Tuberculous Uveitis: Distribution of Mycobacterium tuberculosis in the Retinal Pigment Epithelium

The diagnosis of intraocular tuberculosis has been a challenge until the introduction of polymerase chain reaction (PCR) to detect the mycobacterial-specific DNA sequence in the intraocular fluids. Such investigations have confirmed diverse manifestations of intraocular tuberculosis including retinal vasculitis and serpiginous-like choroiditis. The latter may manifest with multifocal lesions involving the inner choroid, which coalesce to form a geographic pattern simulating serpiginous choroiditis. Although, clinically, the choroiditis appears to involve primarily the inner choroid and retinal pigment epithelium (RPE), the presence of mycobacterium at these anatomic sites has not been documented. Herein, we describe a case of panuveitis that was clinically of unknown cause but for which histopathologic examination of the globe disclosed selective distribution of acid-fast organisms in the RPE. These were confirmed to be Mycobacterium tuberculosis by microdissection of the RPE, followed by real-time PCR.

Report of a Case. The enucleated left eye of a 48-year-old woman was submitted to the Doheny Eye Pathology Laboratory with a brief clinical history, revealing a progressively worsening intraocular inflammation in the left eye despite treatment with topi-

Figure 1. Histologic examination of the globe. A, Necrotic retinal pigment epithelium (RPE) with granulomatous inflammation involving the choroid (hematoxylin-eosin). The insert reveals several acid-fast bacteria in the RPE (Ziehl-Neelsen). B-D, The immunohistochemistry of the necrotic RPE cells. The cells are negative for CD68 (B) and positive for neuronal-specific enolase (C) and cytokeratin (D).
The eye showed keratic precipitates, vitreous "snowballs," and haze and retinal exudates. Laboratory studies included normal chest radiography results, purified protein derivative induration of 4 mm, and negative VDRL and Toxoplasma antibody test results while she was taking systemic corticosteroids. The clinical diagnosis was panuveitis.

Histologic examination of the globe revealed granulomatous inflammation of the uvea with necrosis of the retina and RPE (Figure 1A). Ziehl-Neelsen acid-fast stain revealed several acid-fast bacteria localized in the necrotic RPE cells, which stained positive for cytokeratin and neuronal-specific enolase and negative for CD68 (Figure 1B).

The DNA was extracted from the RPE by microdissection of 5-µm-thick, paraffin-embedded

**Figure 2.** Amplification of *Mycobacterium tuberculosis* IS6110 multicopy element (GenBank accession No. X52471) insertion sequence with SYBR Green I. Fluorescent vs cycle threshold values from tuberculosis (TB) DNA from standard strain (genomic DNA from *M tuberculosis H37Rv* (A) and from test samples (C). Melting curve analysis of *M tuberculosis* amplification products from standard DNA (B) and test samples (D) indicating the specificity of the reaction product. Standard curve generated by analysis of known amounts of standard *M tuberculosis* genomic DNA with the Bio-Rad iCycler optical detecting system (Bio-Rad Laboratories, Hercules, Calif) (E). Purified DNA from the paraffin sections of test, control, and standard DNA were assayed in parallel. PCR indicates polymerase chain reaction; CF, curve fit; RFU, relative fluorescence units; dT, derivative of temperature; and Exp, experimental.
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’ AGGCCAACCTGCAGCG-3’) and reverse primer IS7 (5’ GATGCTGATCCGGCCA-3’), which amplified a 122–base pair fragment (GenBank accession No. X32471). 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 X 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 panuvesitis 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. 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. 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. 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. 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. 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 opsins, 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. TUNEL staining was performed to identify apoptotic cells.