Chapter 115

Cellular Effects of Detachment and Reattachment on the Neural Retina and the Retinal Pigment Epithelium

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INTERFACE BETWEEN RETINA AND RETINAL PIGMENT EPITHELIUM

Early in development of the eye, the neural retina and the retinal pigment epithelium (RPE) become closely apposed and remain so throughout the normal life span of an individual. During very early development of the human retina (until sometime after the eighth week of development), the undifferentiated cells of the neural retina are connected to the differentiating cells of the pigment epithelium by both adhering and gap junctions.[1] In other cell types the latter allow a free transfer of small molecules (less than about 1.5 kDa) between cells.[2] The significance of coupling these two cell layers is not known, but as the neural retina begins to differentiate, the junctions disappear. Shortly thereafter, the apical surface of the RPE elaborates numerous villous and sheet-like processes that interdigitate with the photoreceptor outer segments in the adult eye, and the rod and cone outer segments grow into the extracellular region that becomes the interphotoreceptor space.

The mature RPE is a polarized monolayer of epithelial cells that rests on Bruch's membrane, between the choriocapillaris and the neural retina.[3] The relationship of the apical surface of the RPE to differentiated photoreceptors is anatomically complex. There are no actual cellular junctions between the two layers in the mature eye, but the two are adherent with the degree of adhesion varying among species. Photoreceptor outer segments are ensheathed by specialized arrays of RPE cell microvilli and microplicae that are organized differently for primate rods and cones.[4,5] In human and rhesus monkey retinas, the apical surface of the RPE extends villous-like processes toward the photoreceptor outer segments, where some of them expand into cytoplasmic sheets or lamellae that surround the outer segments.[4–6] For rods, these surround the distal portion of the outer segment with two or three partially overlapping layers. Foveal and extrafoveal cones are usually ensheathed with more layers of apical processes than are the rods, and the ensheathment usually extends farther along the outer segment, about two-thirds of the distance to its base. Typically cone outer segments do not reach the apical surface; for extrafoveal cones, the apical processes may traverse 10 to 20 μm before reaching the distal tip of the outer segment. The apical processes that will ensheath a single cone, however, group together in the supracone space, where they are easily recognizable. The anatomic arrangement of the apical processes surrounding cone outer segments is known as the *cone sheath*.[6] Outside the fovea they are so distinctive that they are easily recognized by light microscopy, as was first described by Walls[7] in 1934. The apical projections of the RPE participate in the phagocytosis of membranous disc packets periodically shed from the apical tips of the rod and cone outer segments.[8–12]

The extracellular space between the apical surface of the RPE, the photoreceptor cells, and the apical microvilli of Müller's glia defines a specific anatomic compartment known as the *interphotoreceptor*, or *subretinal, space*. In this context, we use the term *subretinal space* only when the retina is detached from the RPE. The interphotoreceptor space contains both aqueous soluble and aqueous insoluble components,[13–15] only a few of which have been characterized to date. The extracellular compartment surrounding cone outer segments, termed the *cone matrix sheath*, is biochemically distinct from the rest of the interphotoreceptor matrix;[16,17] additional, specialized domains may also be associated with rod outer segments[18] and with the apical surface of the RPE.[19] At the apical RPE border, tight junctions between adjacent cells constitute a complete diffusion barrier for virtually all matrix molecules in the interphotoreceptor space.[20] Along the retinal aspect of the interphotoreceptor space, however, adhering junctions between the photoreceptor inner segments and Müller cells (the outer limiting membrane) present a less restrictive barrier to diffusion.[21] The RPE mediates the transfer of ions and molecules between the choroidal capillaries and the neural retina. Because there are no cellular junctions between the RPE and photoreceptor layers, and because the extracellular space between adjacent RPE cells is sealed by tight junctions near the apical border, this transfer must take place across the plasma membranes and cytoplasm of the RPE cells and through the interphotoreceptor matrix.

Retinal detachment

Detachment of the retina from the RPE surface results not only in separation of the photoreceptor cell layer from the apical surface of the RPE but also in an expansion of the interphotoreceptor space (i.e. the subretinal space) and a concomitant change in the composition of the interphotoreceptor matrix.[14] In the detached state, extracellular material in the expanded subretinal space is usually referred to as subretinal fluid to distinguish it from normal interphotoreceptor matrix. Retinal detachment initiates a complex

series of cellular and molecular changes in both retinal and RPE cells.[22,23] It may impede the normal transfer of ions and metabolites back and forth between the retina and the RPE-choroid.[24] It may also liberate or activate regulatory molecules sequestered in the interphotoreceptor matrix.[25,26] The severity of the resulting degenerative changes is clearly related to detachment duration and, under many circumstances, has serious adverse consequences for vision in the affected eye. Prompt reapposition of the retina and RPE layers can result in at least partial restoration of vision, implying that some of these abnormal changes can be arrested or even reversed by reattachment.[27] This chapter reviews both the many changes that occur in retinal cells and the ensuing process of morphologic recovery, as revealed by studies of experimental retinal detachment and reattachment.

EXPERIMENTAL RETINAL DETACHMENTS

Animal models of detachment

Here we emphasize the results of the most recent studies using animal models of detachment and reattachment. These studies show that the responses of the RPE and neural retina to detachment fall into several general categories: (1) partial dedifferentiation of RPE cells with mounding of the apical surface and retraction of the apical processes; (2) proliferation and migration of RPE cells into the subretinal space; (3) degeneration of photoreceptor outer segments and synaptic terminals; (4) death of a variable population of photoreceptors; (5) structural remodeling of second- and third-order retinal neurons; (6) proliferation of all non-neuronal cell types within the retina[28]; and (7) Müller cell hypertrophy with eventual glial scar formation. These changes are accompanied by significant biochemical changes in the retina ranging from the increased expression of some molecules to the loss of expression of others.[29–31]

Our understanding of the cellular events that occur when the retina and pigment epithelium are separated or reattached derives principally from the study of experimental animal models. Because of the scarcity of human retinal tissue suitable for such studies, only recently has the histopathologic study of retinal detachments in humans been reported.[32,33] Experimental models of retinal detachment have been developed in a variety of mammalian species from rodents to primates and although they may differ in the method of detachment and in exact details of outcome, they have yielded similar results that constitute a relatively detailed profile of the changes that occur after detachment. Although fewer, experimental studies of reattachment have provided insight into the regenerative capacity of photoreceptor cells as well as the ability of reattachment to slow, stop, or reverse changes induced by detachment. Models of detachment and reattachment also provide opportunities to test the ability of various treatments to affect cellular outcome. Two of these (neurotrophins and hyperoxia) will be discussed.

Studying the cellular and molecular changes that occur after detachment and/or reattachment will eventually lead to a more precise understanding of the degenerative processes within the retina that lead to visual impairment and mechanisms underlying the serious complications of detachment, such as proliferative vitreoretinopathy. Ideally the characteristics of an experimental detachment should closely mimic those found in human patients while allowing for precise control over the extent of separation between the two layers (detachment height), the location of the detachment, its surface area, and the onset of detachment (or reattachment).

Because the retina and the RPE are tightly adherent, producing a detachment is not a simple process. Earlier work on experimental detachments was aimed at mimicking "rhegmatogenous detachment" (from the Greek *rhegma*, rupture or tear; and *genos*, origin) by creating a fairly large retinal tear and then repeatedly aspirating liquefied vitreous into the subretinal space.[34–39] Enlargement of the detachment away from the tear occurs spontaneously so that the time of onset of detachment at sites distant from the retinal tear cannot be defined precisely. Subsequent studies have used a somewhat different procedure, in which fluid is injected between the retina and the RPE through a glass micropipette inserted into the subretinal space.[40] This produces a bullous retinal detachment that can be either very small or expanded to encompass the whole retina. The major disadvantage of this technique is that, in most cases, the retina tends to flatten spontaneously unless a viscous substance such as sodium hyaluronate is used; a graphic illustration of the adhesive "force" between retina and RPE. Although there is no large retinal tear, recent evidence obtained by studying human detachment tissues indicate that the cellular changes are the same in the animal model and human rhegmatogenous detachment.[41] This technique does allow for precise control over the onset of detachment and provides the investigator with the capability to control its size and location.

Photoreceptor–RPE interface

The earliest structural effects of retinal detachment are seen at the interface of outer segments and the RPE. These changes include alterations in the RPE apical surface, proliferation of RPE cells, migration of cells into the subretinal space, degeneration of photoreceptor outer segments, and changes in photoreceptor outer segment renewal.

RPE apical surface

The long and elaborate sheet-like and villous processes that normally ensheath the outer segments are lost within a few hours of detachment and replaced by a "fringe" of short microvilli (Fig. 115-1).[22] During this same time, the overall surface morphology of these cells changes into a rounded contour, with cytoplasm protruding past the normal limits of the apical surface into the subretinal space[42] (Figs 115-1, 115-2). Experiments have shown that this "mounding" of the RPE begins within minutes of detachment in rabbit retinas.[23]

Proliferation of RPE cells

Both tritiated-thymidine labeling and antibodies that label cells synthesizing DNA show that the RPE begins to proliferate within 24 hours of a detachment[43] (Fig. 115-3).[28,44] This proliferative response transforms the RPE's uniform monolayer into a heterogeneous morphology in which strands of cells extend from

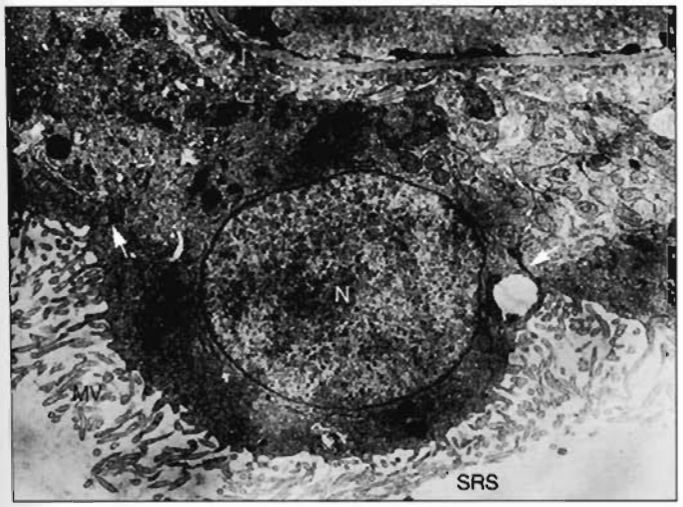

Fig. 115-1 An electron micrograph of the retinal pigment epithelium (RPE) 1 day after production of a retinal detachment. Compared with normal RPE cells, the apical surface is mounded. The sheet-like apical projections that normally ensheathe the outer segments have been replaced by a homogeneous fringe of short, microvillous processes (MV). In this particular cell, the nucleus (N) is displaced into the mounded region. The cell's lateral junctions are indicated by arrows (×6750). SRS, Subretinal space. (From Anderson DH, Stern WH, Fisher, SK et al. Invest Ophthalmol Vis Sci 1983; 24:909.)

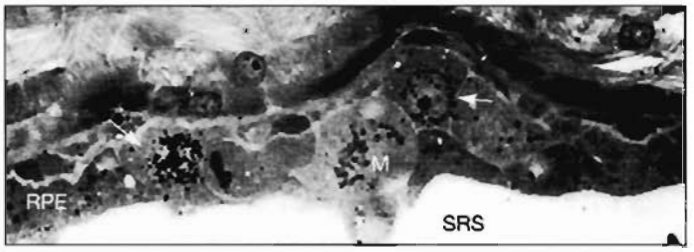

Fig. 115-3 Light microscopic autoradiogram of cat retinal pigment epithelium (RPE) 2 days after detachment. Tritiated thymidine was injected intravitreally 3 hours before fixation. Two labeled nuclei (arrows) and an adjacent mitotic figure (M) indicate that some RPE cells are proliferating at this stage (×850). SRS, Subretinal space. (From Anderson DH, Stern WH, Fisher SK et al. Invest Ophthalmol Vis Sci 1983; 24:911.)

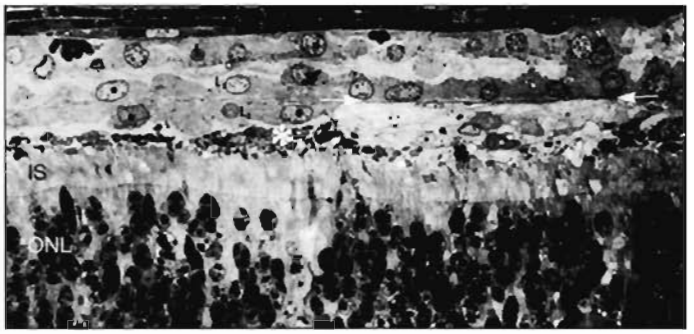

Fig. 115-4 A light micrograph of an area of RPE cell proliferation in a cat retina detached for 14 days and reattached for 30 days. Three monolayers of RPE cells are present (L_1, L_2, and L_3), each displaying different surface polarity. The apical surfaces of L_1 and L_2 face each other, as do the basal surfaces of L_2 and L_3. The basal lamina of L_2 is clearly evident (arrow). Only outer segment fragments (asterisk) appear near the inner segment (IS) tips (×800). ONL, Outer nuclear layer. (From Anderson DH, Guerin CJ, Erickson PA et al. Invest Ophthalmol Vis Sci 1986; 27:174.)

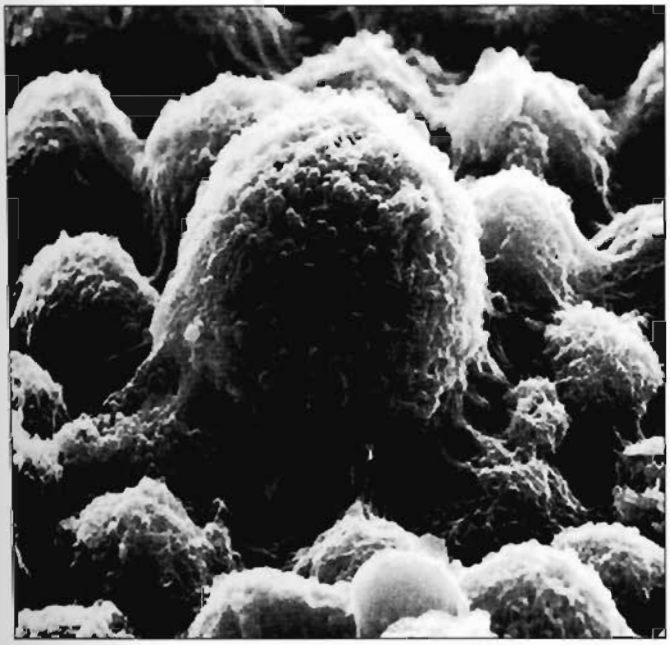

Fig. 115-2 Scanning electron micrograph of the apical surface of the retinal pigment epithelium 6 weeks after production of an experimental detachment, demonstrating the pronounced mounding response of the epithelial cells (×4800). (From Anderson DH, Stern WH et al. Invest Ophthalmol Vis Sci 1983; 24:910.)

the original monolayer into the subretinal space or result in the formation of multiple layers of cells whose polarity does not necessarily match that of the original monolayer (Fig. 115-4). This effect is limited to the region detachment; in attached regions the RPE remains mitotically quiet. These data strongly suggest that attachment of the RPE to the neural retina acts to keep the RPE mitotically inactive and its apical surface highly differentiated. It does appear that the proliferative response of the RPE is self-limiting. In experimental detachments of both owl monkey and cat retinas, proliferation (measured by tritiated thymidine incorporation) is at very low levels after long detachment intervals (e.g. 12 to 14 months).[28,42,45] However, the significance of a continued low level of proliferation over a long period of time is not known. The proliferation of the RPE cells in the subretinal space is probably the basis of pigmentary changes often observed during ophthalmoscopic examination of retinal detachments in humans. It is likely that the demarcation lines noted in human retinal detachments represent zones of proliferated RPE occurring at transitions between detached and attached regions of the eye. Current experimental evidence also suggests that such proliferation may be one of the factors adversely affecting regeneration of the photoreceptor outer segment after reattachment, especially if the polarity of the additional layers is reversed.[6,37]

Migration of cells into the subretinal space

In mammalian retinas the interphotoreceptor space is normally free of cells. Within 24 hours of an experimental detachment,

periodic acid–Schiff-positive cells of diverse morphology appear in the subretinal space. Monocytes are sometimes found between adjacent RPE cells, apparently in the process of migration from the choriocapillaris into the subretinal space. These cell types appear to mature into tissue macrophages within the subretinal space, where they phagocytose cellular debris (including membrane from degenerating outer segments).[42,48] RPE cells can also be found free within the subretinal space after a detachment where they appear to continue scavenging material from the degenerating photoreceptor outer segments. Photoreceptor cell bodies are sometimes extruded into the subretinal space after detachment, and Müller cells frequently migrate into this region where they form subretinal scars.[49] Large subretinal scars formed by Müller cells often have large numbers of photoreceptor cells distributed among the Müller cell processes, as if these photoreceptors were dragged along with the glial processes as they grew into the subretinal space.

Degeneration of photoreceptor outer segments

Like the RPE cells in the detached retina, the photoreceptor outer segments also project into the expanded subretinal space. Under experimental conditions, most outer segments show structural damage within 12 hours, although a few may maintain a relatively normal appearance. Mechanical damage seems likely to play some role in this initial degeneration although its contribution is not understood. The most common signs of cellular damage reported during the first few hours of detachment are a vacuolation or distortion of the distal end of the outer segment. Between 24 and 72 hours after detachment, essentially all rod and cone outer segments show evidence of degeneration. Besides being significantly shortened, they also appear distorted, with discs improperly oriented. The degeneration of outer segments may proceed until those in the zone of detachment appear only as empty sacs of membrane attached to the connecting cilium (Fig. 115-5). In other cases, severely truncated but recognizable outer segments can be present even after detachments of several weeks' duration.[50] During this same time, outer segment debris appears within the subretinal space. This is an abnormal process because in the normal eye the shedding of discs from the photoreceptor distal end is accompanied by immediate phagocytosis by the adjacent RPE cell.

Photoreceptor outer segment renewal

Rod and cone outer segments are renewed by a continual process of disc addition at the base and loss at their tips.[8,10–12,51,52] Thus, any process that interrupts either the production of new discs, their shedding at the distal end, or their subsequent phagocytosis by the RPE will affect the integrity of the outer segment. There is experimental evidence that when photoreceptors are detached from the RPE, disc shedding cannot be induced by treatments that stimulate massive shedding in attached retinas.[53] It also appears from ultrastructural studies that the process of disc morphogenesis must slow down considerably while the retina is detached.[47,54] In long-term detachments, often the ciliary stalks with a few rudimentary discs or membrane evaginations are all that remain. In other cases, up to 25% of the outer segment may remain, although its structure is always disorganized. These disc membranes are found long after membranes synthesized before detachment would be expected to persist, based on the turnover rates for outer segment membranes in normal retinas. Auto-

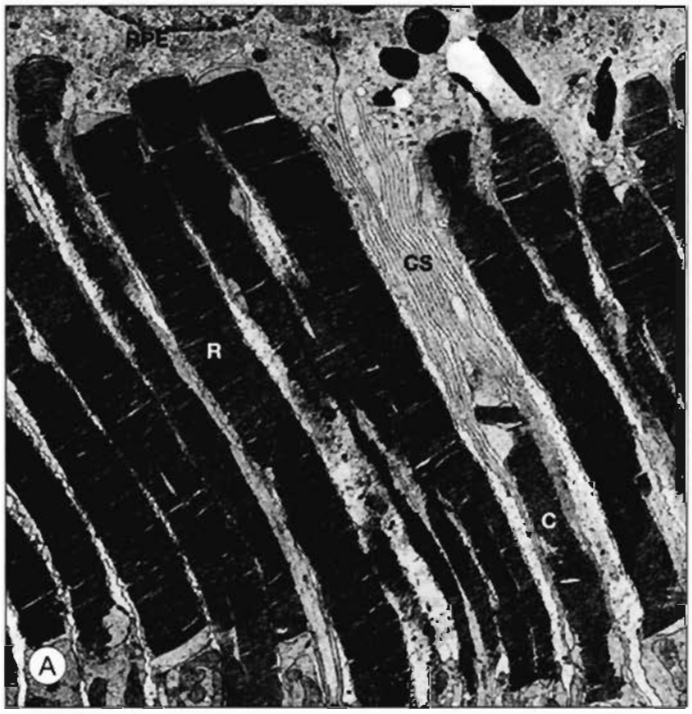

Fig. 115-5 A, An electron micrograph of the photoreceptors and retinal pigment epithelium in a normal cat retina. The outer segments are oriented along their longitudinal axes in a uniform, densely packed row. Cone outer segments (C) are shorter than those of rods (R) and are ensheathed by a complex array of apical processes known as the cone sheath (CS). B, An electron micrograph showing the photoreceptor outer segment structures that remain in a cat retina detached for 13 days. IS, Photoreceptor inner segment; CC, photoreceptor connecting cilium. Arrow indicates a few disc-like structures in the degenerated outer segment (×28 000). (A, From Anderson DH, Guerin CJ, Erickson PA et al. Invest Ophthalmol Vis Sci 1986; 27:173.)

radiographic studies show that newly synthesized proteins continue to be transported and incorporated into these rudimentary outer segments.[55]

Immunolabeling experiments indicate that these discs contain, among other outer segment specific proteins, opsin and peripherin/rds, two important structural components (Fig. 115-6). These results suggest that the process of disc membrane formation and opsin biosynthesis does not cease even after lengthy detachment episodes. Opsin, which is normally concentrated in the outer segment, begins to accumulate in the plasma

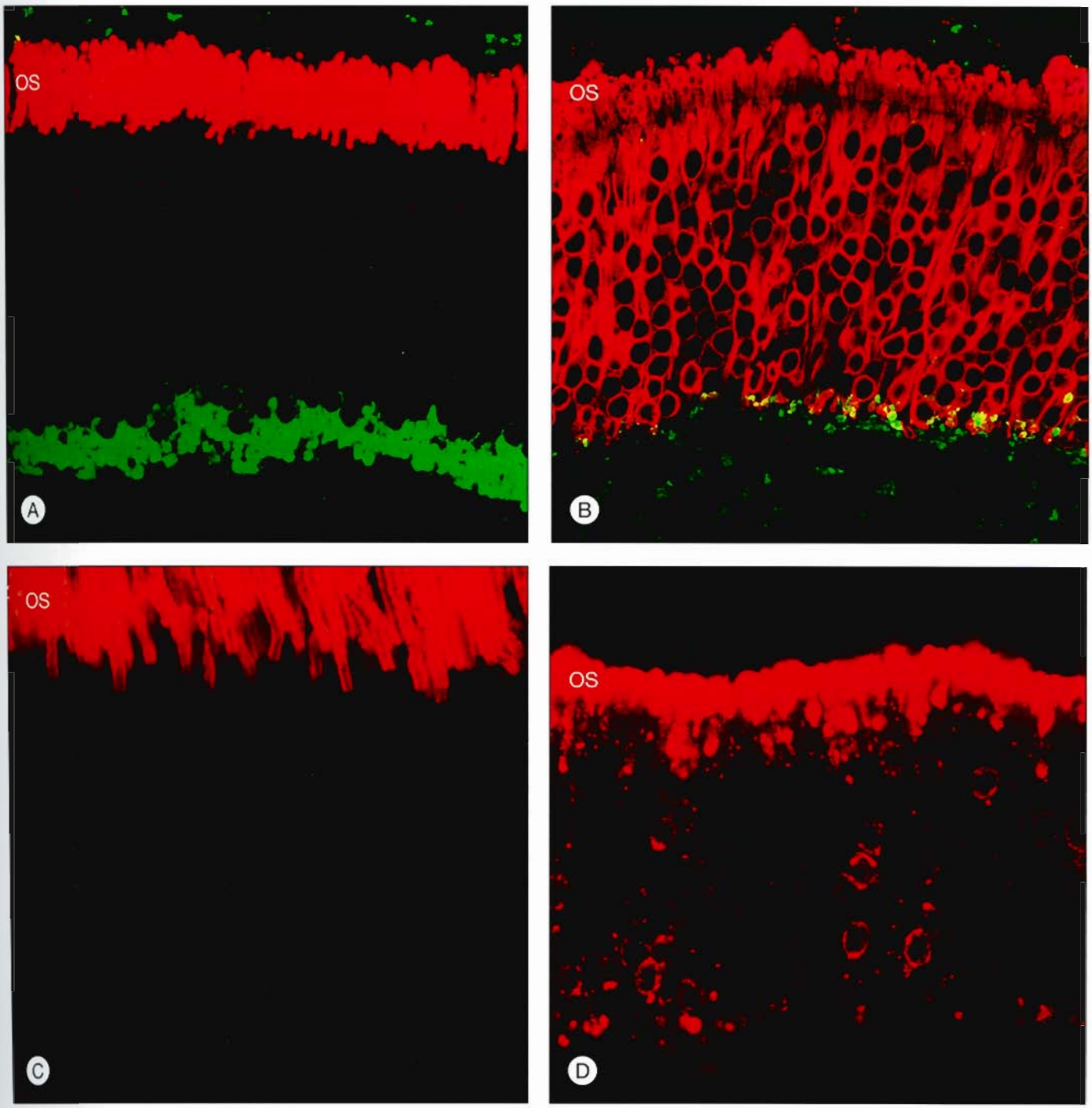

Fig. 115-6 Immunofluorescence images illustrating the changes in labeling with antibodies to rhodopsin, synaptophysin (A and B), and peripherin/rds (C and D) that occur after detachment. A, An antibody to rhodopsin intensely labels the rod outer segments in normal retina (red), while the antibody to synaptophysin labels the terminals of rods and cones (green). B, After 28 days of detachment, the antibody to rhodopsin now labels the entire rod photoreceptor plasma membrane (red). The labeling with the antibody to synaptophysin shows the extent of disruption of the photoreceptor synaptic terminals in the 28-day detachment (green). C, The antibody to peripherin/rds labels the edges of the outer segments in normal retina. D, After detachment this antibody labels intracellular structures (as well as the shortened outer segments) but not the plasma membrane.

membrane vitread to the connecting cilium within a day after an experimental retinal detachment. As outer segment degeneration proceeds, the number of cells showing this new pattern of rhodopsin distribution increases, as does the intensity of labeling in individual cells (see Fig. 115-6). After detachment, peripherin/rds, which, like rhodopsin, is an integral membrane protein but specific to the disc rims, begins to appear in cytoplasmic vesicles. As in the case with the shift in rhodopsin localization, the number of these vesicles increases with detachment time and correlates with outer segment degeneration. Peripherin/rds never appears in the plasma membrane, however.[56] Data from in situ hybridization experiments suggest that mRNA levels for rhodopsin remain at about the same levels as in normal retina 28 days after an experimental detachment. These data, along with results showing that newly synthesized protein continues to be transported and incorporated into degenerating photoreceptor outer segments, suggest that rhodopsin synthesis and transport (and probably that of other outer segment components) persist after detachment but that, in the absence of an organized outer segment, membrane proteins such as opsin and peripherin/rds may be inserted at inappropriate cellular locations.[55] A similar phenomenon occurs early after detachment in cones labeled with cone opsin antibodies, although the plasma membrane pattern is usually short-lived, occurring for only about a week after detachment. After this time the expression of both cone opsin proteins and mRNA is limited to only occasional cells.[57,58] In general, biochemical and immunocytochemical studies have shown that the expression of most photoreceptor-specific proteins declines with detachment time, although the expression of phosducin increases dramatically after detachment.[57] A major difference between rods and cones emerged from studies of protein expression. In general rod proteins continue to be expressed even in detachments of a month in duration, while the expression of proteins associated with cones go to levels that are undetectable by current immunocytochemical or immunosorbent (ELISA) types of assays.[30] Thus detecting the presence of cones in detached retinas based on protein expression is problematic.[59]

Disorganized lamellar debris rather than a discrete packet of discs is found in the subretinal space in long-term detachments. This provides additional evidence that discs are not shed in the normal manner. Thus it appears highly likely that both the normal shedding process and disc formation are affected adversely by the separation of the two tissue layers. Once the two layers are reapposed and outer segments begin to regenerate, a normal balance between disc addition and disc shedding must be reestablished if the outer segments are going to reattain normal length. Recent evidence indicates that this process may occur over months or even years.[60]

Summary

The effects of detachment on the photoreceptor–RPE interface are rapid and extensive. The retinal pigment epithelial apical surface morphology changes abruptly, and cells begin to proliferate and then migrate into the subretinal space. Other cells of hematopoietic origin also enter the subretinal space. All of these probably function to some degree as phagocytic cells, which serve the purpose of removing debris left from degenerating photoreceptors. The photoreceptor outer segments degenerate, leaving most photoreceptors with only a rudimentary cilium that may have a few disorganized discs associated with it. Finally, the change in patterns of protein expression differs in rods and cones; rods continue to express their proteins while cones do not.

Inner retina

Although the earliest and most obvious effects of detachment are seen at the neural retina–RPE interface, they are by no means limited to that region of the retina. A number of changes occur in the retina proximal to the outer segments, especially in detachments lasting more than a day. There are species differences in the ability of the outer nuclear layer to survive retinal detachment. Rabbits and ground squirrels, species with very different patterns of retinal vascularization and photoreceptor distributions, show extensive and rapid degenerative effects in the layer of photoreceptor nuclei, often leading to degeneration of the entire layer.[38,39,61] The photoreceptor layer of species such as cats, dogs, humans, and monkeys is apparently spared from total degeneration during detachment.

Photoreceptor inner segments

During the first day of a detachment, the inner segments appear essentially normal, but between the first and third days, they begin to show signs of degeneration: most commonly swelling, disruption, and loss of mitochondria (and loss of anti-cytochrome oxidase labeling),[27,75] in the ellipsoid region, an overall disruption of the organized rough endoplasmic reticulum and Golgi apparatus in the myoid region, and, within a few days, an overall size reduction of the inner segment. It is interesting to note that the connecting cilium is retained even in severely affected inner segments in long-term detachments. This is a crucial point because the connecting cilium is essential for production of the outer segment. The loss of mitochondria also has the potential to significantly affect the photoreceptors' ability to regenerate, because the metabolic rate in these cells is among the highest of any in the body.

Outer nuclear layer and outer plexiform layer

The outer nuclear layer contains the cell bodies of the photoreceptor cells. These cells extend a process toward the outer plexiform layer, where they form the characteristic synaptic terminals of rods (spherules) and cones (pedicles).[62] The outer plexiform layer also contains the processes of second-order neurons, the cell bodies of which lie in the inner nuclear layer. These processes synapse with each other and with the photoreceptors. The photoreceptor cell bodies and synaptic terminals show a rapid response to detachment in feline retinas. Some of these cells will die by apoptosis[63] (see below), and those that remain show extensive structural change. By 3 days after detachment the cell bodies and terminals show extensive vacuolization and can also show distention of the nuclear envelope and endoplasmic reticulum. The cells often show a loss of cytoplasmic ground substance and degeneration of their mitochondria. Multi-

vesicular bodies occurring in both the cell body and synaptic terminals are also indicators of degenerative changes in these cells. Some of the most striking structural changes in the surviving photoreceptors occur in the cytoskeleton of these cells. Both microtubules and filamentous-actin, which are highly organized in normal photoreceptors, show profound changes after detachment,[64] and since these structures are associated with the transport of molecules within cells and the maintenance of cell shape, their disruption would seem to have significant effects on these cells.

Not all photoreceptor cells degenerate (or die) at the same rate. Some show extensive signs of degeneration, whereas adjacent cells look relatively intact. It does appear that rod cell bodies react more quickly to detachment than do cones. In a region in which nearly all of the rod cell bodies show signs of degeneration and even cell death, neighboring cone cell bodies may look relatively intact. Consistent with this observation, the rod spherules appear to be particularly susceptible to the effects of detachment. These synaptic terminals are normally filled with synaptic vesicles and contain one or two large presynaptic ribbons. When the retina has been detached for 3 days, many of these terminals appear depleted of vesicles except for a few that remain as a halo around a greatly truncated ribbon. Many terminals appear as if they have "retracted" into the cell body, and some synaptic structures generally associated with the outer plexiform layer now occur within the outer nuclear layer (Fig. 115-7).[65] As with the cone and rod photoreceptor cell bodies, the cone synaptic terminals seem to survive the early effects of detachment better than do the rod terminals. Although their shape can change fairly dramatically,[30] they do not appear to retract and by electron microscopy they remain filled with synaptic vesicles.[49]

Neuronal remodeling as a result of detachment

Recent studies using antibody probes have demonstrated that the magnitude of the rod spherule response to detachment is much larger than suggested by the electron microscopic data, and that some unexpected changes occur in both the second- and third-order neurons that form the inner retinal circuitry.[65] Beginning by 1 day after detachment the outer plexiform layer exhibits disorganization of the photoreceptor synaptic terminals when they are labeled with an antibody to the protein synaptophysin. Synaptophysin-positive terminals now begin to appear deep in the outer nuclear layer (Fig. 115-7). At this same time, processes from the rod bipolar cells and horizontal cells (labeled with antibodies to protein kinase C, and neurofilament protein, respectively) begin to grow beyond the normal layer of photoreceptor synaptic terminals and into the outer nuclear layer (Figs 115-7, 115-8).[60] Similarly, ganglion cells, the 3rd order neurons in the retina are affected by detachment (Fig. 115-8).[31] A subpopulation of ganglion cell begins to re-express the protein GAP 43 in their cell bodies. GAP 43 is expressed in ganglion cell bodies early in development, but in the adult retina, after the formation of synaptic connections between ganglion cell axons and their targets in the brain, its expression is limited to some ganglion cell dendrites in the inner plexiform layer. After detachment, GAP 43 positive ganglion cell bodies reappear (Fig. 115-8, asterisks) and GAP 43 positive processes become extensive, often growing into unusual locations such as epiretinal membranes lying on

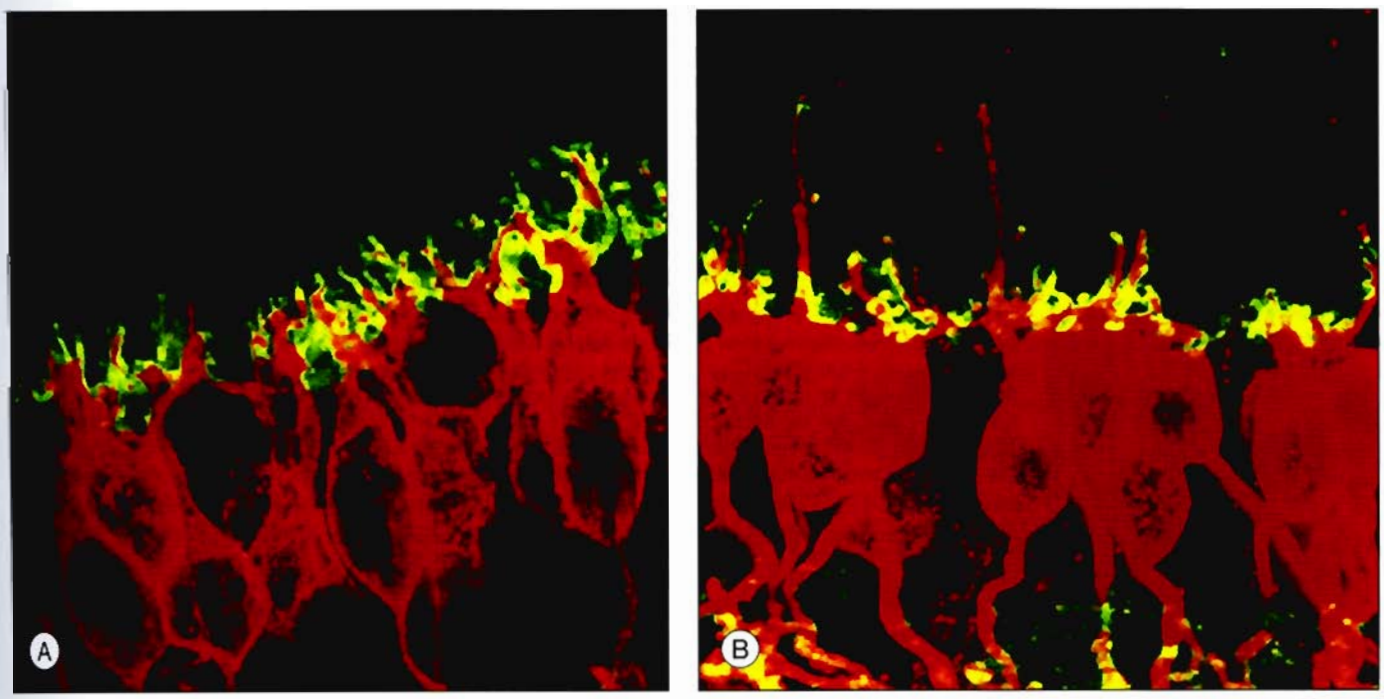

Fig. 115-7 Immunofluorescence images of normal (A) and 3-day detached (B) feline retinas labeled with antibodies to protein kinase C (red) and synaptophysin (green). The antibody to protein kinase C labels only the rod bipolar cells and their dendrites, which connect to the terminals (green, synaptophysin labeled) of the rod photoreceptors. After detachment, the synaptic terminals degenerate, often withdrawing into the outer nuclear layer, and the rod bipolar cells sprout neurites that grow into this layer (B).

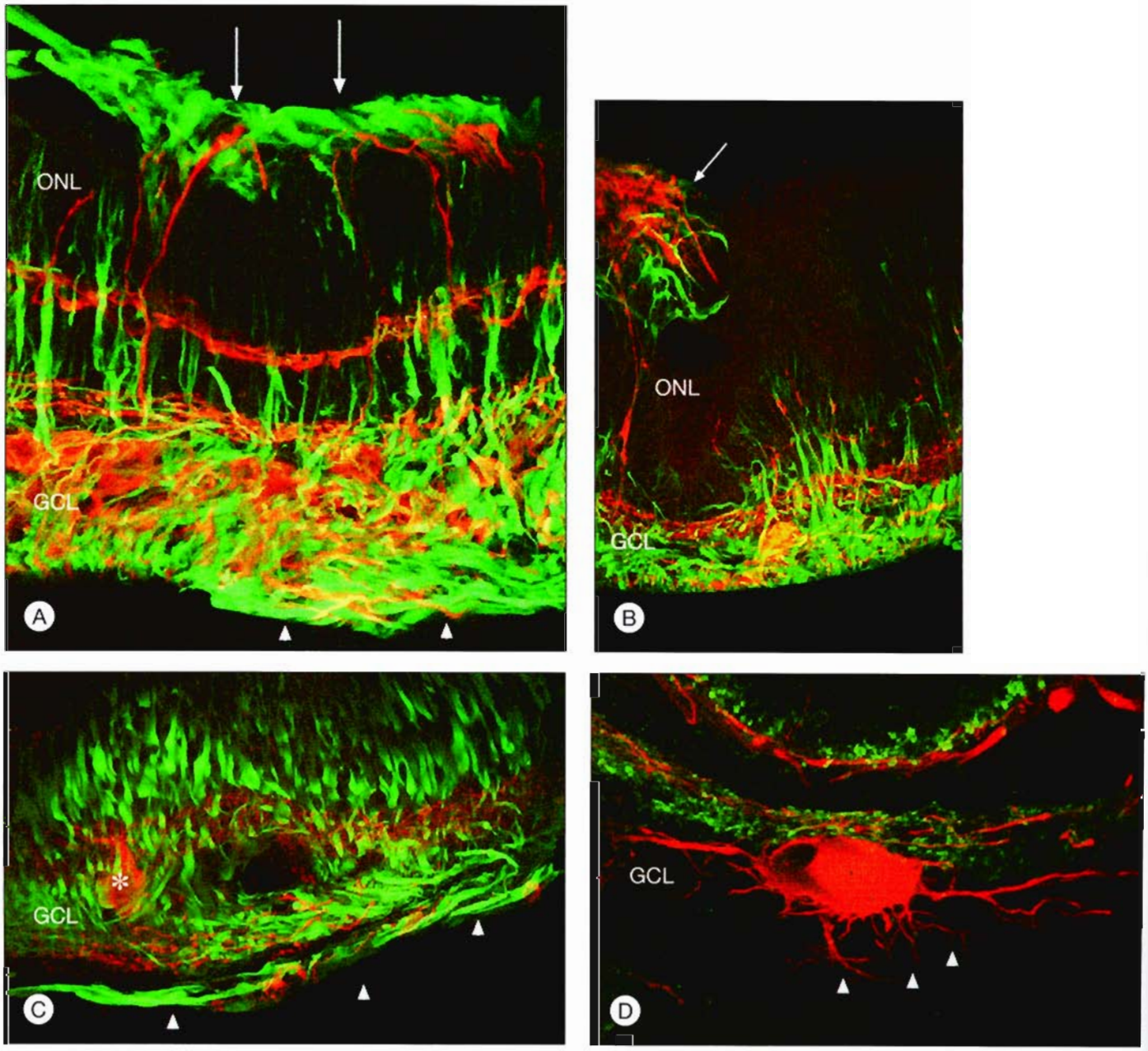

Fig. 115-8 A series of immunofluorescence images illustrating the neurite sprouting of second and third order neurons as well as the formation of sub- and epi-retinal Müller cell scars in the feline retina following a 28-day detachment. A, An antibody to neurofilament protein (red) labels ganglion cell bodies in the GCL and neurites from ganglion cells within an epiretinal membrane (arrowheads) as well as horizontal cell bodies and neurites from these cells extending through the ONL into a subretinal glial scar (green; arrows). An antibody to glial fibrillary acidic protein (GFAP; green) labels intermediate filaments in Müller cells throughout the retina as well as those in the subretinal (arrow) and epiretinal membranes. B,C, An antibody to GAP 43 (red) labels activated ganglion cell bodies (asterisks), dendrites in the inner plexiform layer and neurites that extend through the retina into a subretinal (green; arrow, B) and epiretinal glial scar (arrowheads, C). An antibody to GFAP (green) labels intermediate filaments in Müller cells throughout the retina and in the subretinal scar. D, An antibody to neurofilament protein (red) labels an activated ganglion cell and newly formed neurites extending from the base of its cell body (arrowheads). An antibody to synaptophysin (green) labels synaptic terminals in the outer and inner plexiform layers. E, An antibody to neurofilament protein (red) labels horizontal cell neurites within a glial scar (green; labeled with anti-GFAP) in the subretinal space in a retinal flat mount preparation. "ToPro" labeling (blue) shows the presence of photoreceptor nuclei just below the glial scar. ONL, outer nuclear layer; GCL, ganglion cell layer. (B, From Lewis GP, Fisher SK. Int Rev Cytol 2003; 230:263.)

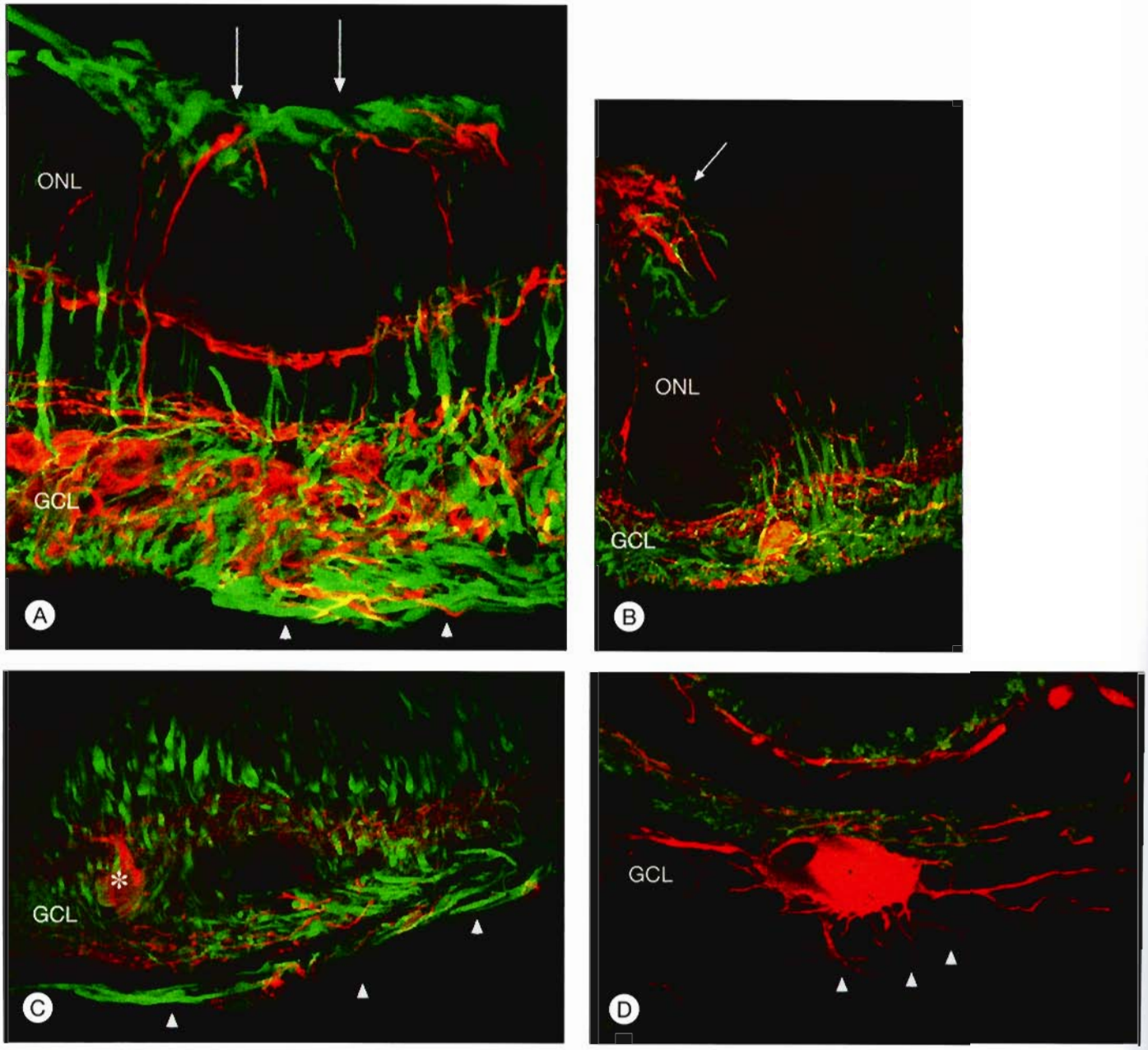

Fig. 115-8 A series of immunofluorescence images illustrating the neurite sprouting of second and third order neurons as well as the formation of sub- and epi-retinal Müller cell scars in the feline retina following a 25-day detachment. A, An antibody to neurofilament protein (red) labels ganglion cell bodies in the GCL and neurites from ganglion cells within an epiretinal membrane (arrowheads) as well as horizontal cell bodies and neurites from these cells extending through the ONL into a subretinal glial scar (green; arrows). An antibody to glial fibrillary acidic protein (GFAP; green) labels intermediate filaments in Müller cells throughout the retina as well as those in the subretinal (arrow) and epiretinal membranes. B,C, An antibody to GAP 43 (red) labels activated ganglion cell bodies (asterisks), dendrites in the inner plexiform layer and neurites that extend through the retina into a subretinal (green; arrow, B) and epiretinal glial scar (arrowheads, C). An antibody to GFAP (green) labels intermediate filaments in Müller cells throughout the retina and in the subretinal scar. D, An antibody to neurofilament protein (red) labels an activated ganglion cell and newly formed neurites extending from the base of its cell body (arrowheads). An antibody to synaptophysin (green) labels synaptic terminals in the outer and inner plexiform layers. E, An antibody to neurofilament protein (red) labels horizontal cell neurites within a glial scar (green; labeled with anti-GFAP) in the subretinal space in a retinal flat mount preparation. "ToPro" labeling (blue) shows the presence of photoreceptor nuclei just below the glial scar. ONL, outer nuclear layer; GCL, ganglion cell layer. (B, From Lewis GP, Fisher SK. Int Rev Cytol 2003; 230:263.)

the vitreal surface (Fig. 115-8). Both horizontal cell bodies and ganglion cell bodies become strongly immunoreactive for neurofilament protein after detachment and results of labeling experiments with that antibody show the extensive and dramatic remodeling of both cell types that occur after detachment (Fig. 115-8). This response contrasts significantly with that of the rod bipolar dendrites, where the growing processes appear directed to find specifically the retracted terminals of rod photoreceptors (Fig. 115-7). Indeed, both horizontal and ganglion cell processes appear to specifically seek Müller "scars" in the subretinal space (Fig. 115-8) or vitreal surface and may run for long distances in these aberrant locations. As shown by these images the neuronal remodeling that takes place within days of a detachment can be dramatic. The effect of these changes on visual recovery after reattachment is unknown. While reattachment appears to stop much of the neuronal remodeling (see section on Reattachment, p. 2020), it is not known if reattachment results in a reversal of these events. To the best of our knowledge, these events have not been studied in very-long-term detachments (e.g. greater than a month) where photoreceptor terminals in the feline retina can no longer be identified by light microscopy, and ultrastructural signs of synaptic contact between photoreceptors and second-order neurons are rarely found.[49]

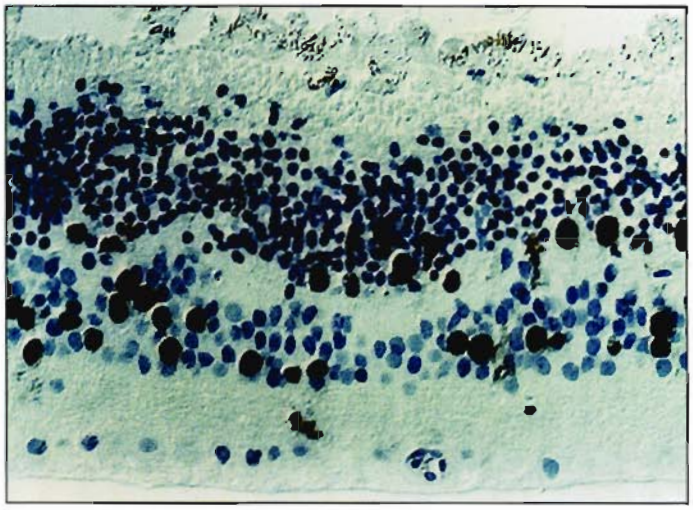

Fig. 115-9 An antibody that recognizes the Ki-67 protein (MIB-1) labels dividing cells (dark nuclei) in a section from a feline retina detached for 3 days. The dividing cells consist of astrocytes, pericytes, endothelial cells, microglia and Müller cells. Labeled Müller cells are present in their normal location but also migrate into the ONL. The tissue is countered stained with toluidine blue. ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer.

Proliferation of cells in the inner retina

In the first week after detachment, there is also a significant proliferative component to the cellular changes in the inner retina. Within 24 hours of the detachment all non-neuronal cell types within the retina, including astrocytes, Müller cells, pericytes, capillary endothelial cells, and microglia, display signs of proliferation as shown by tritiated thymidine autoradiography or when labeled with an antibody that recognizes proliferating cells (Fig. 115-9).[18,22] By 2 days, some labeled Müller cell nuclei are translocated from their normal positions on the vitreal border of the inner nuclear layer into the outer plexiform and outer nuclear layers. This response peaks 3 to 4 days after detachment and declines slowly to very low levels several weeks later.

Müller cell hypertrophy and protein expression

Müller cells begin a process of glial scar formation almost immediately after detachment; changes in protein expression associated with this response are detectable within a day, as is the early growth of Müller cell processes.[29,66,67] Presumably these cells fill the spaces left by degenerating neurons, but they do more than that because they also grow rapidly into the subretinal space. In the feline model, Müller cell processes rarely grow onto the vitreal surface of the detached retina, but readily do so once the retina is reattached, thus producing results similar to that observed in human proliferative vitreoretinopathy.[68] Müller cell bodies migrate, frequently being found in the outer nuclear and outer plexiform layers within 3 days (Fig. 115-9). After detachment, the cytoplasm of these cells fills with 10 nm diameter (intermediate) filaments that, by immunochemical criteria, are composed of glial fibrillary acidic protein,[69a] and vimentin,[66] (Fig. 115-10; see also Figs 115-8, 115-11, 115-13, 115-15). Beyond 3 days of detachment, Müller cell processes often extend into the subretinal space through localized disruptions in the outer limiting membrane. These processes become more commonplace and elaborate as detachment time lengthens. Immunocytochemical labeling and confocal imaging studies demonstrate the unique nature of Müller cell processes extending into the subretinal space. While Müller cell processes within the retina express both vimentin and GFAP, those that grow into the subretinal space appear to preferentially express vimentin in the portion of the cell that occupies the outer retina (Fig. 115-11).[67] These vimentin-expressing processes then grow beyond the outer limiting membrane and into the subretinal space with the appearance of filopodia (Fig. 115-11), retaining their preferential expression of vimentin over GFAP. Microvilli normally extend from the apical surface of the Müller cells, just beyond the outer limiting membrane. These processes are richly decorated with the protein CD-44 (Fig. 115-11). As the Müller cell filopodia intrude into the subretinal space, their surface remains decorated with "microspikes" of CD-44 (Fig. 115-11). The presence of CD-44 on the apical microvilli and subretinal outgrowths may provide for sites of molecular interaction between the growing processes and components of the subretinal space. Interestingly, the Müller cell processes grow beyond the outer limiting membrane preferentially adjacent to cone photoreceptors (Fig. 115-11).[70] The Müller cell processes can grow for long distances on the photoreceptor border, often forming a multilayered "glial scar" within the subretinal space (see Fig. 115-10) that separates the neural retina from the RPE. In spinal cord injuries, astrocytes undergo a similar hypertrophic response where their GFAP-filled processes form glial scars within the neural tissue. Although still controversial, there is evidence that these scars may block the regeneration of spinal neurons.[23] Within the outer plexiform layer the large, hypertrophic Müller cell processes may be comparable to the "glial scar" in spinal cord injury. Because

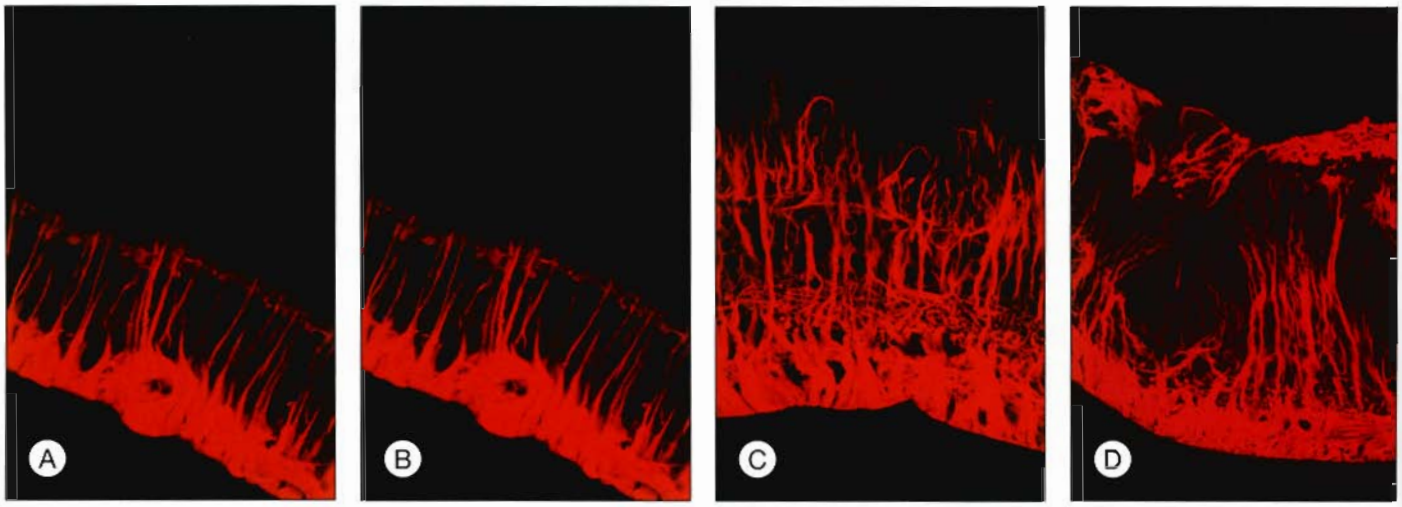

Fig. 115-10 A series of immunofluorescence images of normal (A), 3-day (B), 7-day (C), and 28-day (D) detached feline retinas labeled with an antibody to glial fibrillary acidic protein (GFAP). In normal retina this protein is localized to the endfoot region of the Müller cells bordering the vitreous cavity (and astrocytes in this region, data not shown). After detachment the increase in GFAP within the Müller cells is an excellent marker for the reactivity and hypertrophy of these cells. In this 28-day example a large Müller cell scar occurs in the subretinal space, covering the photoreceptor layer. Labeled Müller cell processes can be seen extending into the subretinal space as early as 3 days after detachment.

of their location, they could have implications for the synaptic connections of the photoreceptors and second-order neurons. The intermediate filament response in Müller cells is even more dramatic than in brain or spinal cord astrocytes because the Müller cells normally have only a small population of intermediate filaments within their endfeet, on the border of the vitreous cavity. Within a day of detachment these filaments begin to extend from the endfoot, rapidly spreading throughout the Müller cell cytoplasm. The immunocytochemical labeling of the intermediate filament proteins is an excellent way to trace the invasion of Müller cell processes into the subretinal space or vitreous[29,64,66,70] (Fig. 115-10; see also Figs 115-8, 115-11).

The presence of Müller cell processes within the subretinal space appears to inhibit the regeneration of photoreceptors after retinal reattachment (see later discussion). What role, if any, their presence within the outer plexiform layer may play in the maintenance or loss of synaptic connections between photoreceptors and second-order neurons or other cellular events during a period of detachment is not yet understood.

In the feline model, Müller cells are apparently stimulated by *reattachment* to grow beyond the inner border of the retina and onto the vitreal surface where they form layers of cellular membranes.[71] An example of such a membrane is illustrated in Fig. 115-8. Müller cell processes that grow into the vitreous have a different structure and a different intermediate filament composition compared to those that grow into the subretinal space.[67] The initial outgrowths into the vitreous occur as thin "wispy" extensions of the endfoot region and have an intermediate filament population that is dominated by GFAP instead of vimentin (compare Figs in 115-11).

Besides the huge shift in intermediate filament synthesis, another metabolic change that occurs in Müller cells after detachment is a decrease in the level of immunostaining with antibodies to different proteins.[29,68] In feline retinas detached for 1 week, the labeling intensity of the Müller cells significantly declines when stained with an antibody to the retinoid-binding protein known as cellular retinaldehyde-binding protein (Fig. 115-12). Similar immunolabeling experiments show a dramatic decrease in the levels of two Müller cell enzymes, carbonic anhydrase II and glutamine synthetase, during detachment (Fig. 115-12). Thus, detachment of the retina clearly produces changes in the metabolism of retinal Müller cells.

Loss of photoreceptor cells

The loss of cells from the photoreceptor layer occurs by cell death and the extrusion of photoreceptor cell bodies past the outer limiting membrane into the subretinal space. Cell death among photoreceptor cells has been shown by the TUNEL technique to occur by apoptosis, and perhaps by necrosis.[61,63,68a] The mechanism by which cells are extruded into the subretinal space is not understood, but these cells have clearly lost their differentiated phenotype. They appear as rounded cells with very little cytoplasm. In cat retinas, there is a significant decrease in the number of photoreceptor cells by 1 month after detachment and this number continues to decline until the outer nuclear layer loses about 80% of its cell population by 90 days after detachment.[40] In regions severely affected by photoreceptor degeneration, the outer nuclear layer can be reduced in thickness to one or two cell layers. The degree of cell death and its timing is species dependent. Of the species used in experimental detachments, rabbits are severely affected, with the retina being reduced to a single layer of cells in detachments of 4 months' duration.[38,39] The cone dominated ground squirrel exhibits rapid and extensive cell death with the ONL often disappearing entirely and being replaced by a meshwork of Müller cell processes.[61] Cell death was not reported in the outer nuclear layer of experimentally detached owl and rhesus

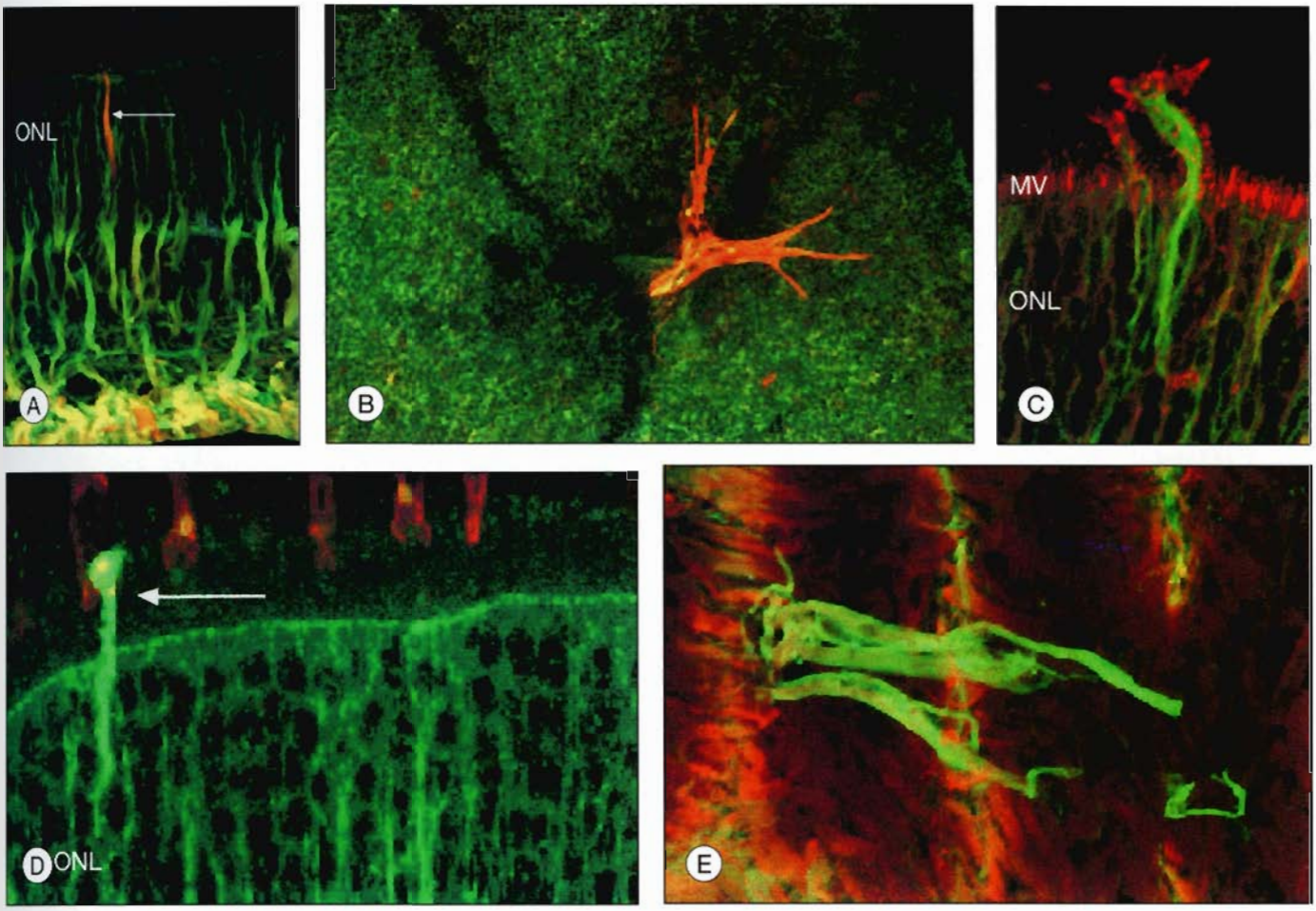

Fig. 115-11 A series of immunofluorescence images illustrating the early stages of Müller cell growth onto the subretinal and epiretinal surfaces of the feline retina. A, Antibodies to GFAP (green) and vimentin (red) label intermediate filaments within Müller cells throughout the retina. The red process (arrow) in the ONL is labeled predominantly with the antibody to vimentin and represents a Müller cell that is just beginning to extend into the subretinal space. B, A flat mounted preparation, viewed from the photoreceptor surface and labeled with antibodies to GFAP (green) and vimentin (red). It shows the predominance of vimentin in a Müller cell process in the very early stages of extending into the subretinal space. C, An antibody to GFAP (green) labels a Müller cell process just entering the subretinal space. An antibody to CD44 (red) labels fine filopodia on this process as well as the Müller cell microvilli (MV). D, An antibody to GFAP (green) labels a Müller cells process entering the subretinal space directly adjacent to a PNA-labeled cone photoreceptor (red). E, A flat mounted preparation, viewed from the vitreal side of the retina, labeled with antibodies to GFAP (green) and vimentin (red). It shows a predominantly GFAP labeled Müller cell process in the earliest stage of extending onto the vitreal surface of the retina. (The vimentin labeling in the background is in the Müller cells' endfeet.) ONL, outer nuclear layer. (Reprinted from Lewis GP, Fisher SK. Up-regulation of glial fibrillary acidic protein in response to retinal injury: its potential role in glial remodeling and a comparison to vimentin expression. Int Rev Cytol 2003; 230:263. Copyright 2003, with permission from Elsevier.)

monkey retinas, even in detachments of 14 weeks' duration.[34,36] Data from the monkey studies suggests that cell death in the outer nuclear layer may not be a factor in human detachments. However, a recent histopathological study by Wilson and Green[33] of retinal detachment in postmortem eyes showed that atrophy of the photoreceptor layer occurred in 26.5% of the retinas examined. And other studies of human detached retinal tissue suggests that this death is via the apoptotic pathway.[69,70a] Thus it appears that cell death in the photoreceptor cell layer could be a significant factor in recovery after reattachment, particularly in detachments of more than a few days' duration. Finding a strategy for preserving photoreceptors may lead to an improvement of the visual outcome after reattachment surgery.[32]

Factors that may affect the outcome of detachment

Although our current level of understanding remains modest, the rapid proliferation of non-neuronal cell types after detachment, as well as the degeneration and eventual death of many photoreceptors, appear to be events very likely to adversely affect the recovery of visual function following reattachment. Any agent(s) that acts safely to promote photoreceptor cell survival, maintain non-neuronal cells in a mitotically quiescent state, sustain the differentiated phenotype of retinal neurons, and prevent secondary events such as subretinal fibrosis and proliferative vitreoretinopathy could be of potentially significant therapeutic value as an adjunct to reattachment surgery. There is evidence from

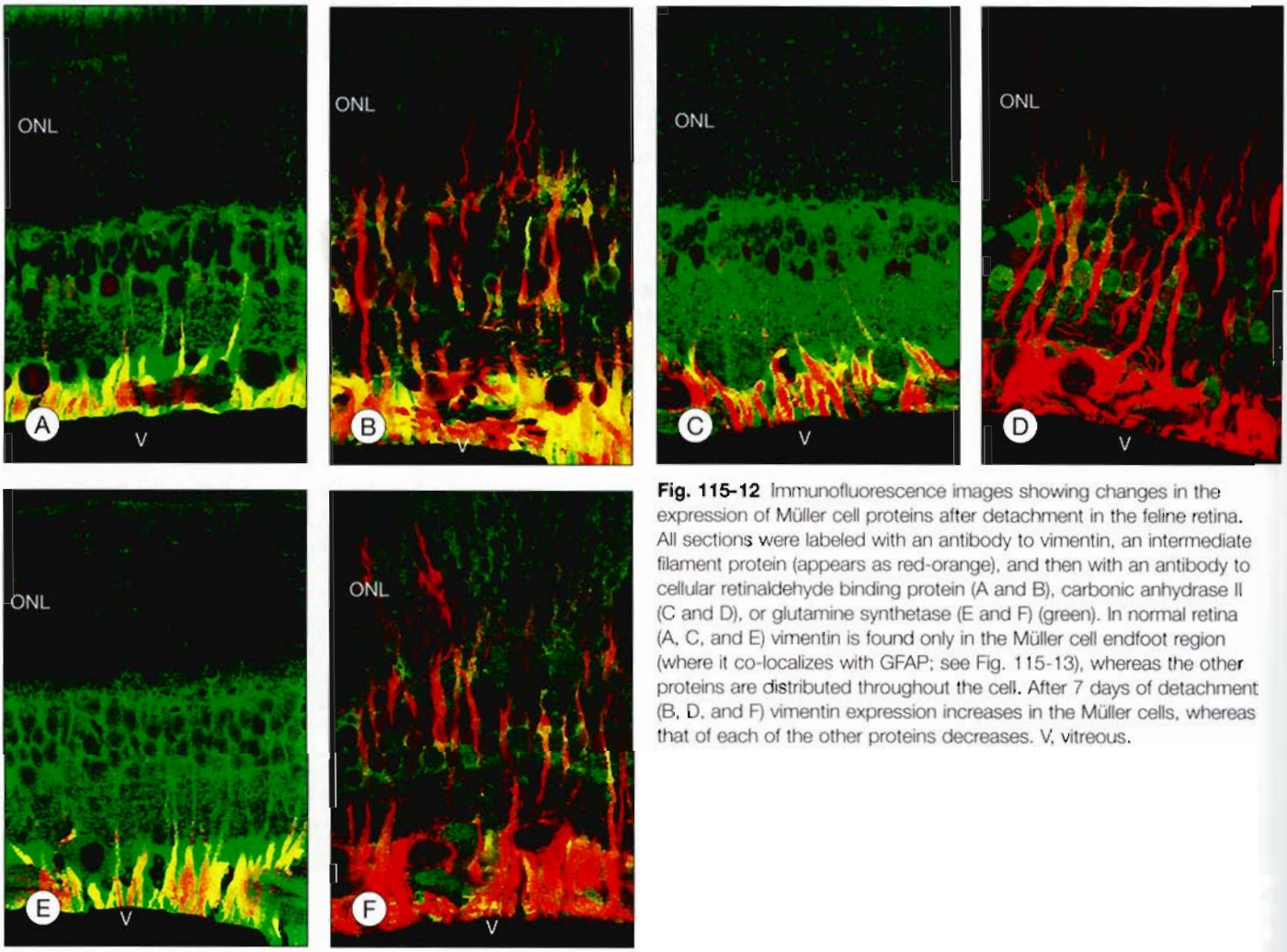

Fig. 115-12 Immunofluorescence images showing changes in the expression of Müller cell proteins after detachment in the feline retina. All sections were labeled with an antibody to vimentin, an intermediate filament protein (appears as red-orange), and then with an antibody to cellular retinaldehyde binding protein (A and B), carbonic anhydrase II (C and D), or glutamine synthetase (E and F) (green). In normal retina (A, C, and E) vimentin is found only in the Müller cell endfoot region (where it co-localizes with GFAP; see Fig. 115-13), whereas the other proteins are distributed throughout the cell. After 7 days of detachment (B, D, and F) vimentin expression increases in the Müller cells, whereas that of each of the other proteins decreases. V, vitreous.

other experimental systems that any one of a number of regulatory molecules may fill this role. A provisional list of such factors includes both acidic and basic fibroblast growth factors (FGF-1 and FGF-2), brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), transforming growth factor-alpha, interleukin-1 beta, neurotrophin-3, neurotrophin-4, and insulin-like growth factor-2.[71a,72] Indeed the intraocular injection of BDNF has been demonstrated to slow or stop many of the "degenerative" events initiated by detachment in the feline model.[40] A much simpler, and highly effective effect can be obtained, however, by simply increasing the concentration of environmental oxygen breathed by the animal after detachment. In the first experiments of this type, animals with experimental detachments were kept in a hyperoxic (70% O_2) environment for 3 days immediately following the detachment.[57] In follow-up studies, the efficacy of environmental hyperoxia was demonstrated in a cone-dominated ground squirrel model.[58] Similar results have also been reported from experiments in which the retina remained detached for a total of 7 days, with hyperoxic therapy delayed until 1 day after the production of a detachment.[71] The cellular results are remarkable in the degree of preservation of both rod and cone photoreceptors (Fig. 115-13). The effect of treatments such as BDNF or hyperoxia on visual outcome after reattachment is still unknown. The use of biological factors is not without risk, however. For example, FGF-2 has been demonstrated to be a powerful rescue agent in other animal models of photoreceptor degeneration[72-74] but when injected into the vitreous it induces the rapid proliferation of Müller cells, astrocytes, and RPE cells as well as the formation of epiretinal membranes.[26] Similarly, even though the molecule CNTF and its analogue, Axokine, have been shown to enhance photoreceptor survival, they also produce retinal folds and rosette formation by photoreceptors,[75] as well as a decline in the ERG.[76,77] Thus, the results are encouraging that adjuncts to surgical reattachment may be forthcoming but they also demonstrate the care that needs to be taken in evaluating such treatments before proposing their use on human patients.

EXPERIMENTAL RETINAL REATTACHMENT

The fact that retinal reattachment restores at least partial vision in most human patients implies that many of the adverse effects of retinal detachment can be reversed successfully. The goal of

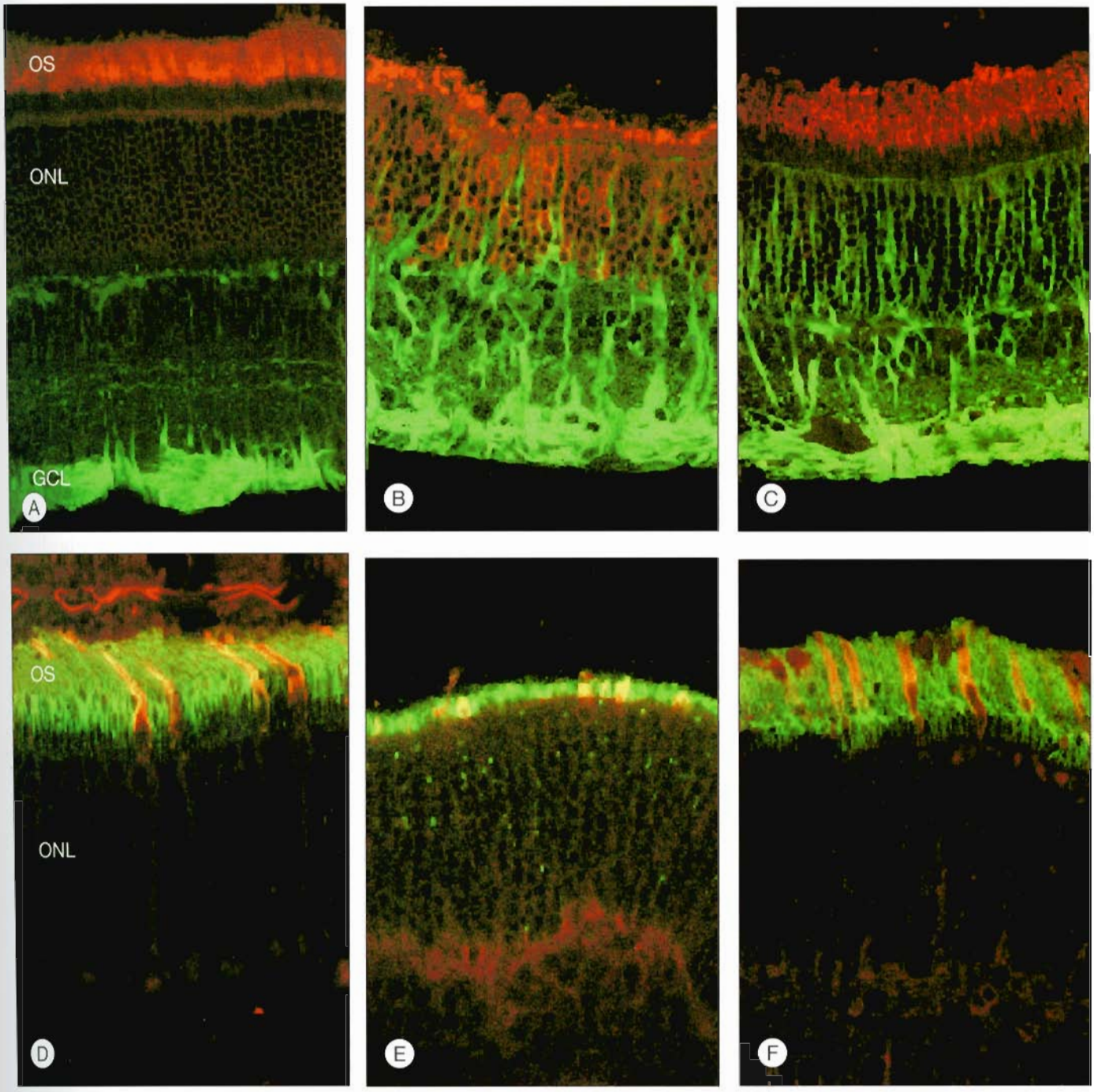

Fig. 115-13 A series of immunofluorescence images showing the effects of hyperoxia (70% O_2) on the outcome of detachment in the feline retina. A,D, normal retinas; B,E, 7-day detached retinas from animals kept in room air; C,F, 7-day detached retinas with hyperoxic exposure beginning 1 day after the detachment (for a total of 6 days of hyperoxia). A–C, Retinas are labeled with antibodies to GFAP (green) and rod opsin (red). B, Following a 7-day detachment in room air anti-GFAP labeling increases in Müller cells, rod outer segments are highly degenerate and anti-rod opsin labeling becomes redistributed to the rod cell bodies in the ONL. C, Following a 7-day detachment with 6 days of hyperoxia, anti-GFAP labeling still increases in Müller cells but there is less opsin redistribution and the rod outer segments are noticeably longer compared to the detachments in animals kept in room air. D–F, Retinas are labeled with biotinylated PNA (red; labels cone matrix sheaths) and anti-C3H (green; labels the rod matrix). E, Following a 7-day detachment in room air the matrix around both rods and cones becomes greatly truncated as the outer segments degenerate. F, Following a 7-day detachment with 6 days of exposure to hyperoxia, the matrix of both rods and cones appears almost normal reflecting the preservation of these structures. OS, outer segments; ONL, outer nuclear layer; GCL, ganglion cell layer. (From Lewis GP, Talaga KC, Linberg KA et al. Am J Ophthalmol 2004; 137:1085.)

experimental studies of reattachment is to understand more fully the cellular and molecular changes that accompany visual recovery. Eventually this should lead to improvements in the ability to manage, optimize, and ultimately manipulate the recovery process.[32,78,79] Discovery of the cellular mechanisms underlying this complex process and the variables that affect the extent of recovery must depend almost entirely on animal models because retinal tissue from human reattachment patients is rarely obtainable.

Most studies of visual recovery in human reattachment patients have been restricted to basic acuity measurements as a function of detachment duration. In general, these studies indicate that detachment duration is inversely related to acuity recovery, with the longest durations correlated with the least recovery. There have been only a few studies clearly demonstrating that improvements in color vision, visual sensitivity, and other visual parameters are also an integral part of the recovery process. In the case of detachments involving the macula, there is no general consensus in identifying a critical period beyond which the prognosis for recovery dims. However, one study provides compelling evidence that visual recovery declines exponentially as a function of macular detachment duration.[78] This conclusion conforms with the morphologic results obtained from experimental models of reattachment. New evidence emerging from studies in human patients suggests that recovery may continue over a very long time, perhaps years after reattachment.[80] Although data on functional recovery in animal models is very limited, visual sensitivity measurements from the two classes of cones found in the ground squirrel suggest that ERG recovery may occur fairly rapidly and not correlate particularly well with the recovery of outer segment length.[80] Indeed, the total number of surviving photoreceptors correlates better with visual recovery in this model than does outer segment length. If a similar situation exists in human patients, then visual recovery may not be a simple function of outer segment growth but a much more complex process, perhaps involving the remodeling of retinal circuitry that occurs after detachment.[81]

Re-establishing the photoreceptor–RPE interface

It is evident that successful re-establishment of the photoreceptor–RPE interface is a critical aspect of recovery after reattachment. The cell–cell interactions that must occur between the photoreceptors and RPE during this recovery period are still not understood, but some relevant concepts have emerged from the experimental models. The cell–cell interaction that must occur after reapposition of the two layers is similar to the interactions that take place during RPE and photoreceptor development. Early in development, the photoreceptor inner segments are apposed to a mounded and undifferentiated apical RPE surface. Unknown metabolic and molecular processes that promote photoreceptor–RPE adhesion tend to maintain this apposition as the photoreceptor outer segments interdigitate with newly forming apical RPE processes and eventually attain their mature configurations. Reattachment of the retina must induce a similar series of molecular events that controls the redifferentiation of the RPE apical surface, the re-ensheathment of the regenerating outer segments (which differs for rods and cones), and probably the resynthesis of interphotoreceptor matrix components as well. Finally, the photoreceptors and RPE must also re-establish a functional relationship. The transport of ions and molecules between the retina and RPE is affected to an unknown degree when the two cell layers are separated from each other. For example, retinoids (chemically distinct forms of vitamin A), coupled with their binding proteins,[82] must be transported back and forth between the neural retina and the RPE as part of the visual cycle. It is not known whether this transport is affected by detachment or reattachment processes.

Photoreceptor outer segment regeneration

Information on the rate of outer segment regrowth after reattachment is sparse and difficult to obtain. In experimentally reattached retinas, regenerating outer segments often appear shortened and misaligned with respect to each other. The stacking of the disc membranes is often abnormal. In addition, there is a high degree of variability in outer segment length from one reattached region to the next, which contributes to the impression that regeneration is not a homogeneous process across the retina (see later discussion). All these factors add to the difficulty in quantifying the rate of outer segment regrowth. However, morphometric and electron microscopic autoradiographic analyses of outer segment regeneration in monkeys whose retinas had been detached for 1 week and then reattached for up to 5 months have revealed some of the dynamics of this repair process.[54] The results suggest an abnormally slow rate of photoreceptor outer segment disc membrane assembly during the first few weeks of reattachment (Fig. 115-14). In monkey retinas detached for 1 week, rod and cone outer segments regain approximately 30% of their normal mean length within 7 days of reattachment, 60% of their length after 30 days, and 100% by 150 days. In the first 30-day interval, the mean disc membrane assembly rate in rods is approximately one third slower than the normal rate (see Fig. 115-14); whether that diminution persists beyond that point remains an open question. Disc shedding, on the other hand, appears to engage after the first reattachment week.

In cat retinas detached for periods longer than 7 days, many outer segments remain shorter than normal several months after reattachment,[46] implying that defects in the assembly or shedding phases (or both) of the renewal process may persist well beyond 30 days in retinas detached for longer durations. Long-term reattachment experiments are expensive because of the cost of maintaining experimental animals, but they may be highly informative given our lack of understanding of long-term recovery and indications that visual recovery may continue for a very long time after reattachment in humans.

Experiments with reattachment after short-term detachment

In the feline model, reattachment after detachments of 3 days or less can be highly effective at preventing or reversing cellular events induced by detachment.[27,28] Fig. 115-15 illustrates the general change in immunocytochemical labeling patterns in retinas detached for 3 days and those detached for 3 days and

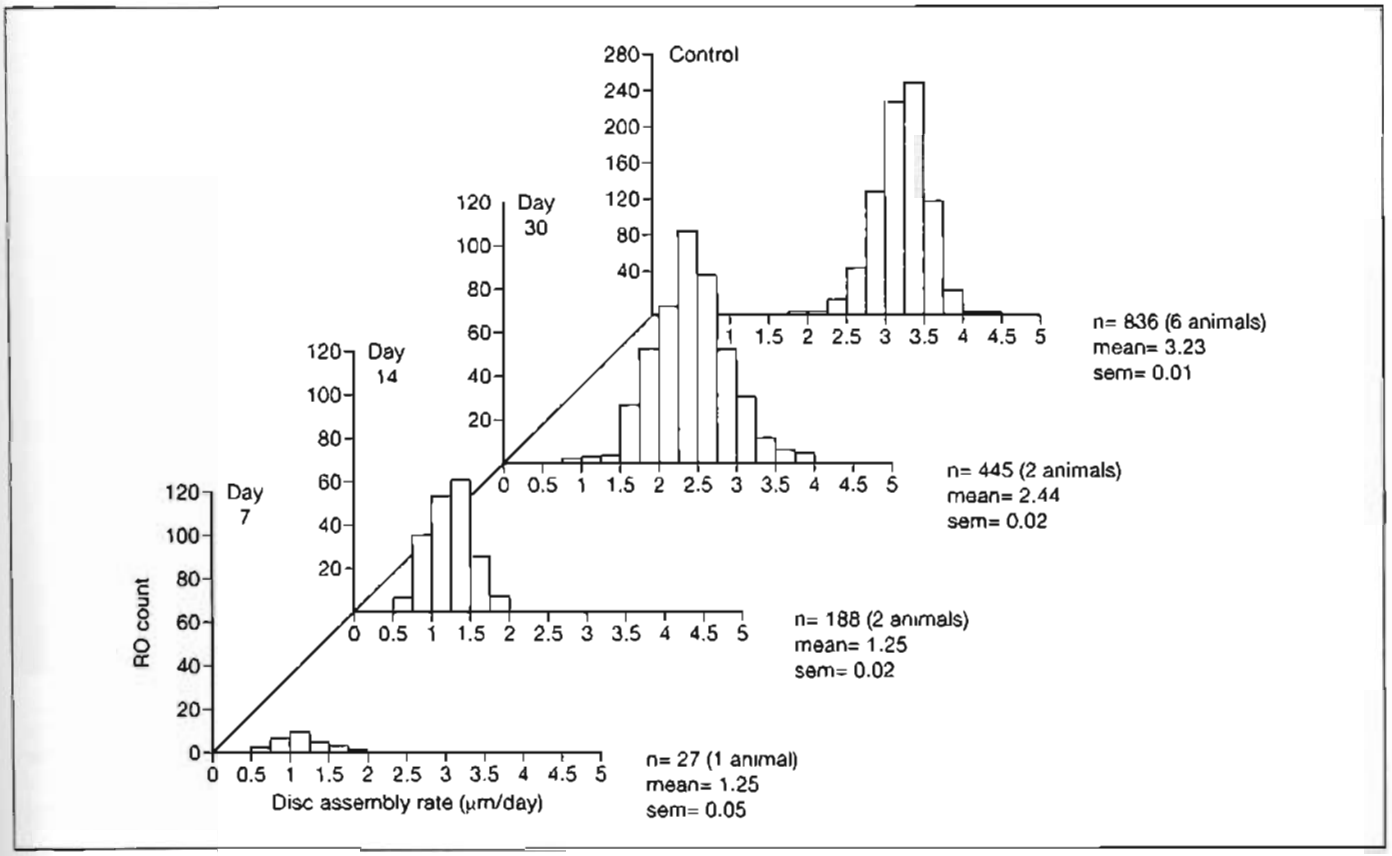

Fig. 115-14 Histograms illustrating the distribution of rod outer segment disc assembly rates after varying periods of retinal reattachment. The macular region in the retinas of rhesus monkeys were experimentally detached for 7 days. At 7, 14, and 30 days after reattachment, rod outer segment disc assembly rates (in micrometers per day) were calculated from electron microscopic autoradiograms in sample populations of macular rods located in the reattached regions. The number of rods was then plotted against assembly rate at several reattachment time points. The mean rate increased from a low of 1.15 μm/day at 7 days to 2.44 μm/day at 30 days. This compares with a mean normal rate of 3.23 μm/day.

then reattached for 28 days. The outer segment disruption and the prominent redistribution of rod opsin observed at 3 days are both virtually absent in the area of reattachment illustrated. In retinas studied on day 3 reattachment after 1 hour or 1 day of detachment outer segments are slightly longer than if the retina remained detached for the whole 3-day period (Fig. 115-16), probably because reattachment is very effective at rapidly stopping the outer segment degeneration induced by detachment. During a 3-day detachment, outer segments degenerate to about 30% of their normal length, and by 28 days they are less than 10% of their pre-detachment length. The outer segments recover to a little more than 70% of their length after 3 days of detachment and 28 days of reattachment (Fig. 115-16). Whether outer segments ever recover their full length after even a relatively short detachment interval remains a compelling question (see above). Reattachment in this model is relatively effective at stopping the loss of photoreceptor cells (Fig. 115-16) and cell death via apoptosis (Fig. 115-16). It is interesting to note, that it is not absolutely effective, because TUNEL positive cells persist in the retinas reattached for 28 days at about half the frequency of a retina that has remained detached for the full 28-day period. Likewise, rapid reattachment is very effective, but not absolutely so, at stopping the cellular proliferation induced by detachment (Fig. 115-16). Given the apparent redundancy in the visual pathways, the loss of a relatively few photoreceptors after reattachment may have no significant effect on vision. However, a continued, low-level of proliferation may not be so benign. Slowly proliferating cells on either retinal surface may eventually produce a cellular membrane.

Patchwork regeneration

One of the most striking features of the reattached retina is the variability in outer segment length from region to region within an individual retina, or between different retinas with the same detachment and reattachment intervals. This may reflect inherent variability in the detachment and reattachment processes themselves as well as the fact that detachments occur at variable heights, that is the distance between the RPE and photoreceptor layer. In addition, certain areas may reattach more rapidly and thus begin the regenerative process more quickly. However, it isn't clear that these variables completely account for the distinctive morphological "patchwork" appearance of photoreceptor recovery in the experimentally reattached retinas. Areas of near-normal photoreceptors may appear adjacent to areas showing almost no regeneration.[46] The results in Fig. 115-17 illustrate this phenomenon for cone outer segments. This "patchwork"

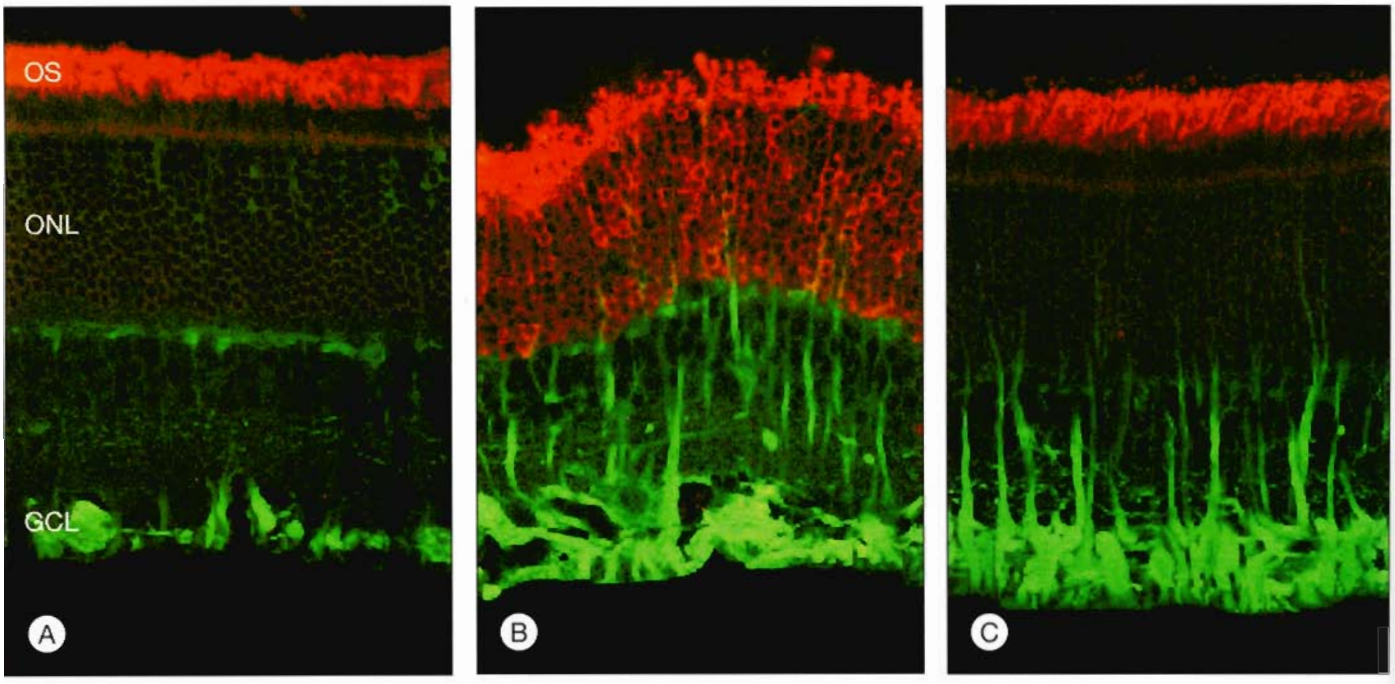

Fig. 115-15 Immunofluorescence images showing the distribution of rod opsin (red) and GFAP (green) in normal (A), 3-day detached (B) and 3-day detached/28-day reattached feline retinas (C). A, Anti-rod opsin immunolabeling is restricted to the rod outer segments and GFAP labeling to the Müller cell endfeet in the GCL in normal retina. B, Following a 3-day detachment rod opsin is redistributed to the rod cell bodies and GFAP increases in Müller cells. C, In retinas detached for 3 days and reattached for 28, rod opsin once again is restricted to the outer segments and GFAP appears "frozen" at the 3-day time point. OS, outer segments; ONL, outer nuclear layer; GCL, ganglion cell layer. (From Lewis GP, Sethi CS, Linberg KA et al. Mol Neurobiol 2003; 28:159.)

recovery is evident at the level of protein expression as well as structurally. Fig. 115-17 also shows a region of reattached retina labeled with the antibody to rod opsin. Although this entire region had been reattached, there is an abrupt transition between an area showing no opsin redistribution to an area with heavy redistribution. Outer segments in the area of opsin redistribution appear shorter and more disoriented than those in the adjoining region of no redistribution. The mechanisms producing this result are not understood. Certainly, the proliferation of either RPE cells or Müller cells into the subretinal space is nearly always associated with poor or nonexistent photoreceptor recovery. In the case of RPE proliferation, recovery seems to depend on the degree of differentiation and the polarity of the proliferated cells. In many instances the apical surface morphology of the proliferated RPE cells bears little resemblance to that of normal RPE. In others, additional layers of proliferated cells may have their polarity reversed, with their basal surface facing the photoreceptors (see Fig. 115-4). In either situation, regeneration is much poorer. Similarly, if the photoreceptors are apposed to glial (Müller) cell processes in the subretinal space, they are unable to regenerate an outer segment. Suppression of proliferation of these two cell types immediately or as soon as possible after detachment may well lead to improved visual recovery after reattachment. However, in Fig. 115-17, there is evidence of neither RPE nor Müller cell invasion of the subretinal space to explain the poor outer segment recovery and opsin redistribution.

The effect of reattachment on Müller cells

As discussed earlier reattachment is effective at greatly reducing the proliferation of non-neuronal cells, including Müller cells. Reattachment appears to halt, but not reverse, the intermediate filament response in Müller cells as well because the level of GFAP expression in the retinas reattached for 28 days is about equivalent to that observed in the retinas detached for 3 days (Fig. 115-15). Thus, the intermediate filament response appears to have "frozen" at about the 3-day level, neither progressing, nor regressing back to the level found in the control retinas. Reattachment is also effective at preventing or at least greatly slowing the growth of Müller cell processes into the subretinal space.[68] As described earlier, however, in some eyes, reattachment appears to induce the growth of Müller cell processes onto the vitreal surface of the retina. These epiretinal membranes are capable of producing wrinkling, and presumably eventual re-detachment of the retina. Intermediate filaments are known to be highly stable components of the cytoskeleton and may provide stability to cellular morphology. It may be desirable to inhibit or reverse this response in order to prevent the growth of Müller cells onto the retinal surfaces. Whether the response is reversed in longer term reattachments is not yet known.

The effect of reattachment on neuronal remodeling

Reattachment appears to stop the retraction of rod terminals. Reattachment also stops the dramatic remodeling of rod bipolar and horizontal cell processes induced by detachment. However,

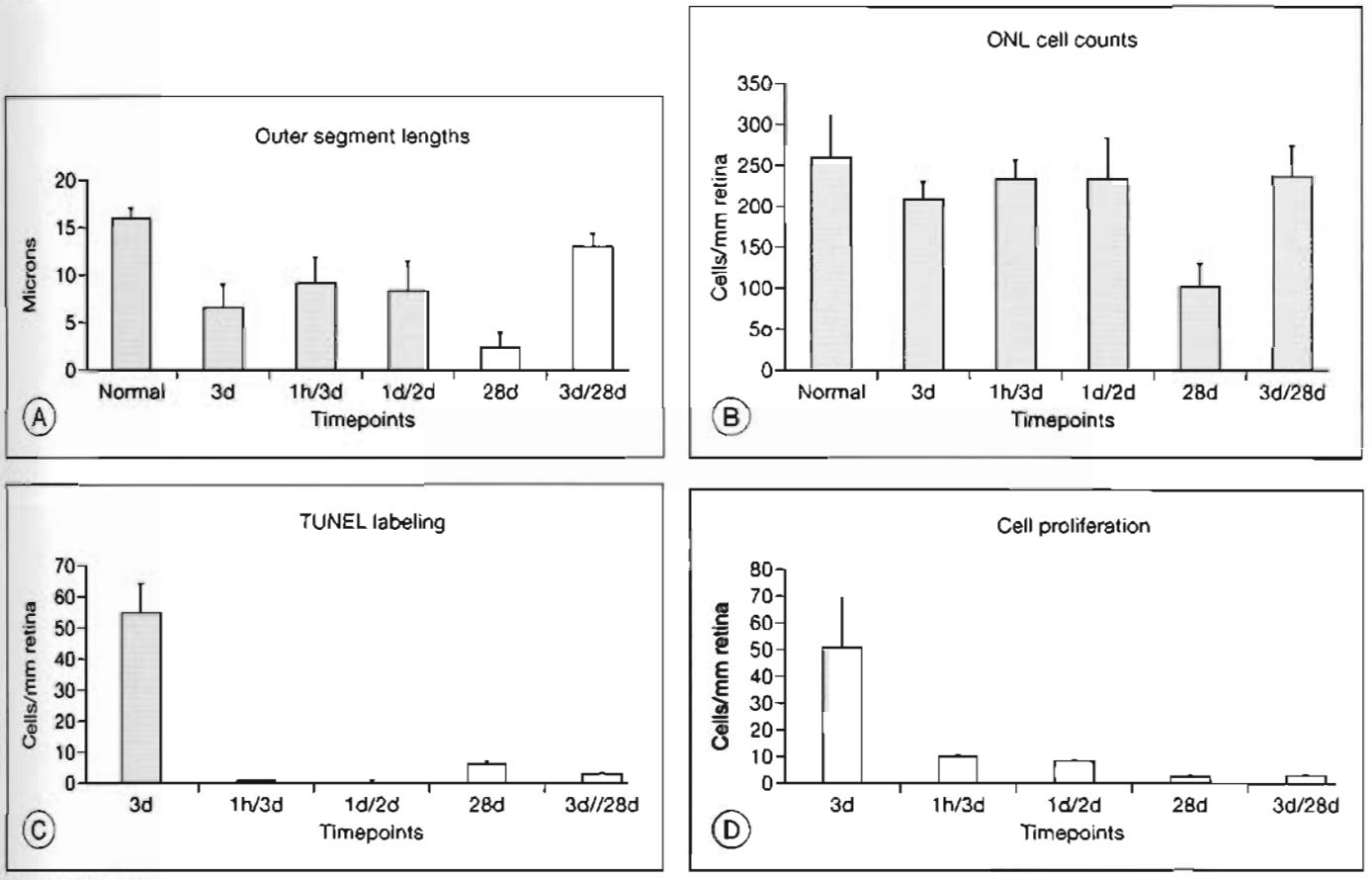

Fig. 115-16 A series of graphs showing outer segment length measurements (A), ONL cell counts (B), TUNEL labeling (C), and cell proliferation counts (D) in normal, detached and detached/reattached feline retinas. (Error bars, ±1 SD from the mean; 3d, 3 day detached; 1h/3d, 1 hour detached/3 days reattached; 1d/2d, 1 day detached/2 days reattached; 28d, 28 day detached; 3d/28d, 3 days detached/28 days reattached.) (From Lewis GP, Sethi CS, Linberg KA et al. Mol Neurobiol 2003; 28:159.)

we do not know if reattachment results in a reversal of the remodeling processes that have occurred at the time of reattachment. Reattachment does appear to create its own neuronal remodeling. Müller cells are induced to grow on the vitreal surface by reattachment where their overlapping processes can form an extensive "epiretinal membrane." Ganglion cell neurites (identified by either anti-neurofilament or anti-GAP 43 labeling) are commonly observed to grow into the resultant epiretinal membranes (Fig. 115-8). Presumably reattachment induces the rod axons to re-extend into their normal location in the OPL because this layer appears much less disrupted and because rod terminals are observed much less frequently in the ONL after reattachment.[68] However, rod axons can now be found extending into the inner retina. These can be identified in the experimental retinas because their plasma membrane labels with the antibody to rod opsin (Fig. 115-18). The fate of these terminals in longer-term reattachments is not known, nor of course is their effect on retinal circuitry in general or on visual outcome. Presently it is not known if they participate in retinal circuitry at all, although their terminals do label with an antibody to the synaptic vesicle protein, synaptophysin.

Retinal reattachment: a new retinal environment

It is no longer accurate to view reattachment as simply returning the retina to its pre-detachment state, at least in the short term. Reattachment induces the growth of Müller cell processes into the vitreous (Figs 115-8, 115-11), where they appear to act as a substrate for ganglion cell outgrowths, and it induces the growth of rod axons into the inner retina (Fig. 115-18). Both of these are indicators of an environment that is changed from that in the normal eye. Exactly what these changes are at the molecular level, for example signaling molecules that may stimulate neurite outgrowth or the vitread growth of Müller cells remains to be determined.

Correlates with human detachments

Although outer segment degeneration has long been accepted to occur in human detachments, the list of events that occur in both human retina and animal models has grown in recent years, largely due to the presence of specific probes that allow for the reliable identification of cellular events in the very small samples of retinal tissue obtained during surgical procedures.[41] Wilson and Green[33] demonstrated over 15 years ago that photoreceptor

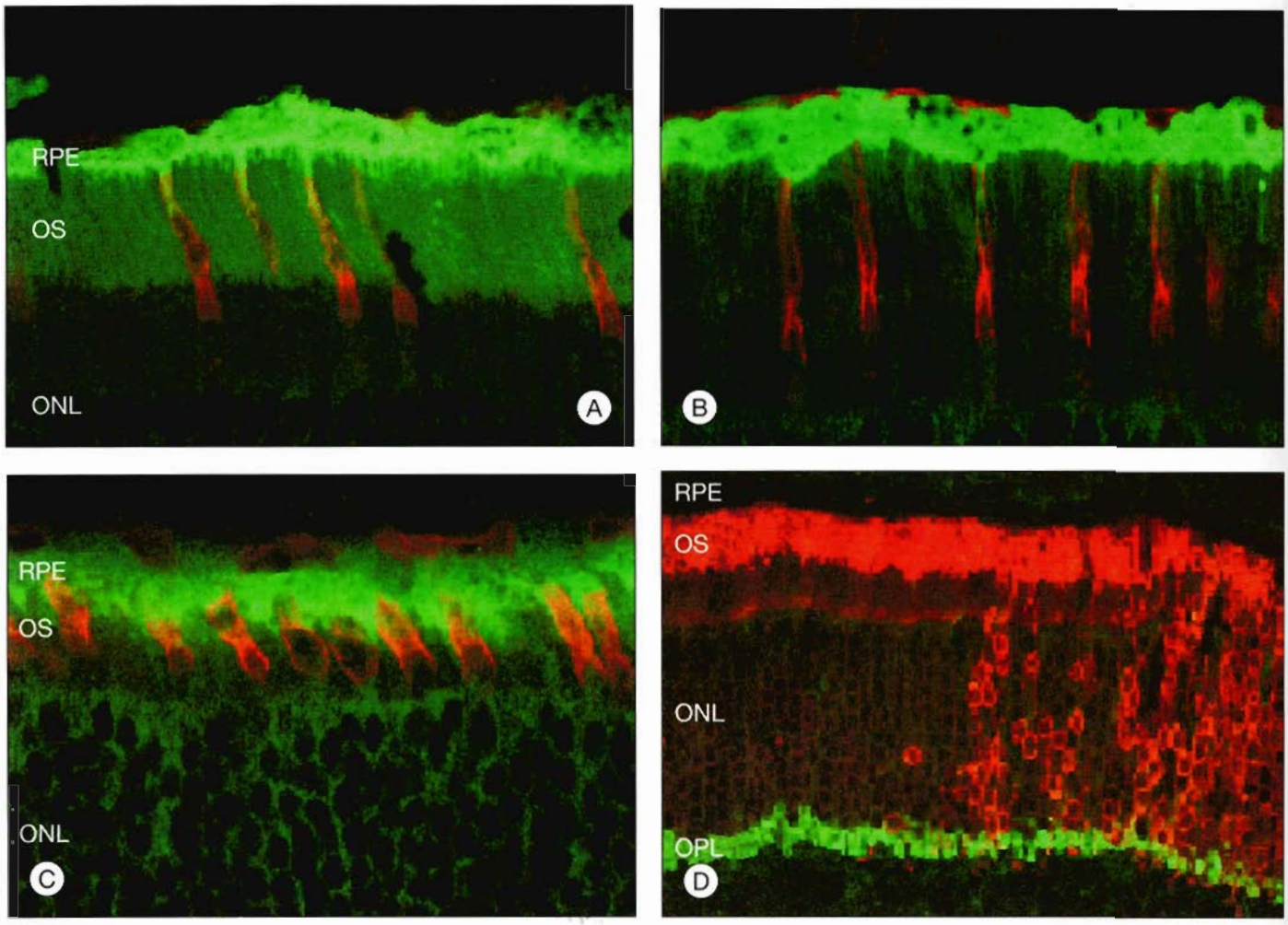

Fig. 115-17 A series of immunofluorescence images illustrating the "patchy" recovery that occurs in the feline retina following reattachment. A,B,C, Retinas are labeled with biotinylated PNA (red; labels cone matrix sheaths) and anti-cellular retinal aldehyde binding protein (green; labels the RPE). A, Normal, control retina. B,C,D, retinas detached for 3 days and reattached for 28 days. B, In many areas of the reattached retina, the cone matrix sheath appears similar to that observed in normal (A) retina. C, In some regions, however, the cone matrix sheaths appear greatly truncated. In these areas the RPE sits adjacent to the inner segments indicating little regeneration of either rod or cone outer segments. D, When the reattached retina is labeled with anti-rod opsin (red), regions within the reattached area can show different levels of outer segment regeneration. In some regions, rod outer segments are quite long and there is no rod opsin labeling in the ONL (left half of micrograph) while in an adjacent region, rod outer segments are truncated and rod opsin is redistributed to the rod cell bodies (right half of micrograph). The green in (D) represents labeling of the photoreceptor terminals with an antibody to synaptophysin. RPE, retinal pigmented epithelium; OS, photoreceptor outer segments; ONL, outer nuclear layer; OPL, outer plexiform layer. (From Lewis GP, Sethi CS, Linberg KA et al. Mol Neurobiol 2003; 28:159.)

cells are lost in human detachments, while Chang and colleagues[40] demonstrated that cell death in human detachments occurs by apoptosis. More recently events such as photoreceptor synaptic terminal retraction, opsin redistribution, neuronal remodeling, Müller cell hypertrophy, and the growth of Müller cell processes into the subretinal and vitreal cavity have all been shown to occur in human detachments (Fig. 115-19).[41] Interestingly, the extension of rod axons into the inner retina is commonly observed in human tissue samples, and many of these samples come from surgery for events secondary to reattachment, exactly the condition that elicits rod axon growth in the feline model. And of course the growth of cellular "membranes" in the vitreous as "PVR" is one of the most serious complications of reattachment surgery. Comparative data provide confidence in the use of animal models, and allow for the selection of models that have similarities to known events in human retina. This is important, since not all species react alike to detachment; as noted earlier in this chapter, feline, rabbit, and ground squirrel retinas all react differently, with each sharing some characteristics with events in humans although to date the feline model shares the most of these. Information is still critically lacking, however, on the reactions occurring within the fovea either in animal models or in human retinas.

Signaling molecules and signaling pathways

It is likely that the long list of cellular events now identified as induced by detachment and reattachment (e.g. photoreceptor apoptosis, neuronal and glial remodeling, proliferation, changes in protein expression) are all mediated through the release of

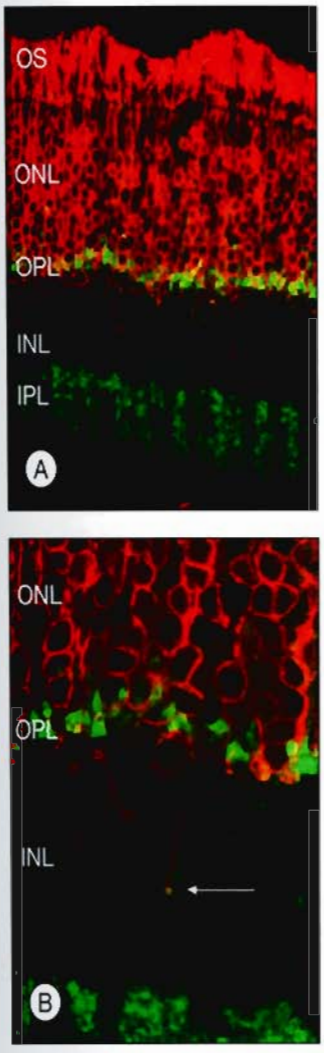

Fig. 115-18 A,B, Immunofluorescence images of feline retinas labeled with antibodies to rod opsin (red) and synaptophysin (green) illustrating rod axon growth into the INL in retinas detached for 3 days and reattached for 28 days. Some of the terminals of the rod opsin labeled processes in the INL are also labeled with the synaptophysin (arrow) indicating the presence of synaptic vesicles. OS, photoreceptor outer segments; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer. (From Lewis GP, Sethi CS, Linberg KA et al. Neurobiol 2003; 28:159.)

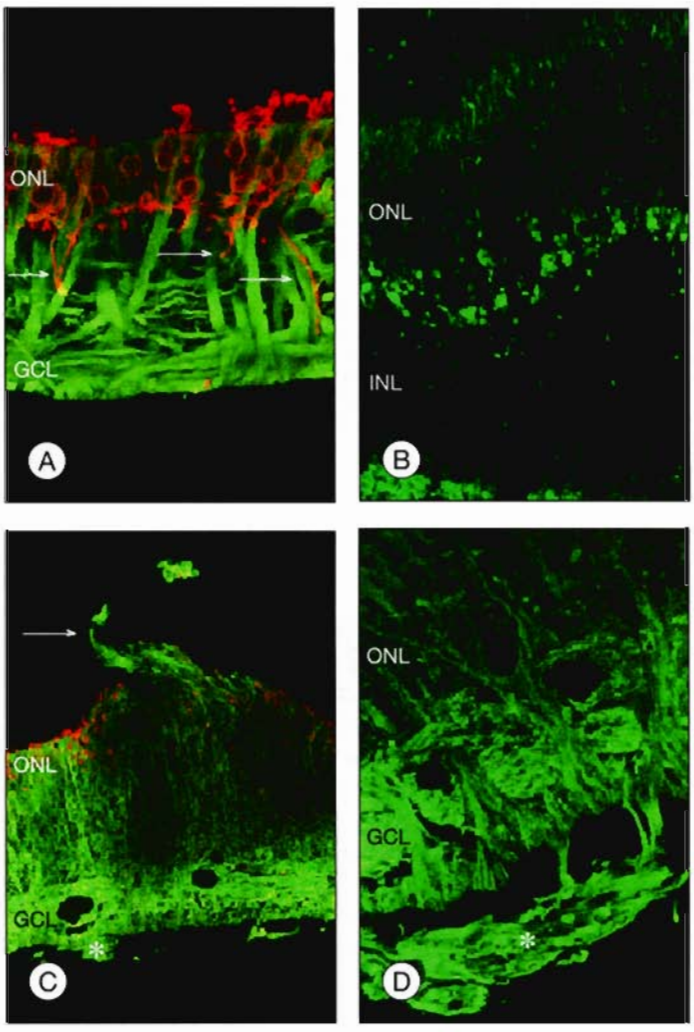

Fig. 115-19 Immunofluorescence images of human retinas that had been previously reattached, illustrating similar responses to detachment as those observed in the feline model. A, Rod axons (arrows; red; anti-rod opsin) extend into the inner retina and Müller cells upregulate their expression of GFAP (green). B, Synaptic terminals (green "dots"; anti-synaptophysin) become scattered within the ONL indicating the presence of retracted rod terminals, while the green "dots" in the INL represent terminals of "overgrown" rod axons. C, Müller cells (green; anti-GFAP) grow onto the subretinal surface (arrow) and onto the vitreal surface (asterisk). In the absence of outer segments, anti-rod opsin (red) labels rod cell bodies. D, Müller cell outgrowths (green; anti-GFAP) grow beyond the inner limiting membrane to form an epiretinal membrane on the vitreal surface of the retina (asterisk). ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. (C, D from Sethi C, Lewis GP, Fisher SK et al. Invest Ophthalmol Vis Sci 2005;46 (in press).)

signaling molecules and the activation of specific intracellular signaling pathways. As indicated earlier, FGF2 injected into the vitreous initiates many of the same events in Müller cells as detachment. Indeed, there is evidence that phosphorylation of the FGF2 receptor (flg) is initiated very rapidly by detachment, occurring within 15 minutes with subsequent dephosphorylation occurring about 2 hours later. This is accompanied by activation of the extracellular signal-regulated kinase (ERK) pathway and an increase in expression of the transcription factor AP-1 in Müller cells and RPE.[83] Experiments in which hepatocyte growth factor (HGF) is overexpressed in the RPE resulted in a serous detachment and subsequent RPE proliferation.[84] Activation of caspase-3, -7, -9 and apoptosis-inducing factor is associated with the wave of photoreceptor cell death that occurs after detachment.[85,86]

LIMITS OF MORPHOLOGICAL RECOVERY

The evidence for complete retinal recovery, even after a brief episode of detachment, is conflicting. Rabbit retinas that spontaneously reattached were described as showing limited and variable outer segment recovery.[39] In cat and primate retinas that were detached and reattached for variable periods of time, it was concluded that "ultrastructural morphology does not return to the pre-detachment state even after brief episodes of detachment coupled with prolonged recovery periods."[46] More detailed analyses have done little to change that conclusion more than 15 years later,[27,68] and have revealed evidence of even greater changes in the retina than were suspected at that time. By comparison to other neurons in the central nervous system, photoreceptors retain a remarkable capacity for recovery from

injury. And although detachment duration is recognized as an important, if not critical, variable in determining the eventual extent of morphologic recovery after reattachment, there still is very little known about the actual recovery process. At present it seems reasonable to conclude that a return to completely normal retinal morphology may never occur or occurs slowly over a time-span of months or years, even after brief episodes of detachment.[40] It also appears, however, that incomplete morphologic recovery may actually be sufficient to subserve near-normal vision under ideal circumstances. The issue of neuronal remodeling and its effects on retinal circuitry and retinal image processing is perhaps an even more complex issue than outer segment regeneration, but one that is critical to determine if we are going to truly understand the regenerative capacity of the visual system.

This raises the issue of whether the neural retina, like other organ systems, has sufficient resilience to sustain an anatomic injury such as detachment without undergoing measurable or perceived deficits in function. This may occur through the redundancy of existing retinal pathways. At present it is simply not known whether some of the structural abnormalities that may persist indefinitely after reattachment have measurable effects on visual capacity. It may be that they do not unless a particularly critical retinal location, such as the fovea, is involved.

Many of the changes described in experimental reattachments occur in small, localized regions, especially when the detachment interval is short. These may have no significant effect on the return of vision unless they occur within the macula, where even a small disruption of retinal structure could have a profound effect. The presence of a macular detachment is known to produce disturbances in acuity, metamorphopsia, and color vision that persist long after the retina is reattached.[78] Damage to or loss of some percentage of the photoreceptor population within the macula, and particularly within the fovea, could account for chronic deficits in visual function. Recent evidence for the structural remodeling of retinal neurons may indicate a greater capacity to compensate for a reduction in the number of photoreceptors in an already established mosaic than previously suspected. Of course remodeling may impose its own limits on the extent of visual recovery in this highly complex process.

ACKNOWLEDGMENTS

Our research was supported by grants EY00888 and EY02082 from the National Eye Institute, National Institutes of Health. The opsin antibodies were gifts from Drs Dean Bok and Robert Molday; the CRALBP antibody was a gift from Dr Jack Saari; the GS and CA II antibodies were from Dr Paul Linser. The authors would like to thank Drs David Charteris, Charanjit Sethi and Ken Linberg for their contributions to the project.

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