sections of the globe. Microdissected RPE from a melanoma specimen was used as a control. The quantitative real-time PCR was performed using the forward primer IS6 (5′ AGGCGAACCCTGCCCAG-3′) and reverse primer IS7 (5′ GATCGCTGATCCGGCCA-3′), which amplified a 122–base pair fragment of the IS6110 multicopy element (GenBank accession No. X52471).[3] The standard DNA used was 10 ng to 10 pg of *M tuberculosis* genomic DNA (Colorado State University). The amount of the test sample product was interpolated from the standard curve of cycle threshold values generated from known starting concentrations of standard *M tuberculosis* DNA. An average of 3 different runs revealed about 1.7×10^6 bacterial genomes in the RPE layer. The controls were negative for DNA amplification (**Figure 2**).

Comment. The present clinicopathologic study combined with microdissection and real-time PCR analysis clearly revealed distribution of the mycobacteria in the RPE even though the retina and uvea were involved with the inflammatory process. Such findings suggest preferential localization of *M tuberculosis* in the RPE in eyes with panuveitis or related intraocular inflammation, including multifocal or serpiginouslike choroiditis resulting from tuberculosis.

In the pathogenesis of pulmonary tuberculosis, the mycobacteria are taken up by the alveolar macrophages that express complement and toll-like receptors.[4] The bacteria are usually destroyed in the phagosomes when they fuse with lysosomes, exposing the bacteria to acid PH, reactive nitrogen species, and lysosomal enzymes.[4] However, the mycobacteria can inhibit the fusion, thus avoiding the microbicidal activity, and may then thrive in the phagosome by avoiding immune surveillance by the T lymphocytes. Since RPE shares several functions with the macrophages, including phagocytosis of bacteria and expression of toll-like and complement receptors, the bacteria noted in the RPE may represent the phagocytosed bacteria.[5] Moreover, the presence of numerous organisms in the RPE may suggest that the organisms thrive in the RPE by preventing phagolysosome fusion. Furthermore, the present report suggests that recurrences in tuberculous choroiditis could result from reactivation of sequestered organisms in the RPE. Prevention of such recurrences and elimination of the sequestered organisms require a longer course of treatment with systemic antimycobacterial agents, preferably at least 6 to 9 months.

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Glial and Neural Response in Short-term Human Retinal Detachment

Histopathological changes following acute retinal detachment have been well documented in animal models.[1-3] To date, however, the changes that follow an acute human retinal detachment are not well characterized owing to the difficulty in obtaining retinal specimens. When retinal detachment is complicated by proliferative vitreoretinopathy, samples obtained from patients undergoing retinectomy provide an insight into the pathologic abnormalities of more chronic stages of retinal detachment. These changes include glial cell intermediate filament up-regulation, glial extensions into epiretinal and subretinal membranes, photoreceptor outer and inner segment disorganization, opsin redistribution, photoreceptor axon retraction and neurite extension, and second- and third-order neurone remodeling.[4] In this histopathological case report, we extend our previous studies to the analysis of a patient with a short-term retinal detachment.

Report of a Case. A 74-year-old woman was admitted to Manchester Royal Eye Hospital with a 10-day history of visual loss in her right eye. She had noted increasing floaters in this eye for approximately 1 month prior to this. She was found to have a bullous superotemporal rhegmatogenous retinal detachment involving the macula. She had no previous history of ophthalmic disease but had asthma and hypertension. Before retinal surgery could be undertaken, she had a cardiac arrest and died on the ward. With the relatives' consent, the eyes were obtained for analysis.

Methods. The right eye was fixed in formalin within 6 hours of death. Retinal tissue was sampled from various sites within the detachment both adjacent to the retinal break and in areas further removed from this region. Samples were embedded in agarose and cut as 100-µm sections. Retinal sections were then double-labeled for immunohistochemistry by using antibodies to retinal glia (glial fibrillary acid protein), photoreceptors (rod opsin, M and S cone opsins), horizontal cells (calbindin D), synaptic vesicle protein (synaptophysin), and retinal pigment epithelium cells (cellular retinaldehyde binding protein). Secondary antibodies were conjugated to Cy2(green) or Cy3(red) and viewed using a BioRad 1024 confocal microscope (Bio-Rad Laboratories, Hercules, Calif). The techniques used and antibody sources have been detailed previously.[4] TUNEL staining was performed to identify apoptotic cells.[5]

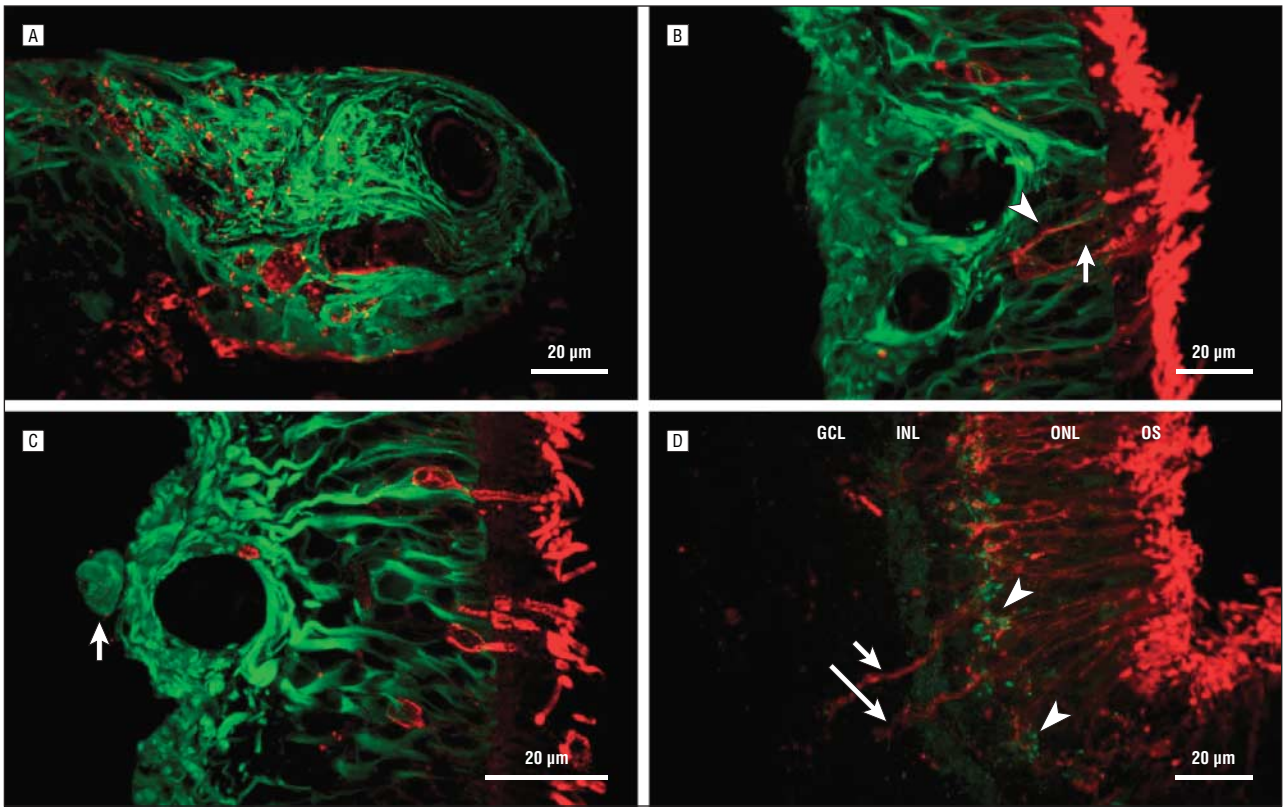

Figure 1. Double-labeled laser scanning confocal images showing the distribution of rod opsin (red), glial fibrillary acidic protein (GFAP) (green; A-C), and synaptophysin (green; D) in areas of detached retina adjacent to a retinal tear. A, Confluent gliosis, glial remodeling (GFAP, green) and scattered disorganized rod remnants (rod opsin, red) at the edge of the retinal break. B, Glial cell up-regulation (GFAP, green) and rod opsin redistribution (red) to a rod cell body (arrow). Early rod neurite extension (arrowhead), usually a feature of more long-term detachments, is also present. C, Area of early glial epiretinal membrane formation adjacent to a retinal vessel (arrow, GFAP, green). The rod outer segments (OSs) show moderate disorganization and redistribution of rod opsin to the cell bodies (rod opsin, red). D, Synaptophysin (green) labeling of clumped rod and cone synaptic terminals in the outer plexiform layer (OPL) (arrowheads). In short-term experimental retinal detachments, antisynaptophysin labeling is also present in the outer nuclear layer, leaving a disorganized OPL. Here, rod neurite extensions (rod opsin, red) pass through the inner and outer plexiform layers to inner retina (arrows). In this specimen, outer segments are more disorganized than in B, and there is a corresponding increase in the redistribution of rod opsin to more proximal cell compartments. Such "patchy" variability in retinal degeneration is a feature of experimental retinal detachment. GCL indicates ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; OS, outer segment.

Results. Macroscopically, retinal breaks were identified within the area of detachment. Samples of retinal tissue selected from foci adjacent to the retinal breaks demonstrated more advanced degenerative changes when compared with tissue from areas more distant from the breaks.

In the retina immediately adjacent to the retinal breaks, there was obvious loss of normal retinal architecture with extensive glial cell hypertrophy forming a confluent scar around the break edge (**Figure 1**). In this area, there was marked loss of neural retinal elements, including photoreceptor inner and outer segments and redistribution of rod opsin to rod cell bodies. There was also evidence of rod axon extensions toward the ganglion cell layer (Figure 1D).

In areas of detachment remote from the retinal breaks, pathological changes were less extensive with residual (though disorganized) rod and cone outer segments (**Figure 2**). Photoreceptor inner segments were preserved, and there was redistribution of opsins to photoreceptor cell bodies. Very occasional apoptotic photoreceptor cells were observed (TUNEL data not shown). Irregular synaptophysin immunostaining was seen in the outer plexiform layer. Marked glial fibrillary acid protein up-regulation and evidence of internal limiting membrane disruption suggested early glial epiretinal membrane formation (Figure 2). Calbindin labeled horizontal cells (and cones).

Comment. This case demonstrates a range of glial and neural retinal pathologic abnormalities. The pathological changes observed in areas of detachment remote from the retinal breaks were consistent with those observed in animal models of acute retinal detachment. In the feline model, a 3-day detachment shows degeneration of the rod and cone outer segments, redistribution of opsins to the photoreceptor cell bodies, withdrawal of rod synaptic terminals toward their cell bodies, outgrowth of rod bipolar and horizontal-cell neurites, and Müller cell proliferation and hypertrophy.[3] Photoreceptor apoptosis has also been demonstrated in acute retinal detachment,[6-8] which peaks at around day 3 or 4 after retinal detachment but then decreases dramatically.[8] This is consistent with our results; we found little evidence of apoptosis, which suggests that at 10 days after detachment the major wave of apoptosis has already passed.

The potential for long-term sequelae following an acute retinal detachment in humans is demonstrated in this case by the observation of areas

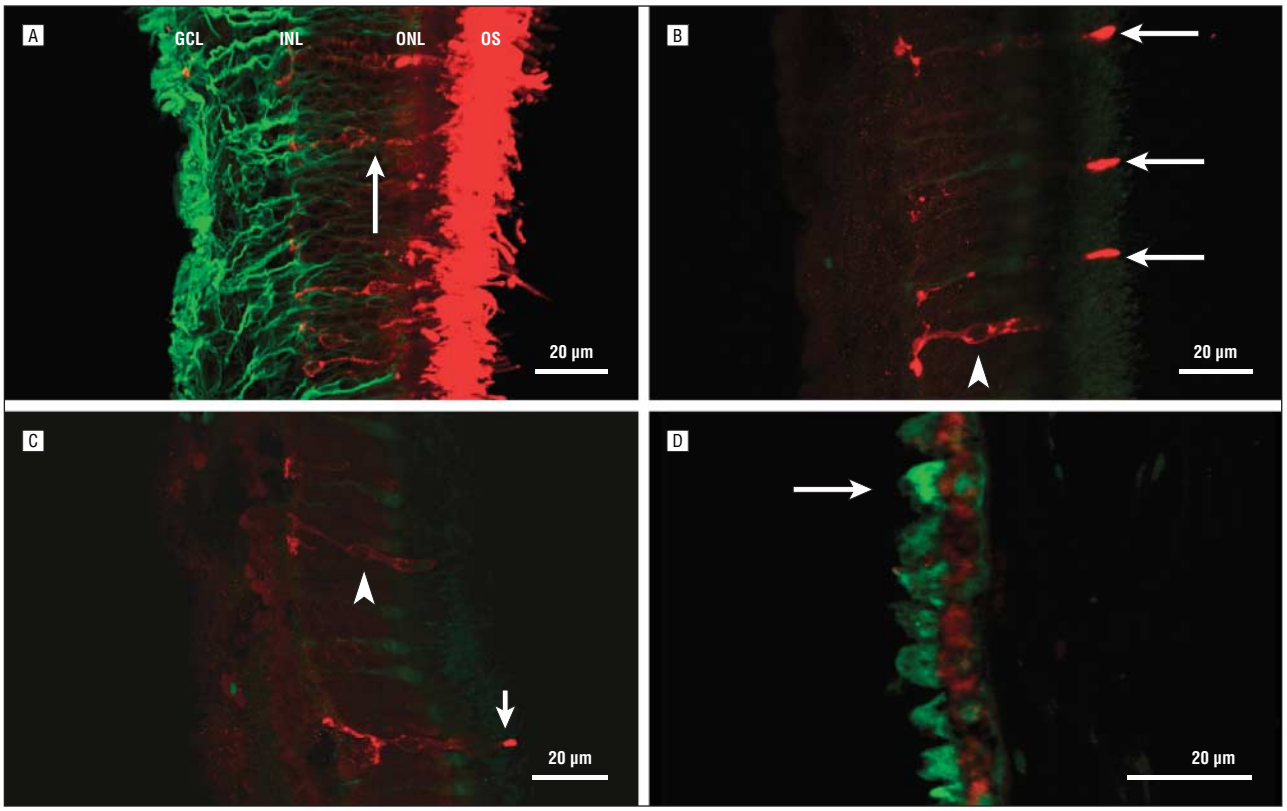

Figure 2. Double-labeled laser scanning confocal images showing the distribution of rod opsin (red; A), calbindin D (green; B and C), S cone opsin (red; C and D), and glial fibrillary acidic protein (GFAP) (green; A) in areas of detached retina distant from the retinal tear. A, Good rod outer segment (OS) preservation (rod opsin, red) and some rod opsin redistribution to cell bodies (arrow) consistent with changes observed in a 3-day detachment in the feline model. Area of glial up-regulation (GFAP, green) and associated irregularity of the internal limiting membrane. B and C, S cones (red) demonstrating preservation of the OS (arrows) together with redistribution of cone opsin to the cell body (arrowhead). Cones begin to lose the expression of many proteins (including cone opsins) at an early stage following retinal detachment as observed within regions (C) exhibiting shortening (arrow) and loss (arrowhead) of OS with redistribution of cone opsin to the cell body. It also shows faint staining with calbindin D (green). D, Cellular retinaldehyde binding protein (green) and variable mounding of retinal pigment epithelium cells (arrow) (similar to experimental retinal detachment[2]). Red indicates lipofuscin autofluorescence. GCL indicates ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer.

of early neural circuitry change (photoreceptor neurite extension and synaptic remodeling), photoreceptor disorganization, and early epiretinal membrane formation (Figure 1B and C)—changes analogous to those seen in animal models.[1]

There was advanced glial scarring and marked loss of photoreceptors adjacent to the retinal breaks (Figure 1). This is more consistent with the findings observed in a 28-day retinal detachment in the feline model.[2] The severity of this pathologic abnormality may be attributed to a localized peripheral retinal detachment having been present around the retinal tear for longer than the 10-day history of visual loss. The initial retinal break may well have occurred at the time of onset of floaters 1 month (or longer because the history was vague) before visual loss. Alternatively, the effect of the trauma of the retinal tear and/or ischemic disruption of the local inner retinal circulation may induce necrotic (including apoptotic) cell death and marked gliosis in the surrounding retina.[9] In animal models, retinal detachment induction is highly controlled with a micropipette; this differs from the clinical pattern of events in which acute retinal tears of variable size are induced by vitreoretinal traction at the time of posterior vitreous detachment. It is possible that retinal tearing may act as a more potent stimulus for cellular disorganization, loss, and remodeling, leading more rapidly to the advanced pathologic abnormalities usually seen following longer periods of retinal detachment in animal models.

The nature of the observed pathologic abnormalities in this case is consistent with those found previously in animal models and is similar to, but less advanced than, the changes seen in human proliferative vitreoretinopathy.[4] The similarities to this and other human studies emphasizes the validity of the animal models currently used in the investigation of the cellular and molecular events that follow retinal detachment.

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Perimacular Retinal Folds Simulating Nonaccidental Injury in an Infant

Ophthalmologic abnormalities are important in the evaluation of infants suspected of being abused. A common form of child abuse is shaken baby syndrome (SBS), in which an infant is violently shaken, producing rapid, abrupt acceleration and deceleration of the cranium. Retinal hemorrhages are frequently found in children with SBS. A particularly severe form of retinal injury in SBS, traumatic retinoschisis, is characterized by a dome-shaped cavity in the macula with elevated perimacular folds at the periphery of the cavity.[1]

The etiology of perimacular folds has been the subject of debate. Massicotte et al[2] reported persistent attachment of the vitreous to the internal limiting membrane at the apices of these folds and suggested that this finding might constitute evidence of violent shaking. A recent report, however, described similar retinal findings in a 13-month-old infant who sustained a skull fracture and intracranial hemorrhage after a television fell on the infant's head.[3] We describe a child with perimacular folds and retinoschisis following a severe crush injury.

Report of a Case. A 4-month-old boy was on the floor when a 12-year-old, 63-kg child fell while running backward over an adult who was changing the infant's diaper, transmitting her entire weight through her buttocks directly to the infant's head. The infant was immediately unresponsive. Evaluation at the hospital revealed a large, comminuted, displaced parietal bone fracture; subdural and intraventricular hemorrhage; and brain herniation. Ocular examination revealed no visual responses and fixed, dilated pupils. Fundus examination revealed a large vitreous hemorrhage in the right eye. In the left eye, there was a macular retinoschisis cavity with elevated perimacular folds (**Figure 1**) and diffuse, 4-quadrant, multilayer retinal hemorrhage. Evaluation by the child protection team revealed no other physical findings or historical information suggestive of abuse. The child was found to be brain dead and he died. Two adults who witnessed the event were interviewed separately shortly after the incident and reported identical details. Forensic investigators determined that the incident was accidental. Histopathologic evaluation of the eyes revealed retinoschisis of the right eye with blood dissecting between the outer nuclear layer and the inner segment and extensive retinal hemorrhage of the left eye with multiple foci of hemorrhage involving all layers of the retina (**Figure 2**).

Comment. Retinal findings similar to our patient's have been described in adults with Terson syndrome. Keithahn et al[4] postulated that this results from a rapid rise in intracranial pressure being transmitted through the optic nerve, resulting in extravasation of fluid from intraretinal vessels and separation of the internal limiting membrane. We postulate a similar mechanism in our patient, who had a very rapid, massive increase in intracranial pressure, resulting in hemorrhage within

Figure 1. Fundus photograph of the left eye demonstrating elevated perimacular fold.

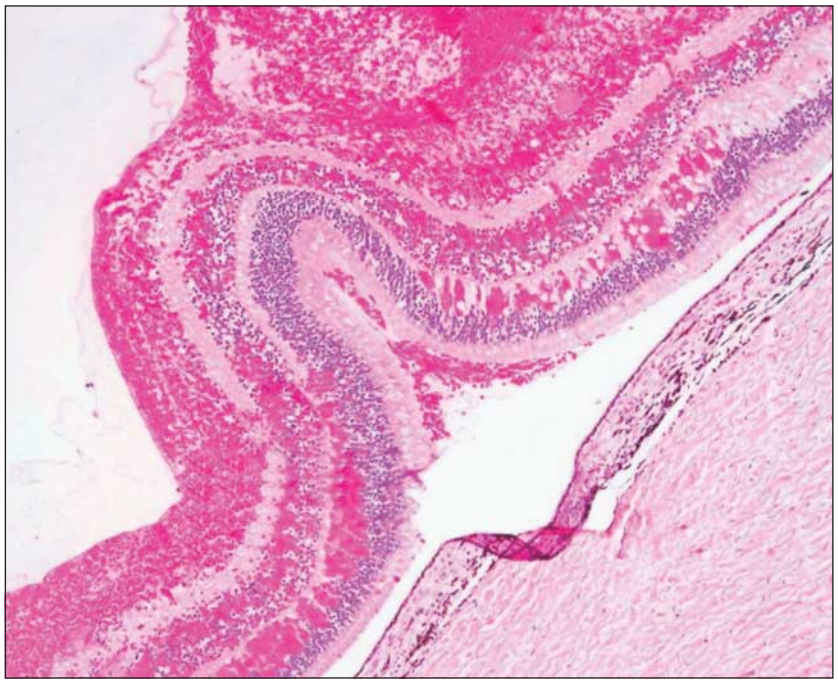

Figure 2. Postmortem photomicrograph of the left eye demonstrating perimacular fold in which the ganglion cells are obscured by severe hemorrhage. Hemorrhage is present in the intraretinal and subretinal layers (hematoxylin-eosin, original magnification ×20).