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Intraretinal Proliferation Induced by Retinal Detachment

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Cellular proliferation after retinal detachment was studied by ^{3}H-thymidine light microscopic autoradiography in cats that had experimental detachments of 0.5–180 days duration. The animals underwent labeling 2 hr before death with an intraocular injection of 200 μCi of ^{3}H-thymidine. The number of labeled nuclei were counted in 1-μm thick tissue sections in regions of detachment, in regions of the experimental eyes that remained attached, and in control eyes that had no detachments. In the normal eye, in one that had only the lens and vitreous removed, and in the eyes with 0.5- and 1-day detachments, the number of labeled nuclei ranged from 0/mm (0.5-day detachment) to 0.38/mm (lens and vitreous removed only). By 2 days postdetachment, the number of labeled nuclei increased to 2.09/mm. The highest levels of labeling occurred in two animals with detachments of 3 (7.86/mm) and 4 (7.09/mm) days. Thereafter, the numbers declined steadily until near-baseline counts were obtained at 14 days. The number of labeled nuclei was slightly elevated in the attached regions of two animals with 3-day detachments. Labeled cell types included: Müller cells, astrocytes, pericytes, and endothelial cells of the retinal vasculature, and both resident (microglial cells) and invading macrophages. In an earlier study RPE cells were also shown to proliferate in response to detachment. Thus, these data show that proliferation is a rapid response to detachment, reaching a maximum within 4 days, and that virtually every nonneuronal cell type in the retina can participate in this response. The data suggest that events leading to such clinical manifestations as proliferative vitreoretinopathy and subretinal fibrosis may have their beginnings in this very early proliferative response. Invest Ophthalmol Vis Sci 32:1739–1748, 1991

Detachment of the neural retina from the retinal pigmented epithelium (RPE) elicits responses from a number of retinal cell types. These responses are cell-type specific and can be placed in a number of overlapping categories: dedifferentiation, degeneration, migration, hypertrophy, and proliferation.[1–11] Both RPE and Müller cells are known to proliferate in response to detachment.[8,9,12–14] It is widely believed that these are the principal cell types responsible for the formation of abnormal cellular assemblages or "membranes" in the vitreous cavity and the subretinal space.[13] Clinically, these two conditions are known as proliferative vitreoretinopathy (PVR)[14–17] and subretinal fibrosis,[8,9,18–20] respectively. The former is the most frequent complication leading to failure of retinal reattachment surgery;[21] the latter can disrupt the normal intercellular relationship between the photoreceptors and RPE, thereby intefering with the regeneration of photoreceptor outer segments after reattachment.[22,23] Proliferation, therefore, is a particularly important component of the retina's overall response to injury.

We identified the cell types that participate in the proliferative response and defined the time course of that response. Our results demonstrate that detachment-induced proliferation is not limited to RPE and Müller cells but encompasses all nonneuronal cell types in the retina. This response reaches a maximum amplitude between 72 and 96 hr and then gradually declines. It is possible that this early intraretinal proliferation is the precursor to the development of more extensive proliferation that, in some cases, eventually manifests itself clinically as PVR and/or subretinal fibrosis.

Materials and Methods

Experimental Retinal Detachments

Details of the experimental retinal detachment procedure were published previously.[22] In brief, cats were anesthetized with 20 mg/kg Ketaset (ketamine HCl; Parke-Davis, Morris Plains, NJ) and 5 mg/kg Rompun (xylazine; Miles Labs, Shawnee, KS). Deep anesthesia was maintained by periodic injections of this mixture. Retrobulbar injection of 0.5 ml of lidocaine

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HCl (2%; Vedco, St. Joseph, MO) was used for local anesthesia. The lens was then removed from the eye, and the eye was allowed to heal for a minimum of 2 weeks. The vitreous was then removed with an Ocutome (Cooper Vision, Irvine, CA), and a glass micropipette (tip diameter, approximately 100 μm) was inserted between the neural retina and RPE. Without large retinal tears, the cat retina tends to reattach spontaneously within a few hours to a few days depending on the size of the detachment. To prevent this, and avoid the necessity of large retinal tears, we injected a 0.25% solution of Healon (sodium hyaluronate; Pharmacia, Piscataway, NJ) through the micropipette into the subretinal space. Sodium hyaluronate in varying concentrations has been identified as a normal component of subretinal fluid in humans.[22] In each case, a large bullous detachment covering most of the superior one half of the fundus was produced. The cats were maintained on a 12-hr light:12-hr dark cycle for a minimum of 2 weeks before detachment surgery, and the detachment surgery was done approximately 4 hr into the light period.

The retinas were detached experimentally from the RPE for 0.5, 1, 2, 3, 4, 5, 6, 7, 14, 30, 60, 90, or 180 days. One animal was used for each time except for 3, 4, and 14 days where two animals were used. Detachments were produced in the right eye only. One normal cat was used as a control. Because the lens and vitreous are removed as part of our detachment procedure, one animal was used in which only that procedure was done. All animals were treated according to the ARVO Resolution on the Use of Animals in Research.

^{3}H-Thymidine Labeling and Autoradiography

We injected 200 μCi of ^{3}H-thymidine (50 mCi/mmol specific activity, Amersham, Arlington Heights, IL) in 0.2 ml of sterile water intraocularly through a 27-gauge needle into the vitreous cavity of both eyes of anesthetized cats 2 hr before death. The cats remained anesthetized during the 2-hr incubation period.

The animals were killed by an overdose of sodium pentobarbital 4 hr after the onset of light (except for the 0.5-day detachment). Then, the eyes were enucleated, and the anterior structures were removed. The posterior portion of the eye was immersed in a mixture of 1% paraformaldehyde and 1% glutaraldehyde in phosphate buffer for 1 hr, dehydrated in a graded methanol series, postfixed en bloc with 2% uranyl acetate during dehydration in 70% methanol, and embedded in LR White resin (Polysciences, Warrington, PA).

Serial 1-μm thick sections were cut from areas of detachment in all animals, from the normal and control eyes with only the lens and vitreous removed (L/V), and from attached regions of the eyes that had detachments for 3 (n = 2), 4, 6, and 60 days. In all except the 7-day detachment and the attached regions from eyes with detachments, sections were cut from the superior retina. Two serial sections were placed near the end of a glass slide until a total of 30 slides were collected per eye. Odd-numbered slides were dipped in a 1:1 mixture of Kodak NTB-2 emulsion (Rochester, NY) and distilled water at 43°C. The autoradiograms were exposed for 7–10 days at 4°C, developed for 2 min in full strength D-19 (Kodak) at 20°C, washed, fixed, and stained with methylene blue-azure II. The even-numbered, undipped slides were stained and used to help identify cells labeled in the autoradiograms. In general, slides 3, 9, 15, 21, and 27 were selected for counting of labeled nuclei. This was done to minimize the chances that one nucleus would appear in more than one section and, hence, be counted twice. All labeled nuclei were counted and classified by cell type according to their morphology and location in a given section, using a Zeiss Universal Research Microscope (Thornwood, NY). The section length was measured with a calibrated reticule, and the data were tabulated as labeled nuclei/mm of retina. Autoradiograms were photographed with a Zeiss Photomicroscope-3.

Results

In the autoradiograms, very lightly scattered labeling occurred over all layers of the retina; such labeling was present only over the tissue specimens. Labeled nuclei were prominent against this low level of background label and were found throughout the neural retina, in the expanded subretinal space, and occasionally, in the vitreous cavity adjacent to the inner limiting membrane.

Labeled Cell Types

The nuclei of different cell types were labeled in the autoradiograms. On the basis of their location in the retina and their morphology in the light microscope, they were classified as: astrocytes, Müller cells, microglia, macrophages, and "vascular cells." The latter included both pericytes and capillary endothelial cells. Microglia are not a type of glial cell; rather, they are resident tissue macrophages in the central nervous system. Nonetheless, because of their special status in the central nervous system,[24,25] they were categorized separately to distinguish them from macrophages in the vitreous or subretinal space. Nuclei of retinal neurons were never labeled above background levels.

Four labeled cells are shown in Figure 1A. Two of

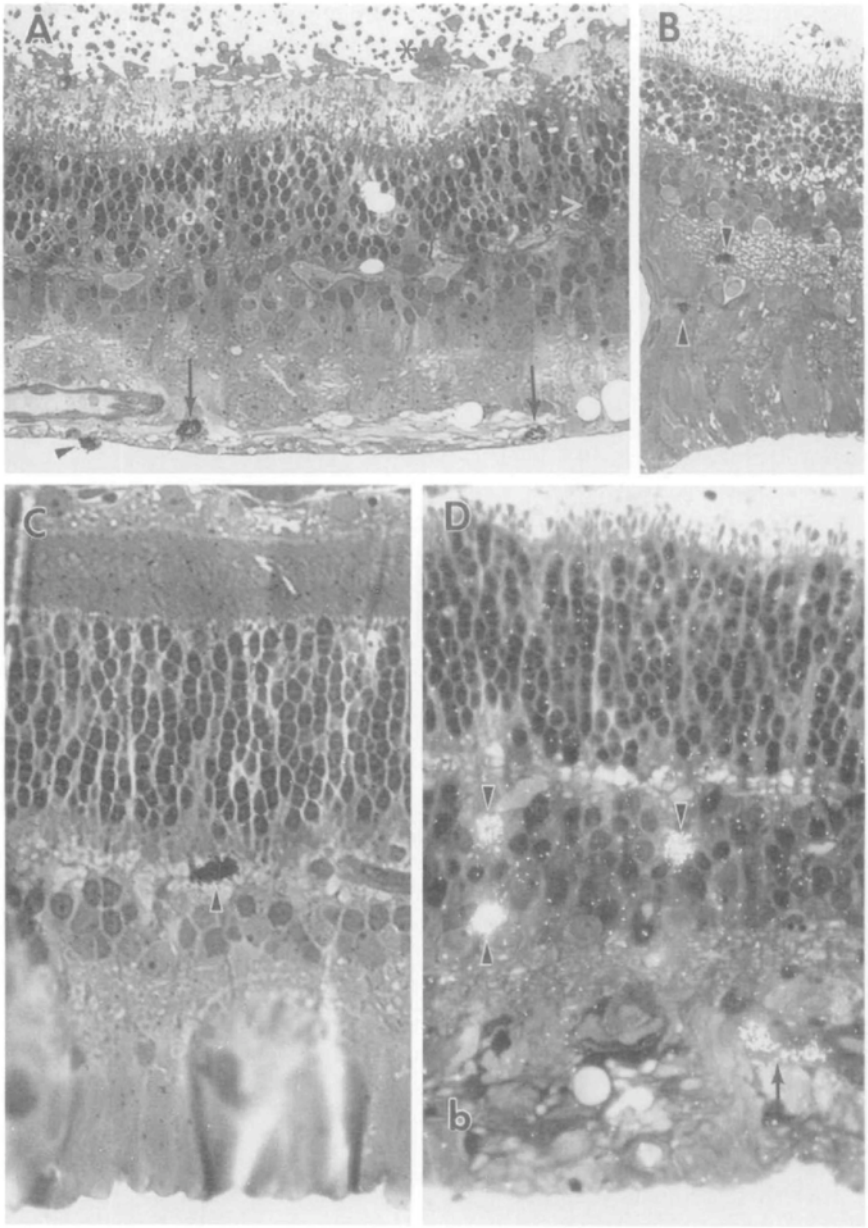

Fig. 1. Examples of labeled cells in the autoradiograms. (**A**) Four-day detachment. Four labeled cells are indicated. Arrowhead = macrophage in the vitreous; arrows = labeled astrocytes in the nerve fiber layer; white arrows = labeled Müller cell nucleus in the outer nuclear layer. The cell labeled with an asterisk is an unlabeled ameboid macrophage in the subretinal space. Both outer and inner segments show signs of degeneration while the remainder of the retina is little affected at this time-point. (**B**) Examples of two labeled astrocytes (*arrowheads*) in a retina detached for 60 days. (**C**) An example of a labeled microglial cell (*arrowhead*) in the midouter plexiform layer of an eye that had only the lens and vitreous removed. (**D**) Superimposed bright- and dark-field exposures. Three labeled Müller cell nuclei (*arrowheads*) in a retina detached for 4 days. One labeled nucleus occurs in its normal location; two have migrated to the sclerad border of the inner nuclear layer. The two labeled nuclei in the lower right (*arrow*) are associated with a retinal vessel, which is out of the field of view Original magnifications: A = ×500; B = ×400; C = ×400; D = ×1200.

these reside in the nerve fiber layer among the axons of the ganglion cells. They are in the appropriate location for, and have the morphology of, retinal astrocytes.[26,27] One labeled cell lies on the vitread border of the outer nuclear layer. Labeled cells of this type were also found in the outer plexiform layer and deeper in the outer nuclear layer. They typically had round, sometimes deeply indented nuclei, a prominent nucleolus, pale cytoplasm, a rounded cell body, and often, elongated processes extending "vertically" through the retina. These features are typical of Müller cells that have translocated as part of the response to detachment.[9,28] The third labeled cell type lies adjacent to the vitreal border of the retina. Such cells were uncommon (only two were found in this study) and had the morphology of macrophages found in the vitreous,[29] but they could also be examples of hyalocytes.[30,31] Both cells were categorized as "macrophages" in our study. A large ameboid cell lies in the expanded subretinal space in Figure 1A. Such cells had the characteristic morphology of phagocytic cells of vascular origin (mononuclear phagocytes[8,24,25]) that commonly invade the subretinal space after detachment.[8] Cells of this type were also labeled (data not shown), making up the majority of cells in the "macrophage" category.

Figure 1B shows two additional labeled cells classified as astrocytes. In this region of the retina the optic fiber layer is greatly thickened (compare with Figure 1A). One labeled astrocyte lies in the layer of optic axons; another lies on the border of the inner plexiform layer adjacent to a large, pale ganglion cell. In serial sections, the latter astrocyte was directly adjacent to a major dendrite of the ganglion cell. Although astrocytes are generally associated with the optic fiber layer and optic nerve, they also commonly occur in the location of this cell.[27,32]

Figure 1C shows a labeled cell lying in the outer plexiform layer. These cells were labeled commonly, and they invariably had oval nuclei about 5.5 μm long with prominent clumps of heterochromatin. They were always elongated in the plane of the outer plexiform layer. Based on these criteria, these cells were classified as "microglia."[33]

Three labeled cells in Figure 1D are classified as Müller cells based on their characteristic morphology. One is in the normal location for Müller cells (on the vitreal border of the inner nuclear layer); the other two have translocated to a location near the outer plexiform layer. Müller cells with displaced, labeled nuclei were first apparent at 2 days' postdetachment, becoming prominent in the 3-day detachments. In one 4-day detachment, 18 of the 43 labeled Müller cells (40%) were translocated from their normal location. In only one instance was a labeled Müller cell found vitread to its normal location. The remainder were in the outer half of the inner nuclear layer, the outer plexiform, or outer nuclear layers.

Figure 2 shows a large inner retinal vessel with six associated, labeled cells. In various sections, labeled cells around the lumen of blood vessels had the location and morphology of both pericytes and endothelial cells (Fig. 1D).[31] Because an accurate distinction was difficult to make by light microscopy, they were both classified as "vascular" cells.

Quantitation

Figure 3 shows the results of counts of labeled nuclei/mm of retinal length in each of the different retinas used in the study. In Figure 4 these counts were compiled according to cell type, and the data were combined for those times with more than one animal. The actual length of retina counted varied with an average of 17 mm, a minimum of 6.8 mm (3-day detachment, #1), and a maximum of 38 mm (control and 7-day detachment). In the retina from the normal control cat, only one labeled nucleus was found in over 9 mm of sectional length examined for the cell counts. This was the only labeled nucleus found in all of the sections from this retina (38 mm of retinal length), and it was associated with a capillary in the nerve fiber layer. In the eye with the lens and vitreous removed, examples of labeled astrocytes, microglia, and macrophages were found but the number of labeled cells was low: 0.38/mm.

No labeled cells were found in the retina detached for 0.5 days, and at 1 day, the number was essentially equivalent to the eye with the lens and vitreous removed (0.29/mm) with only labeled astrocytes identified in the autoradiograms. By 2 days' postdetachment, however, the number of labeled cells began to increase dramatically (to 2.09/mm) with cells from all classes included. The frequency of labeled cells reached its maximum at 3 and 4 days (3.51–7.86/mm). Although there was considerable variability be-

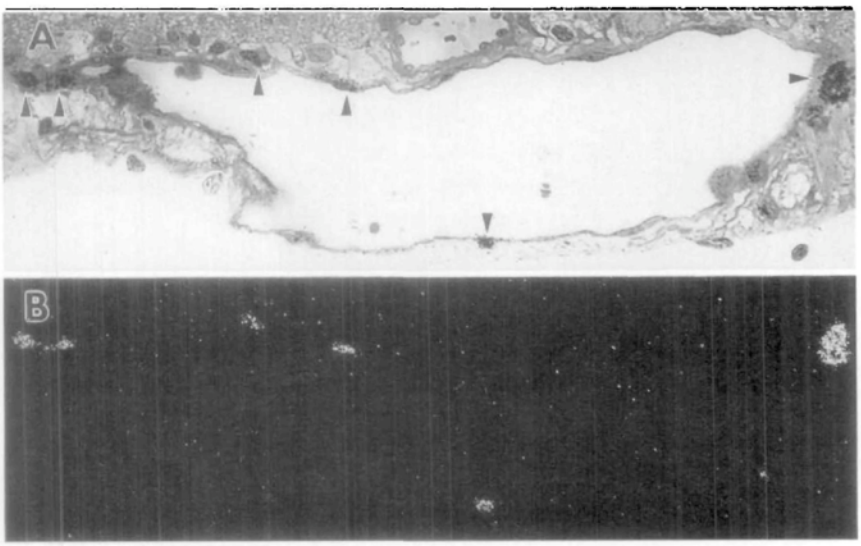

Fig. 2. Bright- (**A**) and dark-field (**B**) exposures of an autoradiogram from a 4-day detachment showing several labeled nuclei belonging to cells lining a large inner retinal blood vessel. The dark-field exposure allows for clearer identification of the corresponding cells (*arrowheads*) in the bright-field exposure (original magnification ×500).

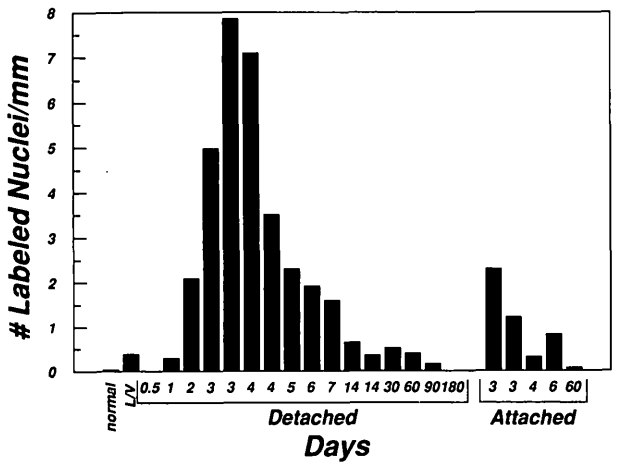

Fig. 3. The number of ^{3}H-thymidine labeled nuclei/mm of retinal length in each of the eyes used for this study. "Normal" refers to a control animal that experienced none of the experimental procedures, "L/V" indicates an eye that had only the lens and vitreous removed. In the 0.5 and 180 day detachments, no labeled nuclei were found. "Attached" refers to retina that remained attached in the eyes with experimental detachment.

tween the four animals at these two times; even the animal with the lowest number of labeled cells (second 4-day detachment) exceeded the number of labeled cells at 2 and 5 days. During this 2-day period, examples of all cell types were labeled, although glial cells (Müller cells and astrocytes) comprised just over 60% of the population. The next largest categories were those of cells associated with the vasculature (17%) and macrophages (16%) with microglia comprising slightly over 4%. After 4 days of detachment, there was a steady decline in the number of labeled cells. From 14 days of detachment through the 90-day period, the number of labeled cells declined to levels near those seen in the L/V eye and at 1 day postdetachment. The combination of astrocytes and Müller cells accounted for most of the labeled cell types found in the detachments extending from 5–90 days (Fig. 4). There were no labeled cells in the 180-day detachment.

We also examined the number of labeled cells in regions adjacent to detached areas of 3, 4, 6, and 60 days' duration. In all cases the number of labeled cells was less than in the detached region of the same eye but greater than in the normal control retina, ranging from 77 times as great in one of the 3-day detachments to 2.3 times as great in the 60-day detachment. Examples of labeled cells from all categories were found in the attached regions, but the combination of Müller cells and astrocytes comprised 54% of the labeled cells in the combined data from all five retinas. In the 60-day detachment, astrocytes were the only cell type labeled in the attached region.

The RPE was mostly unlabeled in this study. Only two convincingly labeled RPE nuclei were identified in all of the sections examined even though there were morphologic signs of RPE proliferation beginning with the 5-day detachment. These results are probably due to the low level of proliferation induced in the RPE,[12] the short labeling time, and the lack of a large retinal tear. The presence of a tear may be necessary for the ^{3}H-thymidine to gain access to the RPE during the short 2-hr labeling time.[34]

Discussion

In several previous studies the morphologic consequences of proliferation have been documented, typically as the occurrence of cell clusters in the subretinal space and less frequently as strands of cells in the vitreous cavity or as mitotic figures in the retina. In general, these studies implicate RPE and Müller cells as the main cell types involved in the proliferative response to detachment.[8,9,12,14,22,35]

Our results provide new information on proliferation in the retina that is probably applicable to many

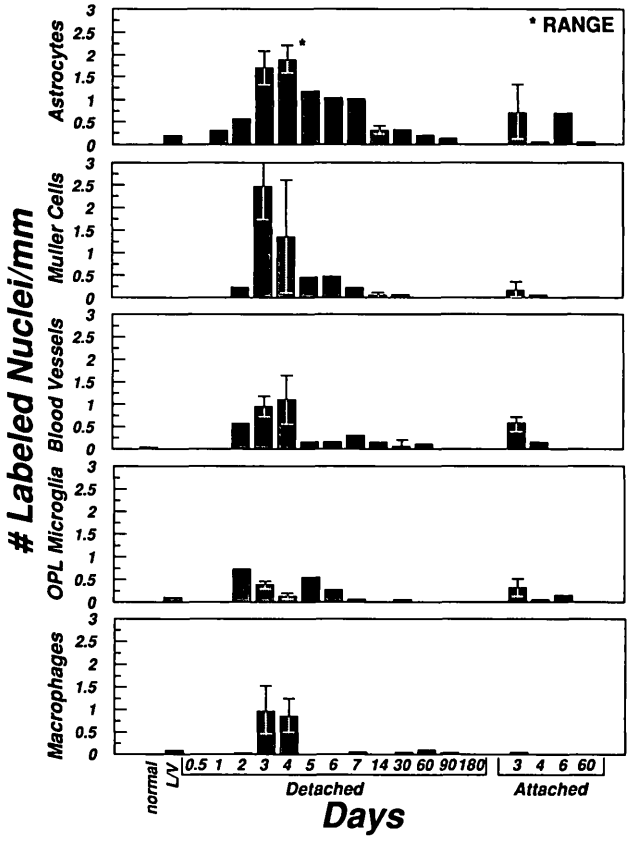

Fig. 4. The number of ^{3}H-thymidine labeled nuclei/mm of retinal length (Fig. 3) grouped according to cell type. In this group the data are presented as averages, with the range indicated, when data were available from more than one animal.

retinal injuries or diseases. We found that all non-neuronal cell types in the neural retina participate in this response including cells associated with the retinal vasculature, glia, and invading and "resident" macrophages (ie, microglia). Our results also indicate that its onset is rapid. It begins within 48 hr after detachment, reaches an apparent maximum after 3–4 days, and then gradually declines to baseline levels even though the retina remains detached. It is important to emphasize that the quantitative results represent only a small proportion of the total proliferative response, because only those cells in the S-phase of the cell cycle during the 2 hr ^{3}H-thymidine "pulse" were labeled. Continuous delivery of ^{3}H-thymidine from the time of detachment to the time of death is the only way to obtain an indication of the true magnitude of the response. An estimate of the total number of labeled cells expected in a 30-day retinal detachment can be calculated, however, by assuming: (1) that the proliferative response follows a time course and pattern similar to that shown in Figure 3 and (2) that the number of proliferating cells identified during a 2-hr labeling interval is directly proportional to the total number expected for a single, 24-hr period. (The original counts (L_o) of labeled cells/mm of retina were adjusted using the standard correction of Abercrombie[36] for estimating nuclear counts from histologic sections. This correction factor adjusts L_o downward to take into account the possibility of counting the same cell twice in successive sections. L_c = the corrected number of cells/mm. The number of labeled cells in one 24-hr period was then estimated to be $12 \times L_c$ based on assumption (2). That number, or $12L_c$, multiplied by 10 gives the number of labeled cells/cm of retinal length. The number of cells/cm of retinal length is based on data from 1-μm thick sections and must be multiplied by 10^4 to estimate the number of cells/cm^2 of retina. The number of cells/cm^2 of retina for days 1–7 was calculated directly from data in Figure 3; the mean of days 7 and 14 was used to estimate the value for days 8–13; and similarly, the mean of days 14 and 30 was used to estimate the value for days 14–30. These data were then summed to provide a cumulative estimate of the total response over a 30-day interval.) By adopting this simple set of assumptions, approximately 6×10^6 cells would be predicted to enter S-phase in 1 cm^2 of retina detached for 30 days. The origin of PVR or subretinal fibrosis, therefore, may lie in the early proliferation of cells in the retina, the migration of some fraction of these cells into the vitreous and/or subretinal space, and their subsequent proliferation at those locations.

The proliferative response identified in this study cannot be attributed merely to the presence of a low concentration of subretinal sodium hyaluronate. Earlier studies in which rhegmatogenous detachments were produced with balanced salt solution also showed evidence of intraretinal proliferation.[9] A recent study in which balanced salt solution detachments were produced in conjunction with a longer ^{3}H-thymidine labeling interval yielded similar results to those reported here.[37]

In addition to detachment, cell proliferation is a common feature of other retinal diseases and injuries including: diabetes,[38–41] photocoagulation,[42] massive retinal gliosis,[43] branch retinal vein occlusion,[44] PVR,[15,20,44–47] and macular pucker.[48] In most of these conditions, identification of the cell types involved and the time course of the proliferation are unknown. The proliferation of capillary endothelial cells is presumed to be a component of the retinal response to diabetes.[49] One study[39] used ^{3}H-thymidine autoradiography to demonstrate endothelial cell (but not pericyte) proliferation in streptozotocin-induced diabetic rats.

Proliferative vitreoretinopathy is the most common complication in retinal reattachment surgery.[50] Both "glial" cells and RPE are generally regarded as the principle cell types in this process,[14,35,51] based on morphologic criteria.[13] Recent immunocytochemical evidence using cell-type specific antibodies indicates that Müller cells are a significant component of human epiretinal membranes of diverse pathologic origin.[52] Our results suggest that the "glial cell" response may eventually be shown to include both retinal astrocytes and Müller cells. Because these two cell types may respond to different chemotactic factors,[13] however, they may not have the same propensity to migrate into the vitreous cavity.

Proliferation in the retina has received relatively little attention compared with the extensive attention it has received in the brain. The proliferation of astrocytes is a common event in many injuries of the central nervous system. Janeczko[53] showed that unilateral injury of the cerebral hemisphere in rats caused astrocytes to begin proliferating within 2 hr, reaching a maximal response at 2 days postinjury. By 4 days the response had declined to the level seen in control tissue. Similar results were reported for other types of brain injury, showing that proliferation of astrocytes reaches a peak at 2–3 days after lesioning.[54–57] Brain ischemia also stimulates proliferation of astrocytes 3–7 days after the ischemic episode with production of a "glial scar" by 10 days.[58] Intraventricular administration of the neurotoxin, kainic acid, also produces the hypertrophy and proliferation of astroglial cells in the rat hippocampus although the time course of the response was not determined.[59]

Müller cells are highly specialized astrocytes, and they react to injuries such as retinal detachment characteristically. Our previous morphologic studies on both cats and monkeys show that these cells hypertrophy and proliferate both in the retina and in the subretinal space.[8,9,22] The proliferative response is accompanied by an upregulation of intermediate filament proteins and a downregulation of other intracellular proteins.[52,60] Data from experimental detachment suggest that some Müller cell nuclei migrate into the outer nuclear layer or subretinal space within a few days of detachment. After reattachment, Müller cells in the subretinal space interfere with outer segment regrowth and, thus, may adversely affect visual recovery.[22] The effect of Müller cell scarring in the retina is unknown although morphologic studies suggest that photoreceptors can withdraw their synaptic terminals during detachment and the presence of hypertrophied cells in the outer plexiform layer may interfere with eventual reestablishing of such connections.[9] A similar process has been hypothesized for astrocytic scars in the central nervous system.[61]

Neovascularization is not usually recognized as a response to retinal detachment although it is common in proliferative diabetic retinopathy[37] and retinal ischemia, where the peak proliferative response of both pericytes and endothelial cells occurs 2–3 days after reperfusion.[62] Our data indicate that detachment is sufficient to initiate the process of both pericyte and endothelial cell proliferation. The response of the vascular cells appears to be self-limiting and thus usually does not proceed to the point of clinically recognizable neovascularization. It may be a pervasive effect, however, eventually leading to the peripheral neovascularization that can occur in chronic rhegmatogenous retinal detachments.[63]

A significant, but previously unrecognized, response to detachment is the proliferation of microglia. The latter were first described as one of the cellular components of the central nervous system by del Rio-Hortega[64] who proposed that there were two types: ameboid and ramified. Ramified cells were generally viewed as quiescent; the ameboid ones were "activated" by various injuries to the central nervous system.[24,65,66] The transformation of ramified microglia to the ameboid, phagocytic phenotype and the proliferation of these cells at the site of central nervous system injuries has been a source of considerable controversy. There is now evidence that microglia proliferate after stab wounds[67] or electrolytic lesions[66] to the brain, injury to the spinal cord,[69] and axotomy.[70] One study showed that the mammalian brain contains two types of mononuclear phagocytes, ramified microglia and blood-born macrophages, that clear debris from sites of injury.[71] These cell types secrete cytokines, including interleukin-1 (IL-1), that stimulate both reactive astrogliosis and angiogenesis at the site of injury.[24,71–74] Moreover, after production of a penetrating wound to the cortex, the greatest number of phagocytic cells appears 2 days after the injury, peak production of IL-1 occurs at 3 days, and a fivefold increase in reactive astroglia and "marked neovascularization" appears at 5 days.[71] Our data suggest that the ramified microglia of the outer plexiform layer[33] are stimulated to proliferate by detachment. By analogy to data from elsewhere in the central nervous system, they almost certainly transform into the ameboid phenotype at which point they become actively phagocytic. We identified phagocytic cells in the detached retina by electron microscopy.[9] Because of the small number of cells, and limited number of animals, we cannot determine whether the proliferation of microglia (and invading macrophages) precedes that of the astrocytes, Müller cells, and vascular cells; certainly they occur in the same general time frame, suggesting a potential role in regulating the proliferation of other cell types. Microglia also occur in the inner plexiform layer of the cat,[33] but most of the labeled cells we found in that layer appeared, on the basis of their close proximity to the ganglion cell layer, to be astrocytes.

In general, the time course of the proliferative response to detachment agrees well with that reported for other central nervous system injuries—major proliferative events appear to occur within 2–5 days in most cases.[54,56,75–78] In recent studies of induced proliferative responses in retina,[79] subretinal vitreous induced a proliferative response within 5 days; others[80] showed that the injection of irradiated autologous fibroblasts into the vitreous of rabbits induced maximum ^{3}H-thymidine incorporation into the retina at 3 days; and the peak mitotic response to retinal ischemia occurred at 4 days.[62] These data support the conclusion that the 2–5-day "window" is critical for initiation of the proliferative response in the retina and elsewhere in the central nervous system.

Of critical importance to understanding, and eventually controlling, the proliferative response to retinal detachment is an understanding of the mechanisms involved. Our data showing that there is a slight increase in labeled cells in the attached region of eyes with detachment agrees with data from other central nervous system injuries that these responses are diminished in magnitude at sites away from the immediate injury. This, in turn, suggests that one or more localized factors may mediate the proliferative response. A number of "growth factors" are potential candidates for that role, including: platelet-derived

growth factor, acidic and basic fibroblast growth factor, epidermal growth factor, glial maturation factor, glial growth factor, IL-1, glial growth-promoting peptides, and thrombin.[13,71,81-83]

Proliferation plays an important role in retinal detachment and in many other central nervous system diseases and injuries. Our results, together with those obtained using other models, identify the onset and overall time course of this response. In addition, our present and previous data[12] demonstrate that detachment is sufficient to elicit a rapid, localized proliferative response, not only from glia, but from all resident cell types except neurons. The nature of this response is consistent with the interpretation that it is mediated by diffusible "factors," secreted in the vicinity of the injury. Identifying these putative factors, the cell types that synthesize them, and their cellular targets will be important in future attempts to inhibit or modulate the proliferative response. The retinal detachment model should provide an excellent in vivo system for achieving these goals.

Key words: proliferation, ^{3}H-thymidine, autoradiography, retinal detachment, retina

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