INTRODUCTION: The presence of dilated, tortuous vessels in ROP (Plus Disease) is a poor prognostic indicator of visual outcomes (1). Our aim was to determine whether alterations in mural cells, pericytes and smooth muscle cells (SMCs), are associated with the pathogenesis of ROP, including Plus Disease.

METHOD: Kittens were exposed at postnatal day (P)1 to 60-70% oxygen for 4 days which results in retinal vaso-obliteration and then returned to air for 0-27 days (hypoxic phase of kitten model of ROP)(2). To more closely mimic human ROP, we modified the kitten model of ROP by subjecting P3 kittens to hyperoxia for only 2 days, which resulted in delayed retinal vascularization and localised vessel loss rather than vaso-obliteration, then returned the kitten to air for 3 or 29 days. Control animals were raised in room air. Retinas were double labelled with antibodies against desmin or α smooth muscle actin (SMA), and Griffonia simplicifolia isolectin B4 histochemistry to label mural cells and the vasculature respectively. The desmin ensheathment ratio (DER), a quantitative measure of vessel stability was determined (3). Control & experimental tissues were examined by electron microscopy.

RESULTS: Neovascularisation occurs during the hypoxic phase of the kitten model of ROP (2). In the neovasculature, radial arterioles and venules were diluted with reduced SMC coverage (see Fig. 1D & E) and SMA and desmin immunohistochemistry (IHC) suggested that the differentiation of these SMCs was delayed. In the modified kitten model of ROP, SMA labelling of surviving venules was markedly decreased (compare Fig. 1A with 1B) and was less even on surviving arterioles (compare Fig. 1A with 1C). The difference normally observed between arterioles and venules at P3 using desmin IHC was lost, consistent with dedifferentiation of SMCs. In addition, the DER of the capillaries that survived the 2 day hyperoxic exposure was reduced.
SMA IHC labelled radial arterioles (a) and venules (v) in the retina of a P6 kitten (A) and the retina of a kitten exposed to 2 days of hyperoxia followed by 3 days in room air (2dO + 3dRA) (B & C). Marked loss of SMA organization was evident on radial arterioles and venules. Ultrastructure of arteriolar wall (D) and venular wall (E) of radial vessels in the neovasculature in retina of a kitten exposed to 4 days of hyperoxia followed by 23 days in room air showed vessel dilation and scant smooth muscle cell ensheathment compared to controls.

**DISCUSSION:** The low DER of surviving vessels is consistent with a highly unstable vascular plexus, receptive to both angiogenic and vessel withdrawal signals. Our results provide compelling evidence of significant changes in arteriolar and venular SMCs in both experimental models of ROP. The delayed differentiation and apparent dedifferentiation of SMCs during the hypoxic phases could result in impaired ability to regulate blood flow, contributing to the vaso-dilatation and tortuosity, hallmarks of Plus disease. The dense vaso-proliferation observed could further compound the vessel dilatation and tortuosity observed due to its effect on venous return and vessel rheology.

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**REFERENCE**