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Clinico-pathological correlation in a patient with previously treated birdshot chorioretinopathy

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Abstract

Purpose—Birdshot chorioretinopathy (BCR) is a bilateral, chronic uveitis primarily involving the posterior segment that often results in progressive vision loss. Histopathology on eyes with BCR has been limited but we had the rare opportunity to study the eyes of a donor with BCR. We sought to compare immunolabeling in the eyes of this donor who was treated with immunosuppression for over 30 years to age-matched controls.

Methods—From each eye, a macular punch and superotemporal regions were used for cryostat sectioning, and immunohistochemistry was performed on the sections using antibodies directed against CD45, ICAM-1, IBA1, and GFAP. The vasculature binding lectin, *Ulex europaeus* agglutinin-I (UEA-I), was also used to perform lectin histochemistry.

Results—At death her visual acuity was 20/25 OD, 20/250 OS with extensive chorioretinal atrophy, vascular attenuation and disc pallor. Compared to controls the BCR donor had extensive degeneration of the outer nuclear layer and RPE as well as choroidal thinning with inner retinal preservation. Loss of UEA-I+ choroidal endothelial cells was extensive and atypical ICAM-1 labeling and IBA+ microglia/macrophages were present along with widespread GFAP labeling throughout the retina.

Conclusion—BCR may cause progressive chorioretinal and optic atrophy with longstanding increased leukocyte abundance throughout the retina and microglial activation especially at the retina-choroid interface.

Keywords

cell biology; human donor; post-mortem; chorioretinal; microglia; retina; choroid; histology

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Birdshot chorioretinopathy (BCR) is a bilateral, chronic uveitis primarily involving the posterior segment that often results in progressive vision loss.¹ Histopathology on eyes with BCR has been limited to three reports detailing hematoxylin and eosin staining and immunoperoxidase studies.²⁻⁴ We performed extensive immunolabeling of the eyes of a donor with BCR who was treated in our retina clinic for over 30 years.

CASE DESCRIPTION

A 40 year old female presented in June 1981 (previously published¹ as case 4 where electroretinogram and Goldmann perimetry results can be found) with trace anterior chamber and 1+ vitreous cells in both eyes (OU), disc edema OU and retinal vasculitis more in the left eye (OS) than the right eye (OD). Best-corrected visual acuity (BCVA) was 20/25 OD, 20/50 OS. Comprehensive systemic work-up (including melanoma) was negative except for positive HLA-A29. Oral prednisone initially resulted in clinical improvement but by August 1982, birdshot lesions were present in both eyes (Fig 1). She was maintained on various dosages of oral prednisone depending on severity of disease (minimum 5 mg per day until death in 2014 from atherosclerotic cardiovascular disease). She refused immunosuppressives until February 1998 when cyclosporine was added. At this time her BCVA was 20/25-2 OD, 20/250 OS with chorioretinal atrophy in the midperiphery and arteriolar attenuation present in the posterior pole. Subtenons corticosteroid injections were given OU. She developed adverse reactions to cyclosporine and azathioprine so methotrexate was started in January 2003. In 2013 she was maintained on prednisone and methotrexate 17.5 mg a day and had 2+ disc pallor OU, extensive chorioretinal atrophy and vascular attenuation OU.

METHODS

Eyes from this BCR donor were compared to those from a large series of age-matched, unaffected donors obtained from the Iowa Lions Eye Bank following informed consent of the donor next of kin, and all experiments were performed in accordance with the Declaration of Helsinki. From each eye, a 6-mm trephine punch (centered on the fovea centralis) and a superotemporal central-to-peripheral retina-RPE/choroid segment was collected and fixed in 4% paraformaldehyde in 10mM phosphate-buffered saline. All samples were cryoprotected in sucrose, embedded in optimal cutting temperature, and stored at -80°C. Samples were cryosectioned to obtain 7µm thick sections. Immunohistochemistry was performed on the sections using anti-CD45 (leukocyte common antigen marker; BD Biosciences, Franklin Lakes, NJ), anti-ICAM-1 (intercellular adhesion molecule-1; leukocyte and endothelial cell marker; R&D Systems, Minneapolis, MN), anti-Iba1 (macrophage and microglial marker; Wako Chemicals USA, Richmond, VA), and anti-GFAP (glial marker; Thermo Scientific, Waltham, MA) antibodies. Biotinylated *Ulex europaeus* agglutinin-I (UEA-I; Vector Laboratories, Burlingame, CA) was also used to perform lectin histochemistry to visualize the retinal and choroidal vasculature. Immunolabeling and lectin histochemistry were performed as described previously.⁵ All sections were counterstained with diamidino-phenol-indole (DAPI; Molecular Probes, Eugene, OR). Sections were washed three times in 1X PBS with 1mM MgCl₂ and CaCl₂ for

5 minutes each. Stained retina-RPE/choroid sections were imaged using fluorescence microscopy with a 20× objective lens.

RESULTS

The most prominent morphological features of the BCR eyes were extensive degeneration of the outer nuclear layer and RPE as well as choroidal thinning (Fig 2). The inner nuclear and ganglion cell layers showed relatively good preservation. Loss of UEA-I+ choroidal endothelial cells was extensive—the normal choriocapillaris had completely degenerated, with preservation of only a few scattered vessels in Haller's/Sattler's layers.

The BCR eyes showed atypical ICAM-1 labeling throughout the neural retina, especially at the interface between the retina and Bruch's membrane, possibly due to a wound healing response by Muller cells, which normally express ICAM1 in the external limiting membrane. Consistent with this finding, strong and widespread GFAP labeling was present throughout the retina for all BCR sections, indicating gliosis in the scarred retina.

IBA+ microglia/macrophages were strikingly elevated in the retina and choroid compared to normal eyes, and cells in the BCR eyes tended to have shorter processes than controls. While not comprehensively quantified in this report, the number of CD45+ cells in the inner choroid of the birdshot donor OD macula appeared much higher than reported in normal eyes, estimated to be about 20 times higher than in normal eyes.⁵

DISCUSSION

Birdshot chorioretinopathy often causes progressive chorioretinal and optic atrophy with severe vision loss; its pathogenesis is not well understood. There is a strong association between BCR and major histocompatibility (MHC) class I antigen HLA-A29, and in light of the high HLA-A expression in the choriocapillaris,⁶ T cell-mediated immunity against targets in the choroid may play a central role in causing disease.^{2,7} This is consistent with the severe damage to the choroidal endothelium observed in this case. Previous histologic analyses of donor eyes with BCR have revealed nongranulomatous nodular infiltration of the choroid^{3,4} and lymphocytic infiltrate mixed with T cells (CD3, CD4, and CD8) and B cells (CD20). Our study expands on these prior studies by showing increased leukocyte trafficking throughout the retina and choroid that appears to persist long after the retinal degeneration. We also noted activation of macroglia (with GFAP and ICAM1 activation) and microglia/macrophage activation (indicated by IBA1 labeling) especially at the retina/choroid interface. It is difficult to know whether these findings would have been similarly observed if she had tolerated the T-cell suppressive medicine cyclosporine. While we did not specifically evaluate T- and B-cells, CD45+ cells (which include all classes of leukocytes) were very abundant and this case suggests the importance of long-term immune suppression for this chronic disease. It is also uncertain whether other mechanisms could have contributed to her vision loss and histopathologic findings. Additional mechanistic studies should provide further therapeutic insights into BCR.

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SUMMARY STATEMENT

Extensive immunolabeling of rare donor eyes with birdshot chorioretinopathy reveals severe damage to the choroidal endothelium as well as increased leukocyte trafficking and microglial activation in the retina.

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Figure 1. Montaged fundus photos of this BCR patient demonstrating typical birdshot lesions in 1992 (A, B) before widespread chorioretinal changes with optic disc pallor and vascular attenuation in both fundi were seen in 2010 (C, D).

Figure 2.

Immunohistochemical comparison of the maculas from a BCR eye (A-D) to age-matched healthy control (E-H). Sections labeled with antibodies directed against IBA1 (A, E; green fluorescence) show increased density of IBA1 positive macrophages in the outer retina and choroid of the birdshot donor (asterisks). Labeling of GFAP (B, F; green fluorescence), and ICAM-1 (C, G; red fluorescence) both showed increased overall labeling for the BCR eye, especially at the outer edge of the neural retina (arrows). Sections with secondary antibody only (D, H) were used as controls. Sections labeled with anti-IBA1 and anti-GFAP antibodies (A, B, E, F) were co-labeled with UEA-I to identify the vasculature (red fluorescence). Blue fluorescence indicates nuclear DAPI counterstain. Orange-yellow fluorescence is due to lipofuscin autofluorescence of the RPE (lost in the birdshot retinopathy eye). Scalebar in H, 100µm. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; CH, choroid.