SCIENTIFIC CORRESPONDENCE

Retinal neovascular markers in retinopathy of prematurity: aetiological implications

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Aim: (1) To determine if expression of the blood-tissue barrier associated glucose transporter GLUT1 is preserved by the neovasculature of retinopathy of prematurity (ROP), in contrast with the reported loss of GLUT1 expression in preretinal vessels of proliferative diabetic retinopathy. (2) To compare the vascular immunophenotype of ROP to juvenile haemangioma, another perinatal neovascular disorder that has recently been shown to express placental type vascular antigens, including GLUT1 and Lewis Y antigen.

Methods: A retrospective case report was carried out. Immunoreactivities for GLUT1 and Lewis Y antigen were assessed in a human eye with stage 3 ROP and compared with those in a control (paediatric) eye. The presence or absence of endothelial GLUT1 and Lewis Y immunoreactivity was determined in preretinal and intraretinal vessels.

Results: Immunoreactivity was positive for GLUT1 and negative for Lewis Y in the intraretinal and preretinal neovasculature of the ROP affected eye and in the normal retinal vessels of the control eye.

Conclusions: Retention of immunoreactivity for GLUT1 distinguishes ROP from proliferative diabetic retinopathy. Furthermore, absence of Lewis Y antigen co-expression distinguishes ROP from juvenile haemangioma, a perinatal form of GLUT1 positive neovascularisation that has recently been linked to placental vasculature.

Retinopathy of prematurity (ROP) is a proliferative disorder of the developing retinal vasculature in preterm infants, multifactorial in aetiology, but at least in part causally associated with prolonged oxygen therapy followed by return to room air. Evidence from both animal models and human specimens suggests that the proliferative activity of the immature retinal vasculature observed in ROP is promoted by exaggeration of physiological molecular mechanisms essential to normal vascular development, including hypoxia induced enhancement of angiogenic factors.^{1–3}

Normal retinal endothelia possess specialised barrier properties similar to those characterising the blood-brain barrier, which are absent in the vasculature of most non-neural tissues. These properties include ultrastructural features (complex tight junctions, absence of fenestrations, paucity of pinocytotic vessels) and specific antigen expressions—most notably that of the glucose transporter protein isoform 1 (GLUT1).⁴ This facilitative transporter is highly and selectively expressed by endothelia at sites of blood-tissue barriers, including brain, eye, testis, and placenta.^{4,5} Furthermore, we recently found that endothelia of juvenile haemangiomas—common, benign, vasoproliferative entities that share certain temporal, epidemiological, and histological features with ROP—also express GLUT1, as part of a unique constellation of several markers of cellular specialisation that also includes Lewis Y antigen (LeY) shared, to our knowledge, only with the vasculature of human placenta.⁶

Figure 1 Haematoxylin and eosin stained section of the temporal retina in the left eye showing marked intraretinal neovascularisation, extending into the vitreous within a thin preretinal membrane (open arrow). Note the focal haemorrhage (black arrow). (Original magnification $\times 200$.)

Figure 2 GLUT1 immunoreaction of the ROP affected temporal retina in the left eye. Strong endothelial GLUT1 immunopositivity was present in both the preretinal (arrows) and intraretinal (arrowheads) neovasculature. GLUT1 immunopositivity was also seen in the nerve fibre and cell body layers. (DAB+ chromagen; original magnification $\times 400$.)

GLUT1 expression is absent in preretinal fronds and vessels of proliferative diabetic retinopathy (PDR), providing a molecular correlate with the loss of barrier function observed in these leaky vessels.⁷ In this study, we assess GLUT1 expression in a human eye affected by acute phase ROP. Additionally, to investigate possible parallels with juvenile haemangioma, we also assessed LeY expression in this specimen.

CASE REPORT

The clinical history and histopathology of this case have been reported.¹ This female triplet was born at 24 weeks' gestation

Figure 3 GLUT1 immunoreaction in uninjured retina, optic nerve, ciliary muscle, and iris of control (A–D) and ROP affected (E–H) eye. Strong capillary endothelial GLUT1 immunoreactivity (black arrows) was present within the uninjured retina (A, E), optic nerve (B, F), ciliary muscle (C, G), and iris (D, H) of both the control and ROP affected eye. Use of red VIP chromagen in B–D, as opposed to brown DAB+ chromagen (A, E–H), allowed clear distinction of specific GLUT1 immunoreaction (red colour) from the brown pigment normally abundant in the iris and retinal/ciliary epithelium. Note the red GLUT1 immunoreaction of the non-pigmented epithelial layer of the ciliary muscle (C, open arrow), overlying the pigmented layer. Intraluminal erythrocytes (E, asterisk) served as internal positive controls for GLUT1. Original magnifications $\times 200$ (A–C, F, H) and $\times 400$ (D, E, G).

by caesarean section necessitated by placenta praevia. Her hospital course was complicated by sepsis, necrotising enterocolitis, and severe respiratory distress requiring intubation for 30 days. She died from multisystem failure at 4.5 months of age.

Indirect ophthalmoscopy of the fundi at 4 weeks of age showed immature retinal vascular development in zone 1 bilaterally, with prethreshold disease by Cryotherapy for Retinopathy of Prematurity Cooperative Group study criteria⁷ noted at age 14 weeks. Stage 3 ROP developed bilaterally, with zone 2 plus disease in the right eye. Diode laser photocoagulation was applied in the right eye as previously described¹; no treatment was given to the left eye. Subsequent zone 2 regression of ROP was observed bilaterally. Examination 5 days before the patient's death showed recurrent stage 3 zone 2 ROP bilaterally of 1–2 clock hours without plus disease, with localised haemorrhage.

At necropsy, the patient's eyes were formalin fixed and paraffin embedded before preparation of 6 μm sections. The untreated left eye was used in the current study. One normal (paediatric) control eye was similarly collected and prepared. Immunohistochemistry was performed as described previously, with minor modifications, using well characterised antibodies to GLUT1 and LeY.⁶ In brief, deparaffinised sections were subjected to citrate buffer antigen retrieval, and protein blocked before incubation with primary antibodies against GLUT1 (MYM, polyclonal, 1:500, Dako, Carpinteria, CA, USA) or LeY (BM-1, monoclonal, 1:100, Dako) for 30 minutes at

room temperature. Bound antibody was detected using a Dako LSAB+ peroxidase kit using DAB+ chromagen (Dako) or VIP substrate (Vector, Burlingame, CA, USA). Negative controls were processed simultaneously without primary antibody. Perineurium and erythrocytes provided normal internal GLUT1 positive controls. A section of LeY and GLUT1 immunopositive juvenile haemangioma was included in each run as an external positive control.

Histopathologically, the ROP affected eye revealed marked neovascularisation within the temporal retina, extending focally into the vitreous, where capillaries lay in a thin preretinal membrane with focal haemorrhage (Fig 1). Retinal capillaries were GLUT1 immunoreactive, including those within areas of neovascularisation and retinal disruption, and within adjacent neovascularised vitreous (Figs 2 and 3E). Normal GLUT1 immunoreactivity was observed in capillary endothelia of the optic nerve, iris, and ciliary muscle in both the patient's and the control eye (Fig 3), but not in the choroid (Fig 3E) or sclera (not shown). GLUT1 immunoreaction was also present in epithelial barriers (retinal pigment epithelium, non-pigmented ciliary epithelium, corneal epithelium, and iris pigmented epithelium), and in the retinal nerve fibre layer, ganglion cell layer, and outer nuclear layer, but not in the photoreceptor inner and outer segments, as previously reported.⁵ LeY immunopositivity was not seen in any portion of the ROP or control eye, including their vasculature (Fig 4A, B), in contrast with consistent GLUT1/LeY co-expression in haemangioma (Fig 4C).^{6,8}

Figure 4 Lewis Y antigen (LeY) immunoreaction of control eye (A), ROP affected eye (B), and juvenile haemangioma (C). Microvessels of both the normal (A, black arrows) and ROP affected eye (B, intraretinal neovascularisation—open arrow; intravitreal neovascularisation—black arrows) lacked LeY immunoreactivity, as did choroidal vessels, shown for the normal eye (A, open arrows). In contrast, strong endothelial LeY immunopositivity was present in this early involutional juvenile haemangioma (C). Original magnifications $\times 200$ (A) and $\times 400$ (B, C).

DISCUSSION

We report two new findings that have implications for the pathogenesis of ROP relative to other types of neovascularisation. Firstly, we found preserved expression of the blood-retinal barrier associated antigen GLUT1 in the preretinal neovascularisation in this eye with severe ROP, in contrast with the loss of GLUT1 expression by preretinal vessels in proliferative diabetic retinopathy.⁵ This new finding implies the possibility of a functional difference in the neovasculature of these two entities. Secondly, ROP neovasculature was LeY immunonegative, in contrast with the consistently LeY immunopositive vasculature of juvenile haemangioma, another form of GLUT1 positive neovascularisation.^{6,8} ROP and juvenile haemangioma, although clinically distinct, share certain striking features, including plump, mitotically active endothelial fronds or sprouts in the early phase that mature and regress with time, exclusive perinatal presentation, and mutual increase in incidence in premature infants. Our finding of LeY immunonegativity in ROP demonstrates a clear distinction in the antigen expression profiles of these two forms of perinatal neovascularisation.

The specific factors responsible for establishing and maintaining high GLUT1 expression in barrier type endothelia are unclear, although astrocytic influences are probably important.⁹ In PDR, loss of GLUT1 expression both in early preretinal fronds and in mature preretinal membranes correlates with permanent loss of barrier properties.⁵ Our finding of preserved GLUT1 immunoreactivity in the preretinal vessels of ROP implies more complete preservation, or perhaps recovery, of neovascular barrier function. This difference between ROP and PDR may reflect age associated differences in plasticity of neurovascular tissues, or inherently different pathogenetic mechanisms. Newly formed intraretinal vessels in the feline model of ROP are leaky, but become “tight” with time, coincident with recolonisation of the retina by astrocytes immigrating from the optic disc.¹⁰ Although our results are unequivocal, they are based upon a single case of ROP and therefore require confirmation with larger studies. Future studies in animal models of ROP, in which microvascular GLUT1 expression during all stages of neovascularisation can be determined, are likewise needed.

We have previously shown that GLUT1 expression is an intrinsic feature of the committed endothelial phenotype of juvenile haemangiomas, one that is independent of proliferative activity and is absent in other benign vascular tumours, reactive proliferations, and vascular anomalies—including granulation tissue, pyogenic granuloma, and vascular malformations.⁸ As noted earlier, GLUT1 expression in haemangiomas is part of a distinct set of tissue specific markers, including LeY, that imply a unique association with placental vessels.⁶ Our present results suggest that this association is not shared by ROP.

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REFERENCES

- 1 Young TL, Anthony DC, Pierce E, *et al*. Histopathology and vascular endothelial growth factor in untreated and diode laser-treated retinopathy of prematurity. *J AAPOS* 1997;1:105–10.
- 2 Stone J, Chan-Ling T, Pe'er J, *et al*. Roles of vascular endothelial growth factor and astrocyte degeneration in the genesis of retinopathy of prematurity. *Invest Ophthalmol Vis Sci* 1996;37:290–9.
- 3 Pierce EA, Foley ED, Smith LE. Regulation of vascular endothelial growth factor by oxygen in a model of retinopathy of prematurity. *Arch Ophthalmol* 1996;114:1219–28.
- 4 Harik SI, Kalaria RN, Andersson L, *et al*. Immunocytochemical localization of the erythroid glucose transporter: abundance in tissues with barrier functions. *J Neurosci* 1990;10:3862–72.
- 5 Kumagai AK, Glasgow BJ, Pardridge WM. GLUT1 glucose transporter expression in the diabetic and nondiabetic human eye. *Invest Ophthalmol Vis Sci* 1994;35:2887–94.
- 6 North PE, Waner M, Mizeracki A, *et al*. A unique microvascular phenotype shared by juvenile hemangiomas and human placenta. *Arch Dermatol* 2001;137:559–70.

- 7 **Cryotherapy for Retinopathy of Prematurity Cooperative Group.** Multicenter trial of cryotherapy for retinopathy of prematurity: one year outcome—structure and function. *Arch Ophthalmol* 1990;**108**:1408–16.
- 8 **North PE,** Waner M, Mizeracki A, *et al.* GLUT1: a newly discovered immunohistochemical marker for juvenile hemangiomas. *Hum Pathol* 2000;**31**:11–22.
- 9 **Risau W.** Induction of blood-brain barrier endothelial cell differentiation. *Ann NY Acad Sci* 1991;**633**:405–19.
- 10 **Chan-Ling T,** Stone J. Degeneration of astrocytes in feline retinopathy of prematurity causes failure of the blood-retinal barrier. *Invest Ophthalmol Vis Sci* 1992;**33**:2148–59.

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