Ultrastructural features and prevalence of tubuloreticular structures in the ocular vasculature of patients with AIDS: a study of 23 cases

Raj K Maturi, Ramon L Font

Abstract

Background—Tubuloreticular structures (TRS) are subcellular inclusions that are most commonly found in endothelial cells and lymphocytes of patients with autoimmune or collagen vascular disorders. In AIDS, TRS have been described in various tissues throughout the body including the lung, kidney, liver, muscle, and skin.

Methods—Ocular tissues from 23 patients with AIDS were examined by electron microscopy. These included 17 post-mortem eyes in addition to three chorioretinal and three conjunctival biopsy specimens.

Results—The overall prevalence of TRS in the ocular and conjunctival endothelial cells was found to be 83%(19/23).

Conclusions—This is the first documented study of the prevalence of these structures in the ocular structures of patients with AIDS. Given the high frequency of their occurrence in AIDS, it is recommended that the presence of TRS in ocular or conjunctival tissues be an indication for obtaining an HIV antibody titre. Additionally, a rheumatological examination for HIV seronegative patients is suggested.

Department of Ophthalmology, Cullen Eye Institute, Baylor College of Medicine, Houston, TX 77030, USA

R K Maturi
R L Font

Correspondence to: Ramon L Font, MD, Ophthalmic Pathology Laboratory, Cullen Eye Institute, Baylor College of Medicine, Houston, TX 77030, USA

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Table 1 Prevalence of tubuloreticular structures (TRS) in 23 patients with AIDS

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<td>17 autopsy eyes.</td>
<td>3 surgical chorioretinal biopsy specimens</td>
<td>3 conjunctival biopsy specimens</td>
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<td>6 eyes with cytomegalovirus (CMV) retinitis</td>
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<td>9 eyes with no clinical or histological retinal lesions</td>
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<td>2 eyes with opportunistic infections*</td>
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<td>6/9 had TRS</td>
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<td>1/2 had TRS</td>
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<td>Overall prevalence of tubuloreticular structures</td>
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<td>3/3 had TRS</td>
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<td>19/23 (83%)</td>
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*Histoplasma capsulatum and Proteus sp were isolated from peripheral blood cultures (one patient each) and demonstrated in the ocular tissues by special stains.

Tubuloreticular structures (TRS) were first described in association with systemic lupus erythematosus (SLE) but have been found in association with other immunological disorders, including AIDS and, rarely, malignant lymphomas and leukaemias. Ultrastructurally, TRS are composed of multiple branching inclusions located adjacent to the cisternae of the rough surfaced endoplasmic reticulum and, occasionally, within the perinuclear envelope. On transmission electron microscopy, these inclusions have a diameter of 20–30 nm and are found most commonly as electron dense cytoplasmic aggregates. Cytotoxic studies have determined that TRS consist primarily of membrane phospholipids and glycoprotein.

An extensive review of the tissue location and cell types containing TRS in humans has been reported previously. One case report has described TRS in the endothelial cell of a choroidal vessel. One of the authors (RLF) has previously described these structures in a conjunctival biopsy specimen of an HIV seropositive patient.

To our knowledge, no published data on the prevalence of these structures in the ocular tissues of AIDS patients have been recorded. The purpose of this study was to document the prevalence of TRS in the retinal, choroidal, and conjunctival vasculature and to demonstrate their ultrastructural features in these locations.

Materials and methods

Postmortem eyes from 17 patients who were HIV seropositive and had a diagnosis of AIDS were examined by both light and electron microscopy. Three surgical chorioretinal biopsy specimens and three conjunctival biopsy specimens were also similarly examined. Paraffin embedded 5 μm sections were stained with haematoxylin and eosin, periodic acid Schiff (PAS), Gomori-methamine-silver (GMS), and Gram methods.

For electron microscopic studies, the tissues were fixed in 10% buffered formalin, postfixed in 2% glutaraldehyde, followed by 1% osmium tetroxide. Thick sections (~1 μm) were stained with toluidine blue and paragon. Ultrathin sections (40–60 nm) were mounted on copper grids, stained with uranyl acetate and lead citrate, and examined with a Jeol 100 CX electron microscope.

We examined an average of 15 grids from each specimen, with special attention to the presence of TRS in the retinal and choroidal vasculature.

Results

The pertinent clinical findings and prevalence of TRS in 23 patients are summarised in Table 1. Nine patients who had no ocular symptoms showed no histological evidence of retinitis. All three surgical chorioretinal biopsy specimens were ophthalmoscopically diagnosed as cytomegalovirus (CMV) retinitis and were confirmed by light and electron microscopic studies. The conjunctival biopsies were performed with a presumptive diagnosis of...
microsporidial keratoconjunctivitis. In these three biopsy specimens, we found intracellular electron dense, lipoidal bodies mostly in the epithelial cells and TRS in the capillary endothelial cells. No microsporida were identified.

We observed that eyes without TRS in the retinal capillaries also failed to demonstrate these structures in the choroidal vasculature.

Representative ultrastructural features of the TRS in the retinal and choroidal endothelial cells are illustrated in Figures 1–3. Figure 4 depicts the presence of TRS in a plasma cell associated with a chronic non-granulomatous choroiditis in a patient with histologically proved CMV retinitis.

Discussion

Our studies demonstrate a high prevalence of TRS in the ocular structures of patients with AIDS. Sidhu and coworkers studied 97 patients with AIDS and found a similar prevalence (85%) of TRS in the endothelial cells from multiple anatomical sites.13 We also note that TRS identified in ocular/adnexal tissues are ultrastructurally quite similar to those observed in other anatomical locations. Thus, their mechanism of formation, while still under some debate, is presumably analogous to that observed in other tissues.

Some observers have suggested that TRS may be of viral origin,1,14 given their close association with viral diseases. TRS are primarily composed of membrane phospholipids and glycoproteins. Evidence against a viral origin includes their lack of nucleic acids, digestion with proteases and pepsin, and insensitivity to digestion with trypsin and ribonuclease. The nucleoprotein strands of myxoviruses and paramyxoviruses have been confused ultrastructurally with TRS. The viral nucleocapsids of the myxovirus/paramyxoviruses have a helical configuration, are not associated with the rough surfaced endoplasmic reticulum, and rarely exceed 18 nm in diameter. In contrast, the tubular structures of TRS measure 20–30 nm in diameter and are contiguous with the endoplasmic reticulum.3

Another hypothesis regarding the origin of TRS suggests that they may be an unusual morphological response of the rough surfaced
endoplasmic reticulum occurring in cells subjected to various insults. Thus, TRS may represent a morphological expression indicative of cellular injury. 1

There is significant evidence that TRS represent a cellular response to increased serum levels of interferon alfa (INF α). 15-18 This association was first noted after the discovery that patients with systemic lupus erythematosus (SLE) had increased serum levels of INF α. 19 Additionally, in vitro studies have demonstrated the induction of TRS by exogenous INF α when added to peripheral blood lymphocytes. 20 It is noteworthy that patients with AIDS, like those with SLE, have high levels of INF α. 15

The presence of TRS in ocular/adnexal structures in a previously HIV seronegative patient may indicate the presence of a collagen vascular disease (typically SLE or scleroderma) or other
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immunologically mediated disorders. If TRS are found in the ocular/adnexal tissues of HIV seronegative patients, we would suggest performing a complete immunological examination (antinuclear antibody, rheumatoid factor, etc) to exclude the possibility of a collagen-vascular disorder. If these results are negative, we believe that periodic HIV antibody testing is indicated to exclude the possibility that the patient might be in the prodromal stage of infection.

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20 Feldman DG, Goldstein AL, Cox DC, Grimpley PM. Cultured human endothelial cells treated with recombi- 
iduate leucocyte A interferon (IFN-a). Tubuloreticular inclusions, anti-proliferative effect and 2'-5' 