Experimental Eye Research 150 (2016) 44–61

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Idiopathic preretinal glia in aging and age-related macular degeneration

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ARTICLE INFO

Article history:
Received 19 March 2015
Received in revised form
11 June 2015
Accepted in revised form 24 July 2015
Available online 26 July 2015

Keywords:
Age-related macular degeneration
Astrocytes
Epiretinal membranes
Muller cells
Glia

ABSTRACT

During analysis of glia in wholemount aged human retinas, frequent projections onto the vitreal surface of the inner limiting membrane (ILM) were noted. The present study characterized these preretinal glial structures. The amount of glial cells on the vitreal side of the ILM was compared between eyes with age-related macular degeneration (AMD) and age-matched control eyes. Retinal wholemounts were stained for markers of retinal astrocytes and activated Müller cells (glial fibrillary acidic protein, GFAP), Müller cells (vimentin, glutamine synthetase) and microglia/hyalocytes (IBA-1). Retinal vessels were labeled with UEA lectin. Images were collected using a *Zeiss LSM 710* confocal microscope. Retinas were then cryopreserved. Laminin labeling of cryosections determined the location of glial structures in relation to the ILM. All retinas investigated herein had varied amounts of preretinal glia. These glial structures were classified into three groups based on size: sprouts, blooms, and membranes. The simplest of the glial structures observed were focal sprouts of singular GFAP-positive cells or processes on the vitreal surface of the ILM. The intermediate structures observed, glial blooms, were created by multiple cells/processes exiting from a single point and extending along the vitreoretinal surface. The most extensive structures, glial membranes, consisted of compact networks of cells and processes. Preretinal glia were observed in all areas of the retina but they were most prominent over large vessels. While all glial blooms and membranes contained vimentin and GFAP-positive cells, these proteins did not always co-localize. Many areas had no preretinal GFAP but had numerous vimentin only glial sprouts. In double labeled glial sprouts, vimentin staining extended beyond that of GFAP. Hyalocytes and microglia were detected along with glial sprouts, blooms, and membranes. They did not, however, concentrate in the retina below these structures. Cross sectional analysis identified small breaks in the ILM above large retinal vessels through which glial cells exited the retina. Preretinal glial structures of varied sizes are a common occurrence in aged retinas and, in most cases, are subclinical. While all retinal glia are found in blooms, vimentin labeling suggests that Müller cells form the leading edge. All retinas investigated from eyes with active choroidal neovascularization (CNV) had extensive glial membranes on the vitreal surface of the ILM. Although these structures may be benign, they may exert traction on the retina as they spread along the vitreoretinal interface. In cases with CNV, glial cells in the vitreous could bind intravitreally injected anti-vascular endothelial growth factor. These preretinal glial structures indicate the remodeling of both astrocytes and Müller cells in aged retinas, in particular those with advanced AMD.

1. Introduction

There are two types of macroglia in retina, astrocytes and Müller cells. Astrocytes normally reside only in the nerve fiber layer where they associate closely with retinal blood vessels and ganglion cell axons (Provis et al., 2000). Within the vascular

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http://dx.doi.org/10.1016/j.exer.2015.07.016
0014-4835/

Table 1
Donor eyes used in this study. This list includes available data regarding ocular and systemic diseases of donors. The preretinal glial structures observed in each are also noted. Abbreviations include: AD-Alzheimer's disease, AF-atrial fibrillation, AMD-age-related macular degeneration, BRVO-branch retinal vein occlusion, CA-cardiac arrest, CHF-congestive heart failure, CVA-cerebrovascular accident, DM-diabetes mellitus, ERM-epiretinal membrane, GA-geographic atrophy, HC-high cholesterol, HTN-hypertension, HC-high cholesterol, HF-heart failure, OD-oculus dexter, OS-oculus sinister, OU-oculus uterque, PDT-photodynamic therapy, PVD-peripheral vascular disease. Visual acuity codes: NA: not available; 996: count fingers, 997: hand motion, 998 no light perception. The number of months since visual acuity was last scored is listed in parentheses.

Donor ID	Age at death	Eye used	AMD classification	AMD treatment	Other ocular disease	Visual acuity	Pretinal glia: Posterior pole	Preretinal glia: outside pole	Other medical problems	Cause of death
1	79	OS	Non-AMD	No	None	NA	Sprouts	Sprouts		Chronic pulmonary disease
2	94	OD	Non-AMD	No	Cataracts removed OU	20/25 (14)	Blooms	Blooms/ sprouts	HTN, CHF, PVD, AF	Cardiogenic shock
3	80	OD	Non-AMD	No	None	NA	Sprouts	No	cardiovascular disorder,	Coronary disease
4	87	OS	Non-AMD	No	Cataracts removed OU	20/40 (8)	Sprouts	Blooms	HTN, HC, DM (II), heart attack	CHF
5	73	OU	Non-AMD	No	Cataracts removed OU	NA	Blooms/ membrane	Blooms/ membrane	HTN	CHF
6	64	OS	Non-AMD	No	None	NA	Sprouts	Nothing		Unknown
7	91	OD	Non-AMD	No	BRVO macular edema, cataracts removed OU	20/20 (26)	Sprouts	Sprouts	HTN, DM, HC	Complete heart block
8	84	OU	Non-AMD	No	Yag laser (2006), cataracts removed OU, laser	NA	Sprouts	Sprouts	AD, HTN, borderline DM	Cardiac arrest
9	91	OS	Early	No	Cataracts removed OU glaucoma-laser surgery	20/80 (12)	Sprouts/ blooms	Sprouts	PVD	HF and AF
10	86	OU	Early	No	Cataracts removed OU	20/25; 20/30 (37)	Sprouts	Sprouts	HC, dementia	Dementia/AD
11	97	OD	GA	No	Cataracts removed OU	996 (8)	Sprouts	Sprouts	HTN, HC, PVD	Spinal stenosis CHF
12	96	OD	GA	No	Cataracts OU	996 (6)	Blooms	Blooms/ sprouts		Respiratory failure/pneumonia
13	92	OD	GA	No	Cataracts OU	20/40 (27)	Blooms	Blooms	HTN, HC	CHF
14	87	OD	Intermediate	No	Cataracts OU	20/30 (3)	Sprouts	Blooms	HTN, AF	Internal bleeding
15	92	OS	Intermediate	No	Retinal embolus, cataracts removed OU	20/30 (12)	Blooms	Blooms	HC, CHF, PVD, HTN	CHF
16	86	OS	Intermediate	No	Cataracts removed OU	NA	Blooms/ membrane	Blooms/ membrane		Unknown
17	90	OD	Intermediate	No	Cataracts removed OU	20/30 (17)	Sprouts	Sprouts	HTN, CHF, AF	CHF, CA, hypoxic respiratory failure, renal failure, pulmonary HTN
18	84	OS	Intermediate	No	Cataracts removed OU	20/30 (9)	Sprouts	Nothing	HTN, heart attack	Influenza/pneumonia
19	100	OS	Neovascular	PDT	Glaucoma	20/400 (17)	Blooms/ membranes	Sprouts	HTN, HC	HF
20	85	OS	Neovascular	Ranibizumab	Cataracts removed OU	996 (8)	Membranes	Sprouts	HTN	Esophageal cancer
21	97	OS	Neovascular	Diode laser	Cataracts removed OU	996 (78)	Large scar: couldn't image pole	Blooms/ sprouts	HTN, CHF	Lung cancer-metastases to adrenal glands
22	86	OS	Neovascular	No for AMD, laser for glaucoma	Glaucoma, ERM OS, subretinal hemorrhage	20/60 (6)	Membranes	Blooms/ sprouts	DM (II), AF, Parkinson's	Acute Respiratory Failure
23	96	OD	Neovascular	Possible laser	Cataracts removed OU	996 (5)	Membranes	Blooms/ sprouts	HTN, DM, HC, heart attack	Unknown
24	94	OD	Neovascular	PDT	Cataracts removed OU	20/25 (2)	Membrane	Sprouts	HTN, Parkinson's disease	CVA
25	78	OU	Neovascular	21 Ranibizumab injections OD, laser OD at border	ERM OD, retinal tears-laser cataracts removed OU	20/60; 996 (4)	Membranes	Membrane	HTN, DM, HC	Pulmonary embolism
26	95	OD	Neovascular	Bevacizumab	Glaucoma OU, ERM	998 (4)	Membranes	Blooms/ sprouts	HTN	CVA
27	81	OS	Neovascular	Focal laser	Glaucoma	20/400 (1)	Blooms/ membranes	Sprouts	HTN, DM	Liver Disease
16B	86	OD	Neovascular	No	Cataracts removed OU	NA	Blooms/ membrane	Blooms/ membrane		Unknown
28	102	OD	Neovascular	No	Cataracts removed OU	997 (5)	Blooms/ membranes	Blooms/ sprouts	HTN	CHF