

Cytokeratin-containing cells in proliferative diabetic retinopathy membranes

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Abstract

Immunohistochemical techniques were used to investigate the relation between retinal pigment epithelial cells (RPE), traction retinal detachment (TRD) membranes, and combined traction rhegmatogenous retinal detachment (CTR) membranes in proliferative diabetic retinopathy. Seven CTR and five TRD membranes were obtained during closed microsurgery. Six of the seven CTR membranes and one of the five TRD membranes contained RPE. Eleven of the 12 diabetic membranes incorporated glial cells. The findings emphasise that the intravitreal membranes of proliferative diabetic retinopathy contain a diversity of cell types and indicate that RPE tend to contribute to CTR, rather than TRD, membranes. The histopathological appearance of CTR membranes is that of a hybrid between TRD and proliferative vitreoretinopathy membranes.

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The epiretinal and posterior hyaloid membranes of proliferative diabetic retinopathy (PDR) are essentially fibrovascular in composition, although it is well established that the membranes contain glial and inflammatory cells.^{1–6} On the other hand, the place of retinal pigment epithelial cells (RPE) in PDR membranes is more contentious. While many investigators have not observed RPE in PDR membranes,^{1,5,7–9} evidence that RPE may contribute to the membranes has been produced by some immunohistochemical and transmission electron microscopic studies.^{2,10–12} For example, an ultrastructural investigation by Hamilton *et al*² identified cells of probable RPE origin in PDR membranes from eyes with previous retinal detachment. The findings of Hamilton and coworkers suggest that RPE become involved in PDR membranes when there is an associated rhegmatogenous retinal detachment.

Rhegmatogenous retinal detachment usually occurs in proliferative diabetic retinopathy when the contractile PDR membranes tear holes in the subjacent retina and convert a purely tractional retinal detachment (TRD) into a combined traction rhegmatogenous retinal detachment (CTR).¹³ CTR membranes are likely to contain RPE because of the association between retinal holes and the release of RPE into the vitreous¹⁴ whereas TRD membranes may not incorporate RPE.

To investigate the relation between RPE, TRD, and CTR membranes we studied CTR and TRD membranes employing immunohistochemical markers to the family of cytoskeletal proteins known as cytokeratins.^{15,16} We used

antibodies which recognise a spectrum of cytokeratins because mammalian RPE have been shown to express a range of cytokeratins in vivo and in vitro.^{15–22} In addition, we compared the distribution of RPE in the membranes with the location of glial cells (detected by immunostaining the glial cytoskeletal protein glial fibrillary acidic protein – GFAP) in the specimens since retinal glia are the other prominent non-vascular cell type found in PDR membranes.^{1–6} The results of the immunohistochemical studies were correlated with the clinical appearances of the retina in the eyes from which the membranes were removed.

Materials and methods

Twelve vascularised PDR membranes were obtained during closed pars plana vitrectomy for tractional complications of proliferative diabetic retinopathy and grouped into CTR (seven) or TRD (five) membranes.

The specimens were fixed for between 4 and 48 hours in 10% formol saline, dehydrated in graded concentrations of ethanol, and embedded in paraffin wax. Sections 6 µm thick of the wax embedded tissue blocks were immunostained for cytokeratins with a keratin wide screening rabbit antiserum (Dako, High Wycombe, UK). This antibody detects a broad range of cytokeratins including cytokeratin 8 (which is present in reactive and in situ RPE)^{17–21} as well as several other cytokeratin subtypes thought to be more variably displayed by RPE (for example, 5, 6, and 16).¹⁷ The antiserum was chosen to minimise false negative staining of RPE cells in tissues. Alternate tissue sections were immunostained for GFAP using an antiserum from Sigma (Poole, UK) or employed in procedural controls, including processing after absorption of the primary antibody with purified antigen. The immunoperoxidase technique was used as previously described^{15,23} with the peroxidase substrate 3-amino-9-ethylcarbazole (AEC) which yielded a pink/red reaction product. Some sections were counterstained with Mayer's haematoxylin. In addition, some sections were stained with haematoxylin and eosin rather than immunohistochemically.

Sections were examined by bright field and differential interference contrast microscopy. The distribution and approximate percentage of cells staining positively for cytokeratin or GFAP were recorded as described previously.^{15,23}

Results

Seven of the PDR membranes contained cells which were immunoreactive for cytokeratins (Table 1, Figs 1 and 2) and this immunore-

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Table 1 Immunohistochemistry of 12 proliferative diabetic retinopathy membranes

Specimen number	Nature of subjacent retinal detachment	CK positive cells (%)	Distribution of CK positive cells	GFAP positive cells (%)	Distribution of GFAP positive cells
1	CTR	+ (20)	Layers/I	+ (10)	Foci/layers
2	CTR	+ (20)	Layers/I	+ (5)	Foci
3	CTR ^a	+ (15)	Foci/layers/I	+ (15)	Foci
4	CTR	+ (5)	Foci/I	+ (20)	Layers/I
5	CTR	+ (5)	Foci/layers/I	+ (10)	Foci/layers/I
6	CTR	+ (5)	Layers	+ (5)	Layers
7	CTR	- (0)	N/A	+ (10)	Layers
8	TRD	+ (10)	Foci	+ (10)	Layers
9	TRD	- (0)	N/A	+ (15)	Foci/layers
10	TRD	- (0)	N/A	+ (5)	Foci
11	TRD	- (0)	N/A	+ (5)	Layers
12	TRD	- (0)	N/A	- (0)	N/A

CK=cytokeratin; GFAP=glial fibrillary acidic protein; CTR=combined traction rhegmatogenous retinal detachment; TRD=traction retinal detachment; I=isolated cells; a=full thickness macular hole; N/A=not applicable; +=present; -=absent.

activity was absent in sections processed in procedural controls: for example, after absorption of the primary antibody with purified antigen (Fig 1b). Six of the cytoke- ratin positive biopsies were from CTR membranes and only one of the membranes which included cyto- keratin positive cells was a TRD membrane

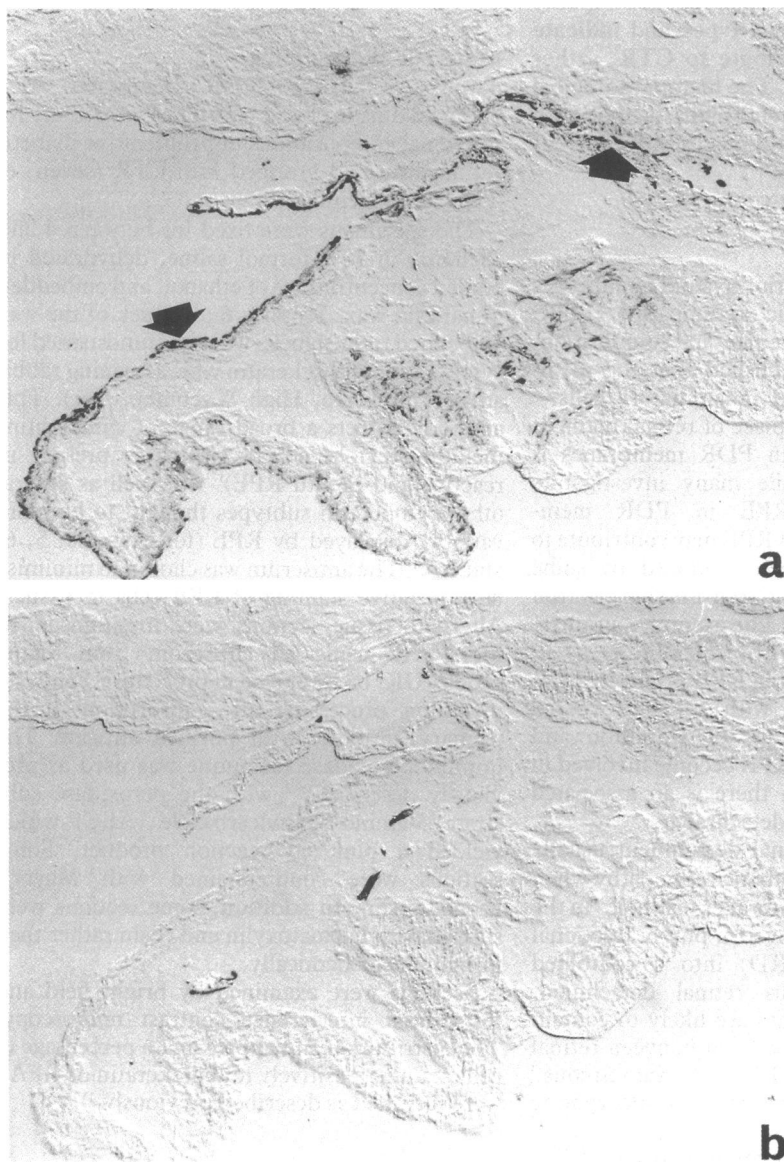


Figure 1 Differential interference contrast micrographs of serial sections of a combined traction rhegmatogenous retinal detachment membrane (a) stained with the immunoperoxidase technique for cytokeratins (no counterstain). Note the prominent layers of cytoke- ratin positive cells (arrows). (b) Labeled with the same method as in (a) but following absorption of the primary antibody against purified antigen. No immunostaining is seen. Magnification $\times 95$.

(Table 1). Conversely, only one of the five PDR membranes which were cytoke- ratin negative was a CTR membrane (Table 1). The association between CTR membranes and the presence of RPE in the biopsies was significant (using the non-parametric sign test which tests the signifi- cance of presence or absence of a parameter; $\alpha=0.1$). The estimated RPE contribution ranged from 20% to 5% of the cells in the cytoke- ratin positive membranes (Table 1). The cytoke- ratin positive cells were aggregated in layers and/or foci (often at apparent tissue surfaces) in the specimens and, in addition, five of the mem- branes also contained scattered, isolated cells which stained for cytoke- ratin (Table 1, Figs 1 and 2).

Eleven of the 12 membranes contained cells which were immunoreactive for GFAP (Table 1, Fig 2). No significant difference could be detected between CTR and TRD membranes with regard to the glial content of the specimens. As with the RPE, the estimated GFAP positive cell contribution ranged from 20% to 5% (Table 1) and glial cells also were arranged in layers and/or foci (again, often at tissue margins). Two specimens incorporated isolated glial cells (Table 1). In some specimens glial aggregates were adjacent to RPE clusters (Fig 2). However, in general, no overall relationship (either in distribu- tion or proportion of cells present) was detected between RPE and glial groups. Colabel- ling of cell aggregates for GFAP and cytoke- ratin was not observed.

In addition to RPE and glia, the membranes contained fibrovascular tissue and a variable number of scattered inflammatory cells.

Discussion

The results emphasise that PDR membranes contain a variety of cell types. In keeping with reports of previous studies,^{1,6} glial cells were present in the vast majority of the diabetic membranes in our investigation. In addition, half of our specimens incorporated RPE whereas RPE were not cited as a component of proliferative diabetic retinopathy membranes in many earlier accounts.^{1,5,7-9} Although the previous studies may have been restricted to TRD membranes, the findings of our investigation suggested that the contribution of RPE to pro- liferative diabetic retinopathy membranes has been underestimated.

We found that the vast majority of RPE containing biopsies were from CTR membranes. Indeed, the correlation between CTR mem- branes and cytoke- ratin positive cell content compares favourably with the association between RPE and membranes of proliferative vitreoretinopathy.¹⁵ However, proliferative vitreoretinopathy membranes tend to include a higher percentage of RPE than do CTR mem- branes: up to 90% of proliferative vitreoretino- pathy membrane cells may be RPE¹⁵ whereas in the present study no more than 20% of the CTR membrane cells are cytoke- ratin positive.

The finding that most proliferative diabetic retinopathy membranes which contain cyto- keratin positive cells are CTR membranes sug- gests that a retinal break is an important factor in

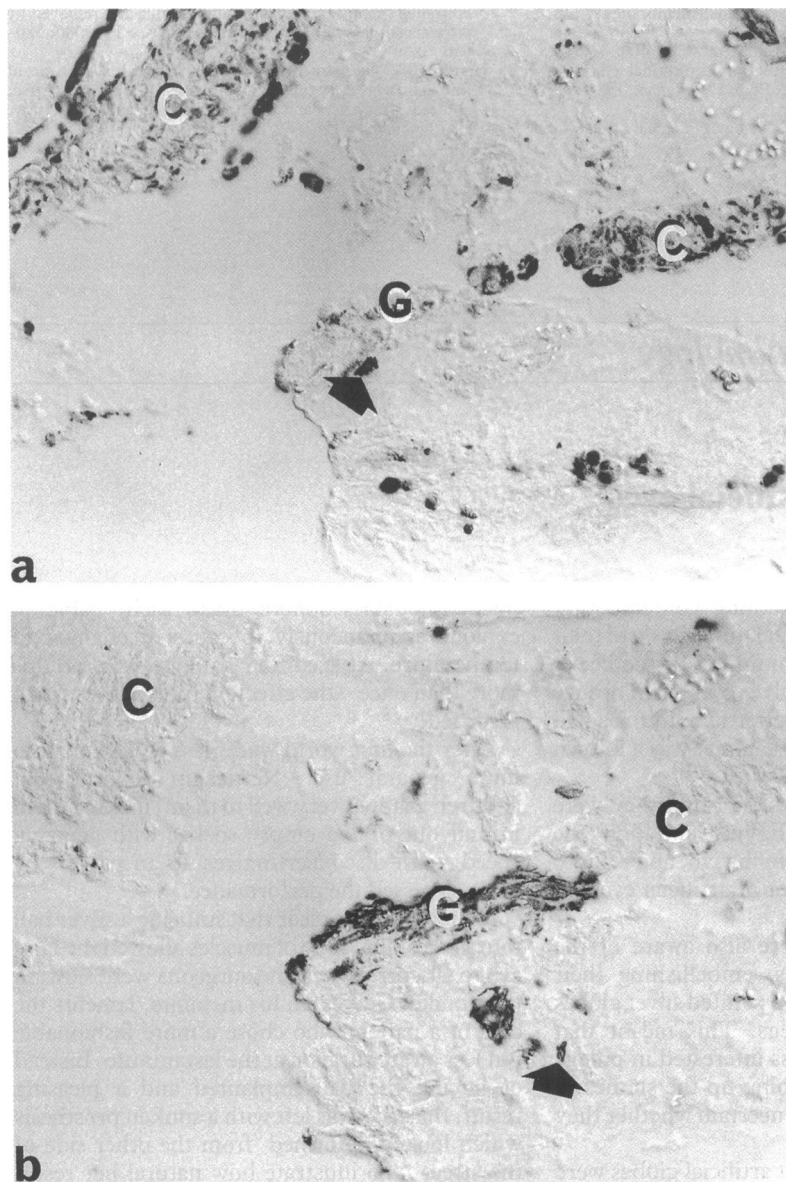


Figure 2 Sections from a combined traction rhexmatogenous retinal detachment membrane stained with the immunoperoxidase method for (a) cyokeratins and (b) glial fibrillary acidic protein (GFAP). Layers and foci of cells are immunoreactive for either cyokeratins (C) or GFAP (G). Pigment is also seen in the tissues (arrows). No counterstain. Magnification $\times 190$.

permitting RPE access to proliferative diabetic retinopathy membranes. Nevertheless, one RPE-containing proliferative diabetic retinopathy membrane was removed from an eye with a purely tractional detachment. Although it is possible that a small retinal hole was not detected at surgery in this TRD membrane, there is evidence that RPE can migrate through intact neuroretina to the vitreous cavity.^{16,24} For example, RPE have been reported in idiopathic and post-inflammatory epimacular membranes²⁴ while vitreous membrane formation in rabbits may be associated with RPE migration through the retina.¹⁶

RPE and glia tended to be clustered into layers or foci of cyokeratin or GFAP positive cells respectively. However, the location of RPE and glial cells appeared very variable with respect to each other suggesting that the distribution of these two cell types was not dependent on each other. In addition, we did not observe colabelling of aggregated cells for cyokeratins and GFAP. Coexpression of the two intermediate

filament proteins has been found in a small subpopulation of epiretinal membrane cells by Viores and colleagues.²² Viores used resin embedded specimens with immunogold techniques²²; methods which were substantially different from our approach and which may have accounted for the differences in results between the two studies.

Presumably the CTR membranes originally were typical fibrovascular TRD membranes which, by virtue of tractional events, caused a retinal tear and subsequently acquired an RPE component. The migration and proliferation of RPE in ostensibly post-contractile proliferative diabetic retinopathy membranes underlines the protracted development of intravitreal membranes compared with skin wounds.²⁵ Moreover, the late acquisition of RPE gives CTR membranes the histopathological appearance of a hybrid between TRD and proliferative vitreo-retinopathy membranes.

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History of ophthalmology

The history of artificial eyes

Artificial eyes – not something contemporary ophthalmologists spend much time on – had their origins in Egypt, where mummies of the better class had enamel covered silver eyes, with bronze lids. (The fact that more geriatric mummies had white pupils confirms that cataract was a known entity.)

This doesn't necessarily mean that they were used for the *living* in Egypt, although the Romans certainly used them *in vivo*, and refer to the 'faber ocularius' (maker of artificial eyes) in inscriptions.

The ancient Greeks were also aware of the principle of artificial eyes, embellishing their more important statues with painted silver globes held in place by gold pegs. This meant that invading Spartans were less interested in pillage and violence than in climbing up the statues to steal the eyes. Again, it is uncertain whether they used them for the living.

We know for certain that artificial globes were being worn by 1561. Pare (who was in disgrace for running out of boiling oil as an army surgeon, until he found that it didn't work anyway) mentions artificial eyes, but gives little detail of their manufacture. And in a play written in 1617, a woman who has rejected a one eyed suitor is admonished with the phrase 'what does it matter – he can have a silver one put in!'. The first English 'faber ocularis' set up in Ludgate Hill in 1681, advertising enamel artificial eyes 'so exact as to look natural', which were also 'very ornamental and commodious'.

But around 1917, the burgeoning 'artificial eye' industry suffered a setback, when its product – greatly needed for injured soldiers returning from the war – came under the Defence of the Realm regulations. They were required to send the Director of Optical Munitions and Glassware 'returns of the number and material of all eyes under their control'.

In point of fact, some of these false eyes proved pretty well *un-controllable*, as the partial vacuum

inside the glass sphere made them liable to explode spontaneously, because of changes in temperature. At least four patients suffered this more than once – the effect on their nerves is not recorded!

After the first world war, glass eyes came into more general use. Nettleship reports that children adapted very well to them, flicking them in and out of the empty socket with amazing speed, with disconcerting results in passers by who witnessed the performance.

Although it was clear that suturing a silver ball into the residual cone of muscles allowed the false eye to sit correctly, some surgeons were slow on the uptake. Graves, in his memoirs, laments the case of a patient who chose a more fashionable but less aware surgeon at the last minute. Instead of having the ball implanted and a pleasing result, this lady was left with a sunken prosthesis which looked deformed 'from the other side of the street'. To illustrate how natural her result could have looked, Graves mentions a nurse who completed the whole of her training without anyone suspecting she wore a prosthesis.

However, the strangest use of a glass eye was reported in the *BJO* in 1911, when at the trial of the Camorrhist secret society in Italy, the accused wished to interrupt his cross examination. 'Becoming wildly excited, he took out his glass eye and threw it on the floor of the court.' His face, apparently, presented a 'horribly disfigured' appearance, and his questioner duly fainted.

FIONA ROMAN

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