Pathological changes in the vitreoretinal junction 1: Epiretinal membrane formation

Article *in* Eye (London, England) · April 2008

DOI: 10.1038/eye.2008.36 · Source: PubMed

3 authors:

David Snead
University Hospitals Coventry and Warwickshire NHS Trust
117 PUBLICATIONS **2,767** CITATIONS
SEE PROFILE

Sean G. James
University Hospitals Coventry and Warwickshire NHS Trust
49 PUBLICATIONS **775** CITATIONS
SEE PROFILE

Martin P Snead
Cambridge University Hospitals NHS Foundation Trust
121 PUBLICATIONS **2,746** CITATIONS
SEE PROFILE

Eye (2008) 22, 1310–1317

www.nature.com/eye

CAMBRIDGE OPHTHALMOLOGY SYMPOSIUM

Pathological changes in the vitreoretinal junction 1: epiretinal membrane formation

DRJ Snead[1], S James[1] and MP Snead[2]

Abstract

Purpose/Background **Epiretinal membrane (ERM) formation is a common change resulting in disturbance of macular vision and predisposing to rhegmatogenous retinal detachment. Current treatment strategies rely chiefly on surgical removal of the membranes from the surface of the retina, allowing the retina to remodel and reattach. Improved knowledge of the pathological process behind the formation of these membranes, particularly knowledge of the cell types involved in their formation, is likely to increase our understanding of the way this group of diseases behave and to improve treatment.**

Methods **We reviewed the histological findings of 109 surgically removed specimens and correlated these to age-related changes seen in a 32 cadaver eyes studied after corneal harvesting. The samples were studied using light microscopy and immunocytochemistry.**

Results **In all cases of idiopathic ERMs, including cellophane maculopathy, macular hole, and vitreomacular traction syndrome, laminocytes were the exclusive cell type present. In cases of macular pucker associated with retinal tears, the membranes contain variable cohesive groups of retinal pigment epithelial (RPE) cells in addition to laminocytes. In cases of proliferative diabetic retinopathy, membranes consist almost entirely of capillaries and hyaline stromal tissue, with or without haemosiderin pigment and RPE cells and in which laminocytes and ILM were not identified. In cadaver eyes PVD was seen in 17/32 (53%) of cases, and the vitreous was attached in 14/32 (43.7%) and in one case no vitreous was present. Isolated laminocytes were present on the retinal surface in 12/18 cases with detached vitreous and in 1/14 cases with attached vitreous. In all cases laminocytes were scanty and confined to the optic nerve head, macular or subjacent macular retina. Immunohistochemistry findings indicate that laminocytes are positive for glial fibrillary acidic protein (GFAP), cytokeratin marker AE1/AE3, type II collagen, and type IV collagen. In some cases novel basement membrane formation was seen. There was a tendency for increased positivity of GFAP and AE1/AE3 with increased cellularity, and where novel basement membrane formation was present.**

Conclusion **Laminocytes are the fundamental cell type in idiopathic ERMs. These cells are frequently found in small and dispersed numbers in eyes containing a PVD. The presence of retinal pigment cells invariable indicates proliferative retinopathy and is only seen in association with a retinal detachment or tear. Diabetic membranes are composed of neovascular stromal tissue, which is most likely to be a response to retinal hypoxia.**

Eye (2008) **22,** 1310–1317; doi:10.1038/eye.2008.36; published online 14 March 2008

Keywords: epiretinal membrane; laminocytes; GFAP; proliferative vitreoretinopathy; proliferative diabetic retinopathy; macular hole

[1]Department of Pathology, University Hospital Coventry, Coventry, UK

[2]Vitreoretinal Service, Addenbrookes Hospital Cambridge, Cambridge, UK

Correspondence:
DRJ Snead,
Department of Pathology, University Hospital Coventry and Warwickhire NHS Trust, Coventry CV2 2DX, UK
Tel: +024 765 388 55;
Fax: +024 765 387 15.
E-mail: david.snead@uhcw.nhs.uk

Received: 19 January 2008
Accepted in revised form: 19 January 2008
Published online: 14 March 2008

Introduction

Epiretinal membrane (ERM) formation characterises a number of pathological changes occurring in the vitreoretinal junction with varying degrees of clinical significance, and upon which a considerable amount of data has already been published. In spite of this there are

relative few accounts accurately characterising the composition of ERMs in different diseases. This knowledge is however important in understanding the pathogenesis of ERM diseases and is potentially useful in guiding management and prognosis.

In their seminal work, Foos and co-workers[1,2] described the structure, composition, and the relationship to the surface wrinkling phenomena of simple ERMs. These membranes are composed of glial cells located on the surface of the inner limiting membrane (ILM). Aside from the issue of macular holes (MH) and vitreomacular traction syndrome (VMTS),[3–6] much of the data published since has addressed the more complex ERMs containing vessels or retinal pigment epithelial (RPE) cells.[7–11] Membranes of cellophane maculopathy (CM) have been largely ignored.

The origin of the cells in simple ERMs was attributed by Foos[1] to accessory glial cells migrating from the nerve fibre layer to the surface of the ILM. Anecdotal evidence suggests that these membranes are relatively common particularly in the elderly;[12,13] however, the precise incidence in the normal eye, and its association with ageing and posterior vitreous detachment (PVD), has not been formerly reported. Previous studies have demonstrated that the cells in simple ERMs show expression of glial fibrillary acidic protein (GFAP) and we have linked expression of this intermediate filament with increasing cellularity and activity of these cells and the production of novel type 4 collagen, suggesting a role in remodelling of the ILM.[14] In respect of this specialised putative role, we have suggested these cells to be termed as laminocytes.

In this paper, we review the histology of a large series of surgically removed ERMs and correlate the findings with the changes seen in normal cadaver eyes, with and without PVD.

Methods

Ethical approval

Ethical approval for this study has been received from both Coventry and Cambridge Local Research Ethical Committees.

Clinical details

Surgical membrane peel specimens

A series of 109 ERM peel specimens, removed during vitrectomy from patients presenting to the vitreoretinal service at Addenbrookes Hospital Cambridge over the period from 1996 to 2006. These patients were classified clinically into proliferative vitreoretinopathy (PVR), CM, MH, VMTS, and proliferative diabetic retinopathy (PDR) groups. In addition, over this period there was a group of four cases of ERM related to specific causes including post-infection and trauma.

Cadaver globes

Thirty-two globes were received from the Bristol Eyebank, following corneal harvesting from patients aged between 43 and 90 years. These specimens were transferred to Coventry and fixed in buffered formalin.

Specimen processing and staining

All surgical membrane peel specimens were embedded in agar gel and processed into paraffin wax blocks as previously described.[14] Globes from cadaver eyes were sectioned in the horizontal plane and processed into oversized paraffin wax blocks. In all cases, 5 μ sections were cut and stained with H&E, type II collagen (TIIC), type IV collagen (TIVC), and GFAP as previously described[14] as well as AE1/AE3 cytokeratin. For AE1/AE3, sections were digested in Trypsin for 10 min and incubated in primary antibody at a dilution 1 : 200. Bound antibody was visualised using the same technique as described for the remaining immunocytochemistry stains.[14] Diastase periodic acid Schiff (DPAS) stain was used on the cadaver globes to assist in visualisation of the ILM.

Results

A total of 109 surgically removed cases were included in this series, the distribution of the different types of membrane is summarised in Table 1.

Cellophane maculopathy, macular hole and vitreomacular traction syndrome

The membranes from these specimens all comprised flattened cells with oval nuclei and pale staining

Table 1 The distribution of different membrane types

Membrane type	*No. of cases*
Cellophane maculopathy	35
Macular hole	15
Vitreomacular traction syndrome	5
PVR	30
PDR	20
Others	4
Total	109

indistinct cytoplasm lying adjacent to ILM (Figures 1 and 3f). The cell density varied between specimens, but in most cases these cells appeared to lie along one side of the stripped ILM. In all cases these cells formed a monolayer. No neural retinal elements, RPE, inflammatory, or fibroblastic cells were identified in these cases. One case of CM contained small vessels lying alongside the cellular membrane (Figure 3f), the remaining cases showed no vessels, and, except for basement membrane, there was no extracellular stromal tissue seen in these membranes. Increased cellularity was often associated with hyperconvolution of the ILM (Figure 1a–c) and novel basement membrane formation, indicated by duplication of the ILM stretching between the hyperconvoluted ILM folds. In all cases, the cells showed positive cytoplasmic staining for GFAP and AE1/AE3 (Figure 1e and f). In cases with high cellularity the laminocytes also showed strong positive cytoplasmic staining for TIIC. Both laminocytes and novel basement membrane in cases with hyperconvolution of the ILM, showed positive staining for TIVC as illustrated previously.[14]

Proliferative diabetic retinopathy

PDR cases showed prominent vessels and stromal tissue, the latter showing weak eosinophilic staining with H&E, and negative staining for TIIC and TIVC. Vessel walls however showed strong TIVC staining (Figure 2). No ILM components were seen in these cases and there were no laminocytes. Many cases contained haemosiderin pigment and macrophages, but only in a minority of cases RPE cells were present.

Proliferative vitreoretinopathy

PVR cases showed heterogeneous membranes. These membranes often comprised ILM and laminocytes similar to the CM group, but with variable numbers of RPE cells, lymphocytes, macrophages, and collagen. The RPE component was usually either arranged in multilayered cohesive sheets with densely eosinophilic cytoplasm and variable amounts of pigment, or as spindle cells within collagenous stroma. These cells stained focally positive for AE1/AE3 and negative for GFAP. In addition, some cases showed silicone oil droplets (Figure 3a and d). No vessels were seen in this group.

Remaining miscellaneous cases

Individual cases of vascular ERMs complicating local radiotherapy for basal cell carcinoma, and one secondary to vasoproliferative tumour were included in this series and have been previously reported.[15–17] In both cases, the predominant component was ILM and small capillaries with some extra cellular stromal tissue. Two post-infective ERMs, one post-cytomegalovirus (CMV), and one post-varicella zoster (VZV), showed small strips of ILM with adherent collagenous stroma and spindle RPE cells (Figure 3b and c). One case of an ERM arising following blunt trauma to the eye showed a hyperconvoluted strip of ILM adherent to which was a thick membrane composed of collagenous stroma and spindle-shaped RPE cells and macrophages (Figure 3e).

Cadaver eyes

In cadaver eyes PVD was seen in 17/32 (53%) of cases, and the vitreous was attached in 14/32 (43.7%) and in

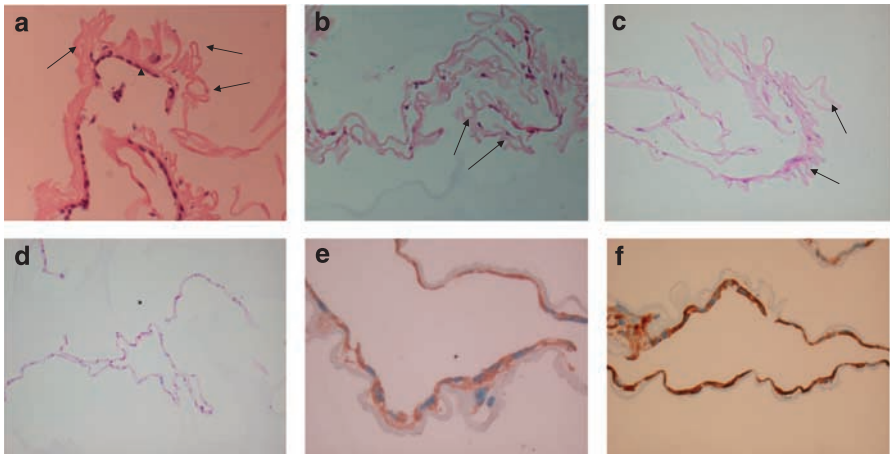

Figure 1 ERMs from CM cases. (a–c) Examples of hyperconvolution of the ILM (arrows), with laminocytes arranged in a monolayer along the vitreal surface (arrowheads). (d) A flat segment of ERM composed of laminocytes in which the ILM is not visible. (e and d) Immunocytochemical staining for GFAP and AE1/AE3, respectively with positive (brown) staining visible for both markers in the laminocytes. Original magnifications (a–d) $\times 200$; (e–f) $\times 400$.

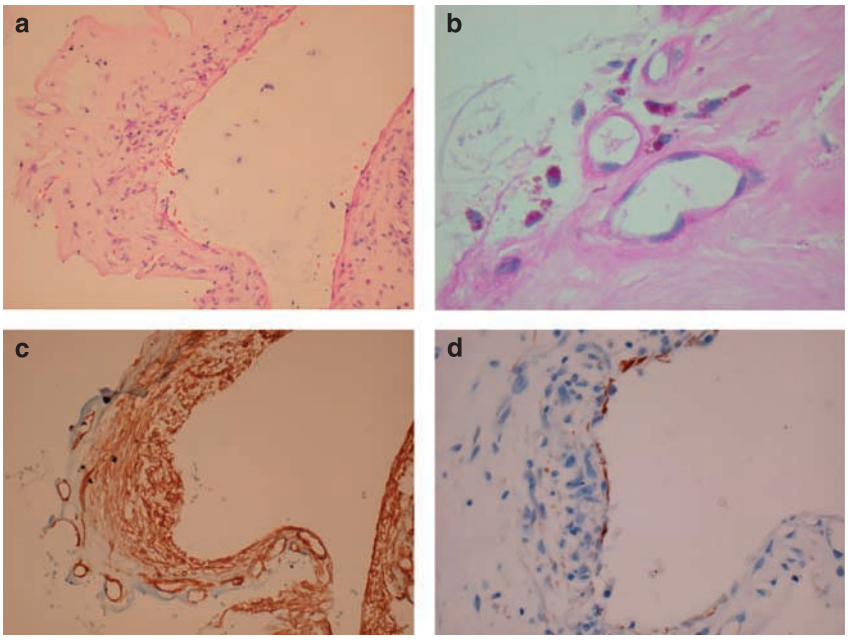

Figure 2 (a) An ERM from a case of PDR showing abundant stromal tissue rich in capillaries, highlighted on high power (b) (H&E). (c) TIVC stain shows smudgy positivity in the stroma and strong staining of capillary walls. (d) Patchy staining for GFAP, note the majority of the membrane is negative for this marker. Original magnification (a, c, and d) $\times 200$; (b) $\times 600$.

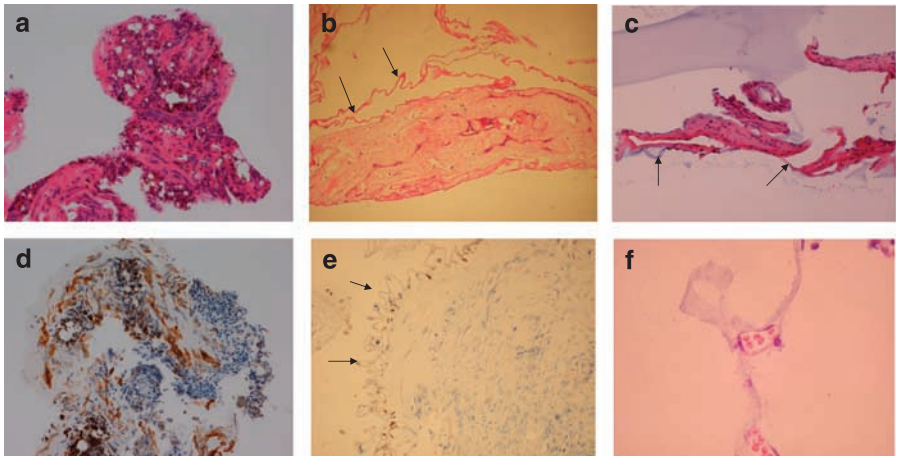

Figure 3 (a) A PVR membrane containing clumps of spindle-shaped RPE cells with abundant melanin pigment. The original ILM is not visible in this field. The rounded clear spaces are lipid 'vacuoles', which is part of a foreign body reaction to silicone oil from a previous retinal detachment repair, (H&E). (d)Immunocytochemistry on this case for AE1/AE3, with brown staining indicating positive staining in the RPE cells. (b and c) ERMs post-VZV and CMV, respectively. The membranes are adherent to the ILM (arrowed) and composed of collagenous stroma containing spindle-shaped retinal pigment epithelial cells (H&E). (e) An ERM complicating blunt trauma. The membrane is composed of a large mass of spindle-shaped RPE cells with abundant stroma and some pigment visible. Note the folded ILM adherent to the membrane border on the left side of the figure (H&E). (f) An unusual CM membrane, in which the laminocyte membrane contains capillaries (H&E), but note the absence of stromal tissue in comparison with PDR (Figure 2). Original magnification (a, b, d, and e) $\times 100$; (c) $\times 200$; and (f) $\times 400$.

one eye the vitreous was absent entirely. In most cases with PVD the ILM was identifiable on the surface of the retina, however two cases appeared to show ILM separating with the vitreous. In the majority of PVD cases where separation had left recognisable ILM on the retinal surface, the posterior hyaloid membrane consisted of a distinct crinkled eosinophilic band covering the posterior surface of the vitreous gel, and on which occasional cells were visible. Laminocytes were easily identified in 12 of 18 eyes with PVD, most frequently in the perimacular

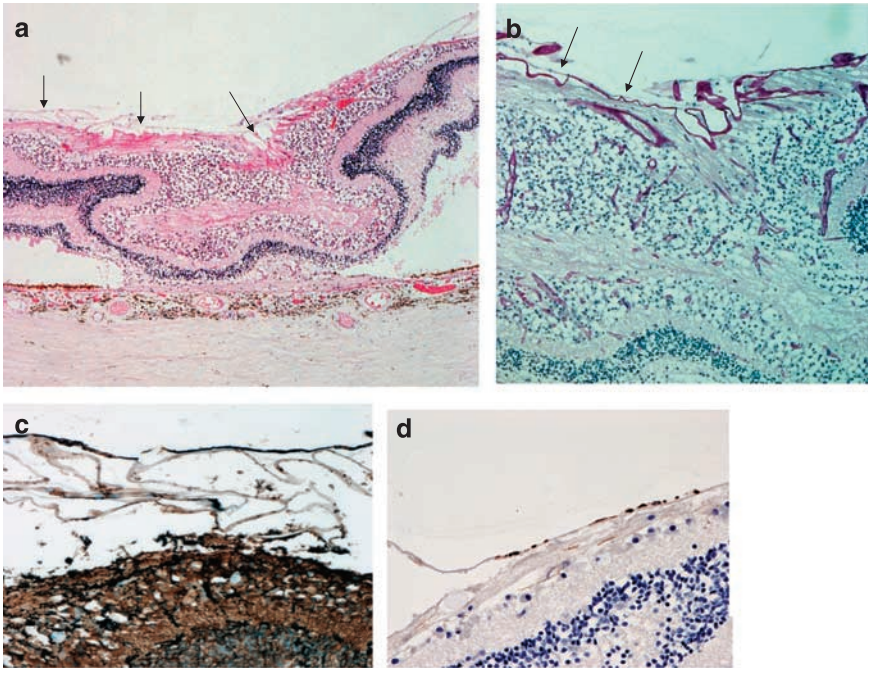

Figure 4 Sections from postmortem globes. (a–c) A case of macular pucker. The retinal surface can clearly be seen in (a) with a typical CM type ERM, including hyperconvoluted ILM present on the retinal surface (arrows) (H&E). The ILM is more clearly demonstrated with the DPAS stain in (b), and the GFAP immunocytochemistry stain shows strong positivity in the laminocytes (c). (d) Another case with a PVD, in which laminocytes are clearly visible on the retinal surface, and staining positively for cytokeratin AE1/AE3. Original magnifications (a) $\times 100$; (b) $\times 200$; and (c and d) $\times 400$.

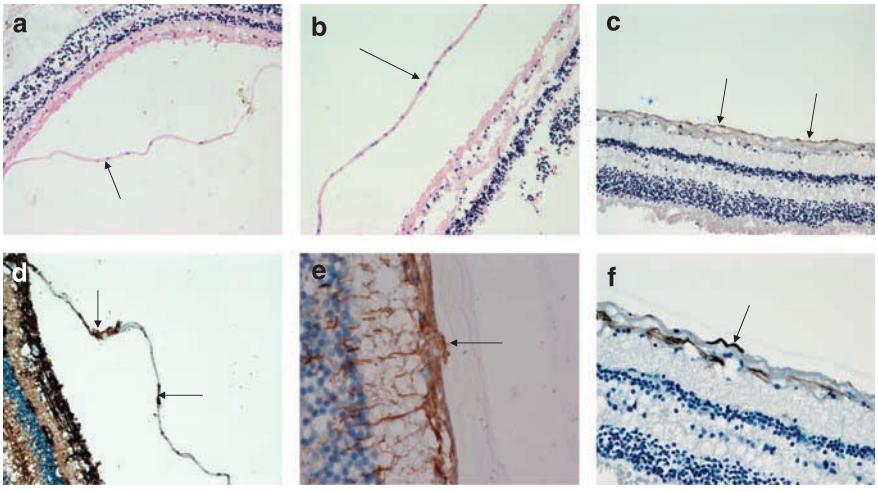

Figure 5 Postmortem globes showing presence of laminocytes on the retinal surface. (a–d) are from globes with PVDs, (e and f) are from globes with attached gel. (a and c) are H&E stain, (c and d) are ICC stains for AE1/AE3 and GFAP, respectively. (e and f) are ICC stains for GFAP and AE1/AE3, respectively. Original magnifications (a and b) $\times 100$; (c and d) $\times 200$; (e) $\times 600$; and (f) $\times 400$.

region and overlying the optic nerve head (Figures 4a–c and 5a–d). In 2 of 14 eyes with attached gel scattered laminocytes were seen lying between the ILM and vitreous cortex (Figure 5e and f). Laminocytes were positive for TIIC, GFAP, and AE1/AE3. The ILM was generally negative for TIVC both in eyes with and without PVD. The ILM did, however, show strong staining with DPAS. One cadaver eye with PVD showed macular pucker complete with an *in situ* simple ERM. This membrane was composed of ILM and laminocytes and showed both hyperconvolutional change of the ILM and novel basement membrane production, the appearances being typical of the type of ERM seen in CM (Figure 4a–c).

Discussion

Since 2004, when we published our last paper on ERMs there have been over 300 publications on this subject. Despite this extensive, largely clinical-based literature, research into the pathology of ERM formation has relied on relatively small number of series.[3,18–20] Ultrastructural studies have tended to predominate and there are very few studies examining large numbers of ERMs from different clinical settings by light microscopy (LM). This study is one of the largest of its type, and shows that the histology of the common types of ERM is quite distinctive and allows classification into different clinical groups by routine LM. The salient distinguishing histological components being the presence or absence of ILM, the presence or absence of RPE cells, and the presence or absence of neovascularisation. With these criteria, it is possible to classify ERMs into three distinct types: simple laminocyte ERMs, which contain only ILM and laminocytes, PVR/tissue repair membranes, which contain ILM, laminocytes, and RPE cells with variable amounts of extracellular stroma, and neovascular ERMs, which are devoid of ILM and contain vessels and hyaline stroma. This classification and aetiology are summarised in Table 2.

The most common ERM type in this series was the simple ERM, seen in CM, MH, and VMTS, the ultrastructural features of which were originally described by Foos.[1] The only cellular components of these ERMs are the GFAP- and AE1/AE3-positive cells. In view of their distinctive laminar arrangement, close association with the ILM and evidence of novel ILM production (Figure 1), we prefer the term laminocytes to describe these cells.[14] We believe that these cells have variously been reported as glial cells, glial fibroblasts, astrocytic cells, and Muller cells in previous studies.[11,27,28] The expression of GFAP, which is a well-characterised intermediate filament specific for astro-glial differentiation,[29] is the most useful marker in their identification. Evidence of proliferation and their ability to migrate on the retinal surface has been demonstrated.[30,31] The ILM in these membranes frequently shows hyperconvolutional change analogous to the surface wrinkling phenomena described by Roth *et al*.[2] In these cases, the density of laminocytes is usually increased and there is evidence of novel TIVC basement membrane production, both as cytoplasmic positivity in laminocytes and also staining of the extracellular basement membrane lying alongside the ILM as previously reported.[14] We believe these changes probably represent an attempt to remodel the ILM. The presence of TIIC positivity in laminocytes suggests an additional role in secreting collagen into the vitreous gel. These features clearly indicate that laminocytes have a central role in the pathology of simple ERMs, and we believe the success of ILM peeling in repairing the pathology caused by these membranes, including VMTS and MH, is due to the removal of these cells from retinal surface, despite this aspect of ILM peeling seeming to have been ignored in the debate over this controversial issue.[32]

While GFAP staining is indicative of an astro–glial origin, the positive staining for AE1/AE3 is surprising. This cytokeratin stain includes a combination of antibodies to both high- and low-molecular-weight cytokeratins to ensure wide spectrum of reactivity to the different cytokeratins expressed in epithelial cells.[33] It seems likely, therefore, that the staining in this instance relates to cross reactivity between one of these antibodies and some of the intermediate filaments expressed in laminocytes, rather than implying an epithelial cell of origin for these cells.

Whatever the reason, positivity for AE1/AE3 has a practical advantage; it enables these cells to be seen more readily on the retinal surface, because the retina is

Table 2 Classification of epiretinal membranes by histology and aetiology

Type	*Predominant histological features*	*Aetiology*	*Mechanism*	*Biological mediators*	*References*
Simple idiopathic	ILM, laminocytes	PVD, laminocyte migration and activation	?Surface tension on retina ?Disruption to the ILM	?GNF, GFRα, RET1	[21]
Tissue repair	ILM, laminocytes, RPE cells, fibroblasts macrophages	Retinal tear, trauma, infection, and blunt injury	Cytokine-driven tissue repair process	NF-κB pathway: TGFβ, IL6, PDGF	[22–24]
Neovascular	Capillaries and acellular stromal tissue	PDR, radiotherapy, and vasoformative tumours	Hypoxia and neovascular cytokines	VEGF, HIFα ANG1	[25,26]

Abbreviations: GNF, glial neurotrophic factor; GFRα, glial-derived neurotrophic factor receptor α; RET1, RNA-editing 3′ terminal uridylyl transferase; NF-κB, nuclear factor κB; TGFβ, transforming growth factor β; IL6, interleukin 6; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; HIFα, hypoxia-inducible factor α; ANG1, angiopoietin 1.

negative for this marker, in contrast to GFAP where there is often strong background staining in the retinal nerve fibre layer. In this study, we used AE1/AE3 on cadaver globes to investigate the incidence of laminocytes in asymptomatic globes. Previous studies provided anecdotal evidence that the cells are commonly seen on the retinal surface in eyes from middle aged and elderly patients.[1,12,27] This study shows that in eyes with attached vitreous laminocytes are rare, but commonly seen in eyes with PVD. Nevertheless, the presence of occasional laminocytes lying between the ILM and vitreous in cases with attached gel Figure 5e and f, and their presence on the surface of the posterior hyaloid after PVD,[34] provides evidence of their arrival at the vitreoretinal junction before PVD occurs. This raises the possibility that PVD may in fact be a cellular event, in which laminocytes are the active cell type. If this is the case, then one might expect therapies modulating their behaviour may be useful in preventing or promoting PVD in clinical practice.

The presence of the PHM as a defined anatomical structure, distinct from cortical gel, has proved a controversial concept. By definition, the PHM can only exist once a PVD has occurred, and therefore, until that point, any cellular activity leading up to separation would occur at the interface between ILM and vitreous. The report by Snead *et al*. in this issue, indicates that the PHM is covered by laminocyte-like cells, particularly concentrated around the Weiss ring, which concurs with the distribution seen in the cadaver globes in this study. The presence of cells both on the ILM surface and PHM in cases of vitreous detachment indicates that separation has probably occurred along a line of laminocyte activity at the vitreoretinal interface. We speculate that the development of simple ERMs is dependent on laminocytes remaining on the ILM when PVD occurs, allowing these cells to form a monolayer across part of the retinal surface, attempting to remodel the ILM surface, and setting up tractional forces in the process. It is quite possible that the signals for such laminocyte activity may be enhanced by incomplete vitreous separation, which might be expected to promote additional tractional forces on the retinal surface.

In PVR cases, the ERM is usually complicated by the presence of RPE cells. These cells are present in variable numbers and can be easily recognised morphologically as multilayered cohesive cells, with opaque eosinophilic cytoplasm and, often, the presence of pigment. These cells stain positively for cytokeratins, and negatively for GFAP. Along with the RPE cells, variable numbers of macrophages and lymphocytes can be present, and the membranes are often thick and contain collagen, indicating a tissue repair process. Laminocytes are also often present in these membranes and show similar TIIC, TIVC positivity and ILM hyperconvolution to that seen in simple ERMs, indicating a similar role for these cells in these two types of ERM. The only difference between these two groups rests on whether the membrane has resulted in a retinal tear or detachment. We therefore suggest that these cases represent a more advanced stage of idiopathic simple ERM formation, complicated by a tissue repair reaction stimulated by retinal detachment or tear. In contrast to other reports,[11] we did not identify RPE cells complicating ERMs in any cases without either a tear or detachment. A very similar tissue repair reaction was seen in a variety of ERMs complicating retinal detachments; in this series we saw isolated cases tissue repair type ERMs in post-CMV, and VZV infection and following trauma.

In PDR the situation is different. These membranes are essentially neovascular in nature and their relationship to the ILM is impossible to assess on surgically excised membrane peel specimens. This issue has however been elegantly addressed on postmortem eyes by Faulborn *et al*.[35] Their celloidin-embedded samples showed these vascular membranes present within the vitreous cortex. In our series, we were unable to identify ILM in any PDR cases. We would conclude that it is quite likely the majority of these membranes invade into the vitreous, from the retina, and must therefore breach the ILM. The absence of any inflammatory cell component is in keeping with a hypoxia, providing the primary stimulus for the formation of these membranes. The only additional instances where we saw similar neovascular membranes was in a case of post-radiotherapy ERM formation and an ERM complicating a vasoformative tumour; both of these cases have been previously reported.[16,17]

Acknowledgements

We thank Professor J Armitage, Director and Dr V Smith of the Bristol Eye Bank for the supply of cadaver globes used in this study, and Dr J McCarthy of Gloucester Royal Infirmary, Wing Cmdr. R Scott of Birmingham and Midland Eye Institute, and Dr C Graham of Stoke Mandeville Hospital who kindly referred some of the cases used in this study.

References

1 Foos RY. Vitreoretinal juncture—simple epiretinal membranes. *Graefes Arch Clin Exp Ophthalmol* 1974; **189**: 231–250.
2 Roth AM, Foos RY. Surface wrinkling retinopathy in eyes enucleated at autopsy. *Trans Am Acad Ophthalmol Otolaryngol* 1971; **75**(5): 1047–1058.

3 Schumann RG, Schaumberger MM, Rohleder M, Haritoglou C, Kampik A, Gandorfer A. Ultrastructure of the vitreomacular interface in full-thickness idiopathic macular holes: a consecutive analysis of 100 cases. *Am J Ophthalmol* 2006; **141**(6): 1112–1119.
4 Ishida S, Yamazaki K, Shinoda K, Kawashima S, Oguchi Y. Macular hole retinal detachment in highly myopic eyes: ultrastructure of surgically removed epiretinal membrane and clinicopathologic correlation. *Retina* 2000; **20**(2): 176–183.
5 Haritoglou C, Gandorfer A, Gass CA, Schaumberger M, Ulbig MW, Kampik A. Indocyanine green-assisted membrane peeling of the internal limiting membrane in macular hole surgery affects visual outcome: a clinicopathologic correlation. *Am J Ophthalmol* 2002; **134**(6): 836–841.
6 Blain P, Paques M, Massin P, Erginay A, Spielmann AC, Santiago PY *et al*. Epiretinal membranes surrounding idiopathic macular holes. *Retina* 1998; **18**(4): 316–321.
7 Hiscott P, Hagan S, Heathcote L, Sheridan CM, Groenewald CP, Grierson I *et al*. Pathobiology of epiretinal and subretinal membranes: possible roles for the matricellular proteins thrombospondin 1 and osteonectin (SPARC). *Eye* 2002; **16**(4): 393–403.
8 Sheridan CM, Magee RM, Hiscott PS, Hagan S, Wong DH, McGalliard JN *et al*. The role of matricellular proteins thrombospondin-1 and osteonectin during RPE cell migration in proliferative vitreoretinopathy. *Curr Eye Res* 2002; **25**(5): 279–285.
9 Harada C, Harada T, Mitamura Y, Quah HM, Ohtsuka K, Kotake S *et al*. Diverse NF-kappaB expression in epiretinal membranes after human diabetic retinopathy and proliferative vitreoretinopathy. *Mol Vis* 2004; **10**: 31–36.
10 Hiscott PS, Grierson I, McLeod D. Retinal pigment epithelial cells in epiretinal membranes: an immunohistochemical study. *Br J Ophthalmol* 1984; **68**(10): 708–715.
11 Smiddy WE, Maguire AM, Green WR, Michels RG, de la Cruz Z, Enger C *et al*. Idiopathic epiretinal membranes. Ultrastructural characteristics and clinicopathological correlation. *Ophthalmology* 1989; **96**: 811–821.
12 McLeod D, Hiscott P, Grierson I. Age-related cellular proliferation at the vitreoretinal juncture. *Eye* 1987; **1**: 263–281.
13 Foos RY. Vitreoretinal juncture; epiretinal membranes and vitreous. *Invest Ophthalmol Vis Sci* 1977; **16**(5): 416–422.
14 Snead DR, Cullen N, James S, Poulson AV, Morris AH, Lukaris A *et al*. Hyperconvolution of the inner limiting membrane in vitreomaculopathies. *Graefes Arch Clin Exp Ophthalmol* 2004; **242**(10): 853–862.
15 Snead M, Snead D, Mahmood A, Scott J. Vitreous detachment and the posterior hyaloid membrane: a clinicopathological study. *Eye* 1994; **8**: 204–209.
16 Shankar P, Bradshaw SE, Ang A, Rennie IG, Snead DR, Snead MP. Vascularised epiretinal membrane associated with vasoproliferative tumour. *Eye* 2007; **21**(7): 1003–1004.
17 Addison A, Snead DR, Snead MP. Radiation retinopathy secondary to radiotherapy for a lower lid BCC. *Eye* 2004; **18**(12): 1273–1274.
18 Green WR, Kenyon KR, Michels RG, Gilbert HD, De La Cruz Z. Ultrastructure of epiretinal membranes causing macular pucker after retinal re-attachment surgery. *Trans Ophthalmol Soc UK* 1979; **99**(1): 65–77.
19 Charteris DG, Downie J, Aylward GW, Sethi C, Luthert P. Intraretinal and periretinal pathology in anterior proliferative vitreoretinopathy. *Graefes Arch Clin Exp Ophthalmol* 2007; **245**(1): 93–100.
20 Smiddy WE, Michels RG, Green WR. Morphology, pathology, and surgery of idiopathic vitreoretinal macular disorders. A review. *Retina* 1990; **10**(4): 288–296.
21 Harada C, Mitamura Y, Harada T. The role of cytokines and trophic factors in epiretinal membranes: involvement of signal transduction in glial cells. *Prog Retin Eye Res* 2006; **25**(2): 149–164.
22 Cassidy L, Barry P, Shaw C, Duffy MJ, Kennedy S. Platelet derived growth factor and fibroblast growth factor basic in vitreous of patients with vitreoretinal disorders. *Br J Ophthalmol* 1998; **82**: 181–185.
23 Bochaton-Piallat ML, Kapetanios AD, Donati G, Redard M, Gabbiani G, Pournaras CJ. TGF-beta1, TGF-beta receptor II and ED-A fibronectin expression in myofibroblast of vitreoretinopathy. *Invest Ophthalmol Vis Sci* 2000; **41**(8): 2336–2342.
24 Hiscott PS, Unger WG, Grierson I, McLeod D. The role of inflammation in the development of epiretinal membranes. *Curr Eye Res* 1988; **7**(9): 877–892.
25 Caldwell RB, Bartoli M, Behzadin MA, El-Remessy AE, Al-Shabrawey M, Caldwell RW. Vascular endothelial growth factor and diabetic retinopthy: pathophysiological mechanisms and treatment perspectives. *Diabetes Metab Res Rev* 2003; **19**: 442–455.
26 Abu El-Asrar AM, Missotten L, Geboes K. Expression of hypoxia-inducible factor-1alpha and the protein products of its target genes in diabetic fibrovascular epiretinal membranes. *Br J Ophthalmol* 2007; **91**(6): 822–826.
27 Hiscott PS, Grierson I, Trombetta CJ, Rahi AH, Marshall J, McLeod D. Retinal and epiretinal glia—an immunohistochemical study. *Br J Ophthalmol* 1984; **68**(10): 698–707.
28 Kampik A, Green WR, Michels RG, Nase PK. Ultrastructural features of progressive idiopathic epiretinal membrane removed by vitreous surgery. *Am J Ophthalmol* 1980; **90**(6): 797–809.
29 Eng LF, Ghirnikar RS, Lee YL. Glial fibrillary acidic protein: GFAP-thirty-one years (1969–2000). *Neurochem Res* 2000; **25**(9–10): 1439–1451.
30 Kase S, Saito W, Yokoi M, Yoshida K, Furudate N, Muramatsu M *et al*. Expression of glutamine synthetase and cell proliferation in human idiopathic epiretinal membrane. *Br J Ophthalmol* 2006; **90**(1): 96–98.
31 Hisatomi T, Enaida H, Sakamoto T, Kanemaru T, Kagimoto T, Yamanaka I *et al*. Cellular migration associated with macular hole: a new method for comprehensive bird's-eye analysis of the internal limiting membrane. *Arch Ophthalmol* 2006; **124**(7): 1005–1011.
32 Williams GA. Relationship between macular hole size and the potential benefit of internal limiting membrane peeling. *Br J Ophthalmol* 2006; **90**(10): 1216–1217.
33 Woodcock-Mitchell J, Eichner R, Nelson WG, Sun TT. Immunolocalization of keratin polypepides in human epidermis using monoclonal antibodies. *J Cell Biol* 1982; **95**: 580–588.
34 Snead M, Snead D, Richards A, Harrison J, Poulson A, Morris A *et al*. Clinical, histological, and ultrastructural studies of the posterior hyaloid membrane. *Eye* 2002; **16**: 447–453.
35 Faulborn J, Boswald S. Microproliferations in proliferative diabetic retinopathy and their relationship to the vitreous: corresponding light and electron microscopic studies. *Graefes Arch Clin Exp Ophthalmol* 1985; **223**: 130–138.