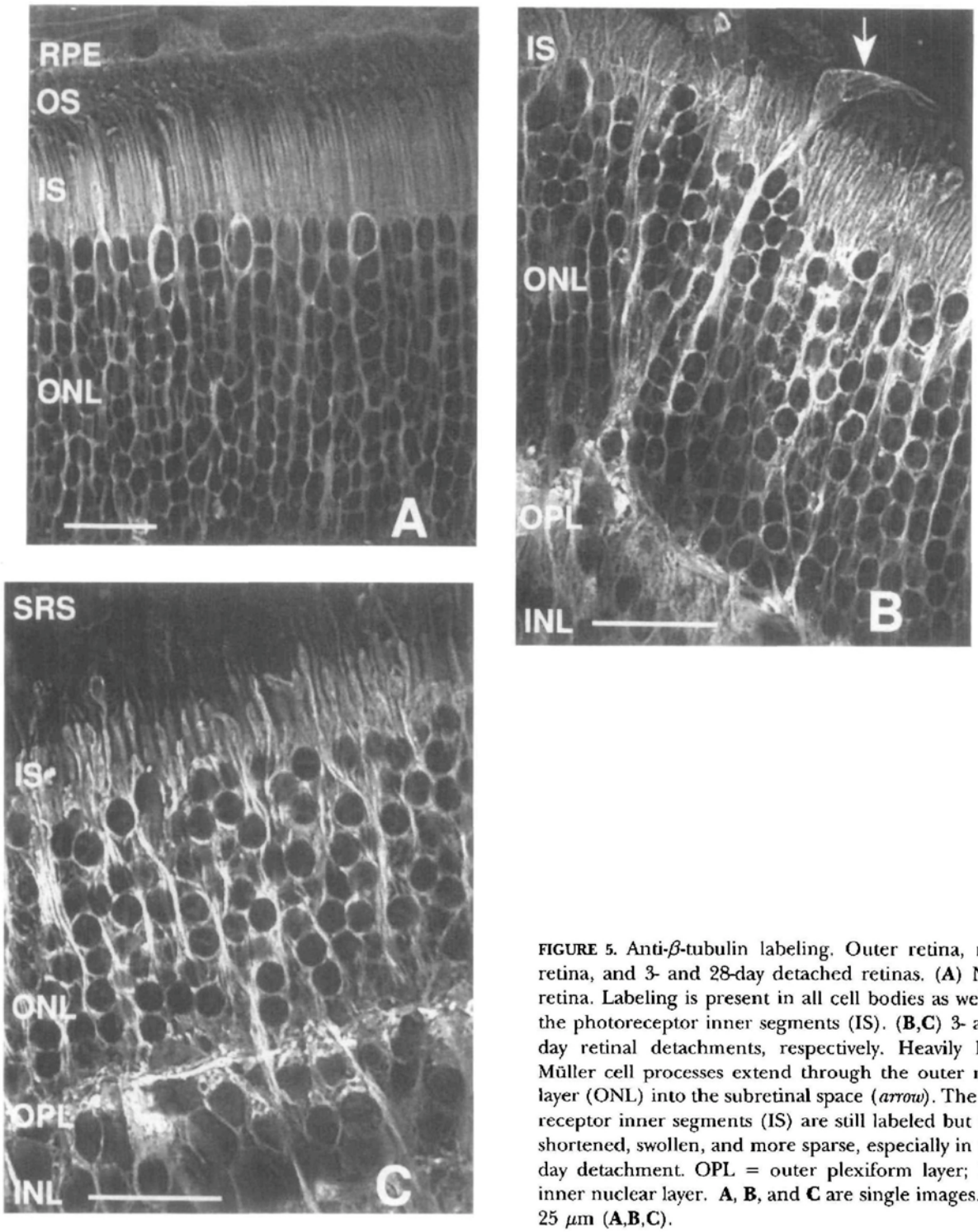

FIGURE 5. Anti-β-tubulin labeling. Outer retina, normal retina, and 3- and 28-day detached retinas. (**A**) Normal retina. Labeling is present in all cell bodies as well as in the photoreceptor inner segments (IS). (**B,C**) 3- and 28-day retinal detachments, respectively. Heavily labeled Müller cell processes extend through the outer nuclear layer (ONL) into the subretinal space (*arrow*). The photoreceptor inner segments (IS) are still labeled but appear shortened, swollen, and more sparse, especially in the 28-day detachment. OPL = outer plexiform layer; INL = inner nuclear layer. **A**, **B**, and **C** are single images. Bar = 25 μm (**A,B,C**).

even at 3 days they appeared greatly shortened and swollen (Fig. 5B). Perhaps the most striking change was not associated with a specific change in antibody labeling but with the overall decrease in the packing of the inner segment, which the antibody labeling allowed us to visualize clearly. In general, the labeling pattern within the OPL assumed a much less organized appearance at 3 days after detachment as the photoreceptor terminals retracted into the ONL (Fig. 6B). At 28 days after detachment, the distinct labeling of photoreceptor synaptic terminals was unrecognizable within the OPL (Fig. 6C).

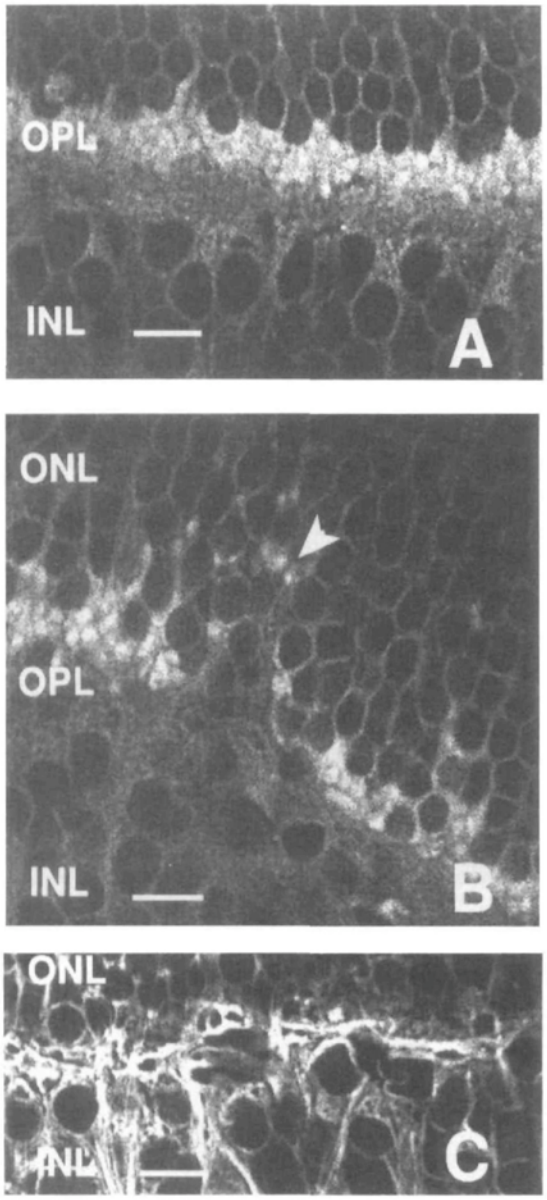

FIGURE 6. Anti-β-tubulin labeling. Outer plexiform layer, normal retina, and 3- and 28-day detached retinas. (**A**) Normal retina. The photoreceptor synaptic terminal labeling in the outer plexiform layer (OPL) appears well organized. (**B**) 3-day retinal detachment. The OPL appears less regular, and there is a retraction of the photoreceptor terminals into the outer nuclear layer (ONL; *arrowhead*). (**C**) 28-day retinal detachment. The OPL is highly disorganized, synaptic terminal labeling is no longer recognizable, and the OPL is filled with anti-tubulin labeled processes. INL = inner nuclear layer. **A**, **B**, and **C** are single images. Bar = 10 μm (**A**,**B**,**C**).

Western blot analysis with this antibody showed a single band at 50 kDa, corresponding to the approximate molecular weight for β-tubulin (Fig. 7). After detachment, the intensity of the band decreased although the immunocytochemical data suggest a significant increase in labeling of Müller cells. The decrease in the intensity of the bands is most likely caused by the degeneration, and subsequent loss, of the photoreceptors.

Actin

Although some level of rhodamine–phalloidin staining occurred in all layers of the normal retinas, the most distinctive staining was present in the inner segments, the OLM, the photoreceptor synaptic terminals in the OPL, the terminals within the IPL, and in the retinal vessels (Figs. 8A, 8C). At 3 days after detachment, there was a significant decrease in labeling of the photoreceptor inner segments (Fig. 8B) even though the inner segments still were detected easily by labeling with anti-tubulin. In addition, the labeling intensity within the OPL appeared to decrease dramatically and to become "disorganized" by comparison to normal retina (Fig. 8D). Although the OLM continued to be labeled to some degree at all detachment times examined, focal areas of disruption were observed in which labeling of the OLM appeared to be displaced sclerad over the distance equivalent to the diameter of several photoreceptor cell bodies (Fig. 8B).

DISCUSSION

The retina is a highly organized, stratified tissue. After retinal detachment, some of this organization rapidly

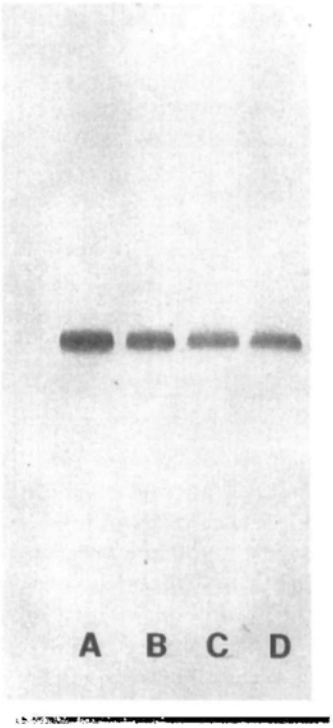

FIGURE 7. Western blot of β-tubulin from normal (**A**), 3-day (**B**), 7-day (**C**), and 28-day (**D**) detached retinas showing a slight decrease in intensity of a 50-kDa band over time.

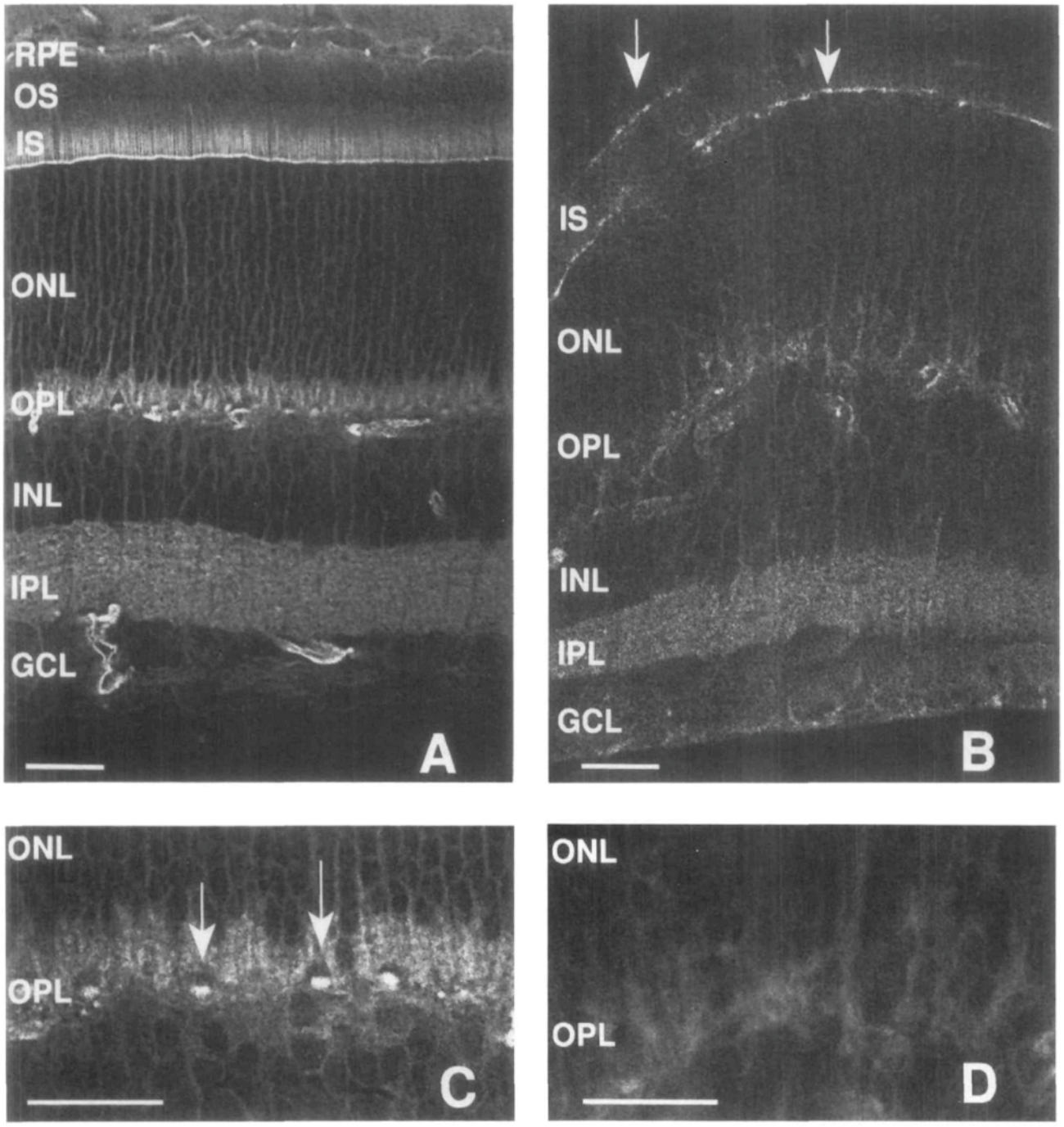

FIGURE 8. Rhodamine–phalloidin staining. Normal and 3-day detached retinas. (**A**) Normal retina. Staining occurs prominently in the photoreceptor inner segments (IS), the outer limiting membrane (OLM), the photoreceptor synaptic terminals in the outer plexiform layer (OPL), the terminals within the inner plexiform layer (IPL), and the retinal vessels. (**B**) 3-day retinal detachment. There is a decrease in staining intensity of the inner segments and the photoreceptor synaptic terminals within the OPL. The outer limiting membrane (OLM; *arrows*) is labeled but is disrupted and displaced sclerad in places. (**C**) Higher magnification of the OPL from a normal retina. Labeling is present in the photoreceptor terminals, with the cone terminals especially prominent (*arrows*). (**D**) Higher magnification of the OPL from a 3-day detached retina. No discrete labeling of the terminals is present, and the OPL is highly disorganized. GCL = ganglion cell layer; INL = inner nuclear layer; OS = outer segments; RPE = retinal pigment epithelium. **A**, **B**, **C**, and **D** are single images. Bar = 25 μm (**A**,**B**,**C**,**D**).

deteriorates as photoreceptor cells die and other cell types proliferate, hypertrophy, and migrate. Although the basis for the loss of vision always has been considered to be the degeneration of photoreceptor outer segments, understanding the cellular and biochemical changes that accompany the dramatic changes in retinal organization may well be necessary if we are to optimize recovery after injury. The results from this study demonstrate that the cytoskeleton in many retinal cells becomes highly reactive in response to injury, suggesting that many of the morphologic changes observed after detachment may be attributable to changes in the cytoskeleton of individual cell types.

One of the first morphologic changes that occurs after detachment is the rapid degeneration of photoreceptor outer segments.[9,35-38] Within 1 day, outer segments become disorganized and truncated. Within 3 days, most outer segments are a fraction of their normal length. Concomitant with this degeneration is the apparent decrease in filamentous actin within the inner segment even though the inner segment itself appears to be more or less preserved in comparison to the outer segment. Because F-actin bundles normally extend the length of the inner segments, a loss of actin filaments from this region could contribute to the destabilization of photoreceptor structure, including degeneration of the outer segment. Indeed, one of the effects of detachment is to cause a breakdown in the normal compartmentalization of organelles within the inner segment so that those associated with the ellipsoid and myoid regions now intermingle.[3] In contrast, the tubulin labeling present in the inner segments is not obviously altered after detachment; it continues to occur at what appears to be approximately the same level as in normal retina, as long as there is an intact inner segment. We do not know if the actual organization of the tubulin cytoskeleton itself is preserved because cat photoreceptors are simply too small to allow us to make this determination. Perhaps actin filaments play a more significant role than microtubules in stabilizing the compartmentalization of organelles in the photoreceptor inner segment.

Filamentous actin at the base of the outer segment is essential for the formation of new disc membranes.[39] Although we could not detect the small concentration of F-actin at the base of the outer segment in the cat retina using indirect immunofluorescence because of the narrow diameter of the outer segments, a dramatic decrease in F-actin expression, as suggested by phalloidin staining, indicates a potential effect on this critical accumulation of F-actin with a subsequent effect on the ability of the cell to assemble properly the new discs. We also examined the levels of actin expression in the detached retina using Western blot analysis, but the results (data not shown) were not terribly informative and showed shifts in the overall actin content that did not correlate with the pattern of phalloidin labeling.

Our data with both rhodamine–phalloidin and the β-tubulin antibody showed profound changes in the organization of the photoreceptor synaptic terminals within a few days of detachment. These cytoskeletal changes correlate well with our previous ultrastructural demonstration that photoreceptor synaptic terminals appear to "retract" toward their cell bodies and lose their distinctive synaptic invaginations.[3] If this signifies a loss of connections to second-order neurons, these changes may have even more significance for the return of vision after successful reattachment surgery than outer segment degeneration and photoreceptor cell loss. The loss of rod and cone cells, or the imperfect regeneration of outer segments,[10] may be compensated for by redundancy built into the visual pathway. It may be much more difficult to reestablish appropriate synaptic connections if they are disrupted in the adult retina. Indeed, if this occurs in human retinal detachments, it may explain why visual defects are reported in patients after successful reattachment surgery accompanied by a reasonable return of visual acuity.[40]

Subtle cytoskeletal changes within Müller cells also can be observed within 1 day of detachment. GFAP, vimentin, and tubulin, all major cytoskeletal components within the Müller cells, are present in different but overlapping subcellular locations. GFAP and vimentin are normally in highest concentration in the endfoot region of the cell, whereas tubulin is present in highest levels within the Müller cell processes that traverse the IPL and INL. Within 1 day of detachment, the intermediate filament proteins appear to condense in the endfoot region. The subsequent increase in these proteins, which ultimately fill the entire cell within a week after detachment, appears to originate from this mass of filaments in the endfoot. This is in concert with demonstrations that the mRNA for intermediate filament proteins is exported from the cell body vitread to the endfoot,[16,32,41] and it suggests that the intermediate filament cytoskeleton expands from an initial "growth region" that exists there. Conversely, although there is little anti-tubulin labeling in the endfoot region of the normal retina, microtubules begin to fill this area within 3 days of detachment. In this case, the microtubules appear to originate from the highly fluorescent cell body. Microtubules often extend out from a microtubule organizing center. Although we could not identify a specific organizing center in these sections, we frequently observed a bright spot of fluorescence in Müller cell bodies that may have represented this structure.

Within 1 week of detachment, the entire Müller cell cytoplasm is filled with intermediate filaments and microtubules. The function of the intermediate filaments is not known, but it has been suggested that they may play a similar role to that of the microtubules—that is, they may be involved in the remodeling and stabilization of cells as they change shape and as they transport molecules within the cell.[42-44] Indeed, intermediate filaments have been proposed to act as tracks for the transport of mRNA in and out of the nucleus.[16,43] Microtubules, on the other hand, have been definitively linked to intracellular transport processes using various "molecular motors."[45] The increase in microtubules extending into the endfoot and expanding processes of these Müller cells may reflect their need for increases in transport and trafficking of various molecular components as the cell undergoes significant remodeling.

The dramatic increase in the expression of cytoskeletal proteins, particularly those making up the intermediate filaments, is a characteristic response of astrocytes during glial scar formation after injury to the central nervous system.[46-49] Within the retina, an increase in these proteins represents an important structural response of the Müller cells to induced as well as inherited retinal degenerations.[11-17] In the case of retinal detachment, as the upregulation of intermediate filament and microtubule proteins occurs, Müller cell processes undergo dramatic three-dimensional changes. The whole cell can migrate or its processes can grow into the vitreous cavity, where they form membranes on the surface of the retina that can contract and detach the retina[50-54]; they can migrate into the newly created subretinal space where the scar tissue has been shown to prevent the regrowth of the photoreceptor outer segments[10]; and, in a somewhat less characterized response, Müller cell processes can hypertrophy within the retina, potentially blocking the reformation of synaptic connections, disrupting existing pathways, or even changing the optic properties of the retinal tissue.[3,9] The end result of this massive hypertrophy of the Müller cells is part of an overall disorganization of the retinal layers: Müller cell nuclei migrate from their original location in the INL and enter the OPL, the ONL, and sometimes even the subretinal space; photoreceptor nuclei appear in the subretinal space as the OLM becomes disrupted; and the OPL, once well defined by both the actin and tubulin labeling present within the photoreceptor synaptic terminal region, almost completely disappears. As a result, the cell bodies of the ONL and INL appear to lay directly adjacent to one another. It appears that disruption of the OLM is directly associated with areas of hypertrophy of Müller cells into the subretinal space. The loss of adherens junctions of the OLM may then result in movement of photoreceptor nuclei into the subretinal space, one of the mechanisms by which photoreceptors are lost after detachment. Although intermediate filaments classically have been thought to be the underlying subcellular change associated with glial hypertrophy,[47-55] this study shows an overall increase in tubulin and an increase in what are probably bundles of microtubules in Müller cells as they undergo hypertrophy. The eventual ability to inhibit this response by Müller cells may have profound effects on the potential for visual recovery by preventing subretinal gliosis, disruption of intraretinal organization, and loss of photoreceptor cells after breakdown of the OLM.

Knowing the changes that occur in specific cytoskeletal components within retinal cells may help in the understanding and potentially in the control of adverse changes that occur after detachment or other retinal injury. The results from this study begin to elucidate several of the steps in that process. Moreover, these studies indicate that very early intervention will be required to interrupt these processes because changes in the retinal cytoskeleton occur rapidly after injury.

Key Words

actin, confocal microscopy, cytoskeleton, glial fibrillary acidic protein (GFAP), Müller cell, photoreceptor, retinal detachment, tubulin, vimentin

Acknowledgments

The authors thank Dr. Christopher Guerin and Maura Jess for their technical assistance.

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