thymosin beta 4 was applied topically to healthy rats and mice in scrape and alkali injury models. Our limited study was not designed to determine the optimal dose or length of treatment.

All patients in this study showed clinically significant reduction in the size of their epithelial defects. Case 1 demonstrated complete healing within the 28-day treatment period (Figure 1). Some fluctuation in the size of the defects was seen in most patients; however, regression to the pretreatment defect size did not occur. Case 3 healed completely by the end of the follow-up period (day 36). These findings are particularly noteworthy given that the defects showed no inclination toward healing in the 6 to 12 weeks prior to initiating treatment. No evidence of stromal thinning or vascular ingrowth of the corneal stroma on the iris surface or retina was observed in any of the patients (2 of 3 patients with diabetes had been treated previously for proliferative diabetic retinopathy).

No patient reported discomfort associated with the drops. Improved ocular comfort and decreased conjunctival injection was correlated with healing.

In conclusion, this study suggests a promising role for thymosin beta 4 drops in the treatment of patients with recalcitrant, nonhealing, neurotrophic corneal epithelial defects. This pilot study is to be continued; if the initial results are supported, then a controlled clinical trial will be warranted to explore the full effects and proper dosing of thymosin beta 4 in the modulation of corneal wound healing in patients with neurotrophic keratopathy.

Steven P. Dunn, MD
David G. Heidemann, MD
Christopher Y. C. Chow, MD
David Crockford, BS
Nabila Turjman, PhD
Janet Angel, BS
Christian B. Allan, PhD
Gabriel Sosne, MD

Author Affiliations: Departments of Ophthalmology, Children’s Hospital of Michigan, Detroit Medical Center, Wayne State University, Detroit (Dr Dunn); William Beaumont Hospital, Royal Oak, Michigan (Drs Dunn, Heidemann, and Chow); RegeneRx Biopharmaceuticals, Inc, Bethesda, Maryland (Mr Crockford, Drs Turjman and Allan, and Ms Angel); and Departments of Ophthalmology and Anatomy/Cell Biology, Kresge Eye Institute, Wayne State University School of Medicine, Detroit, Michigan (Dr Sosne).

Correspondence: Dr Dunn, Michigan Cornea Consultants, PC, 29201 Telegraph Ave, Ste 101, Southfield, MI 48034 (stevenpdunn@ameritech.net).

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Cellular Composition of the Ridge in Retinopathy of Prematurity

In retinopathy of prematurity (ROP), elevated levels of oxygen arrest the normal posterior-to-anterior growth of intraretinal blood vessels; in some patients, the demarcation zone between vascular and avascular retina thickens into an ophthalmoscopically visible ridge that designates stage 2 ROP. The ridge consists of a larger anterior collection of spindle-shaped cells in the nerve fiber layer (the “vanguard”) and a variably present smaller posterior vascularized rearguard. Although the ridge is an important aspect of ROP pathogenesis, its cellular composition has not been well characterized.

Report of Cases. Four eyes were obtained at autopsy from 2 children clinically diagnosed with stage 2 ROP at the Stanford University Medical Center.

Case 1. A male baby was born at 24 weeks’ gestational age and treated with oxygen for respiratory distress from birth. Stage 2 ROP was diagnosed 8 weeks after birth. The child died at age 12 weeks of multiple organ failure.

Case 2. A male baby was born at 27 weeks with hydrops fetalis and an 11p13-15.1 deletion. The patient received supplemental oxygen and died 6 weeks after birth.

Immunohistochemical examination was performed on formalin-fixed paraffin-embedded sections using antisera to identify mature and immature vascular endothelial cells (CD31; Dako, Glostrup, Denmark, or CD34; Becton, Dickinson, and Co, Franklin Lakes, New Jersey), astrocytes (glial fibrillary acidic protein [GFAP]; Dako), astrocyte precursor cells (PAX2; Zymed Laboratories, San Francisco, California), myeloid lineage cells (microglia and macrophages; CD68; Dako), pericytes (desmin; Dako), neurons (neuron-specific enolase; Dako), or proliferating cells (Ki67; Dako).

Ridge Vanguard. A thickened ridge of spindle cells (the vanguard) in the nerve fiber layer just anterior to the most distal blood vessels was verified in eosin-stained sections in the temporal and nasal aspects of all 4 eyes; a smaller vascular proliferation in the rearguard was evident in some cases (Figure 1A). CD34 and CD31 labeled intraretinal blood vessels (Figure 1B and C). No CD34 or CD31 immunoreactivity was detected in ridge spindle cells (Figure 1B and C). Scattered CD68+ cells (presumed resident retinal microglia) were present in the inner retina posterior to the ridge, often adjacent to blood vessels, but were found only rarely within the ridge (Figure 1D and E). Occasional lightly GFAP-immunoreactive cells were detected in the ridges (Figure 1F).
Strongly GFAP-labeled astrocytes were detected in the optic nerve and throughout the vascularized inner retina (Figure 1G). Essentially all cells within ridge vanguards exhibited intense PAX2 immunoreactivity (Figure 1H and I). Desmin immunoreactivity was seen in extraocular muscles and around intraretinal arterioles but not in the ridge; neuron-specific enolase–labeled neurons were absent in the ridges (not shown).

Ridge Rearguard. Most endothelial cells lining rearguard vessels exhibited CD31 and/or CD34 immunoreactivity (Figure 2A and B). Several CD68+ presumed microglia were found among the vessels (Figure 2C). Rare GFAP+ and several PAX2+ glial cells were present in the rearguard (Figure 2D and E).

Ki-67+ cells appeared only rarely in the rearguard (Figure 2F). In case 1, Ki-67+ spindle cells were infrequently detected in ridge vanguards, while in case 2, numerous proliferating spindle cells were scattered throughout the ridges (Figure 3A and B). As a positive control, proliferating cells were found in extraretinal neovascular tufts and basal corneal epithelium (Figure 3C and D).

Comment. In normal retinal development, blood vessels arise at the optic nerve head and extend anteriorly to reach the periphery near term. Astrocytes similarly arise from the optic nerve and extend peripherally in advance of the nascent vasculature. In premature infants exposed to supplemental oxygen therapy, vessel growth arrests and a hypercellular ridge may develop at the border between vascularized and avascular retina. Although the ridge plays a critical role in ROP as the site of vascular shunting, and of either disease regression or progression, definitive description of the cellular composition of the ridge is lacking. Spindle-shaped ridge cells have been suggested to be mesenchymal angioblasts, or a heterogeneous population of vascular precursor cells, glia, and possibly pericyte precursors and accessory cells, that serve a transient developmental function. Two studies concluded that at least some ridge cells are glial, based on poorly defined microscopic features and GFAP labeling. We found that nearly all spindle-shaped cells that compose the ridge vanguard are glial: they are predominantly PAX2+ astrocyte precursors and, to a far lesser extent, mature GFAP+ astrocytes. We found no evidence of immature vascular endothelial cells (CD31+/CD34+) within the ridge vanguard. Only a few scattered CD68+ microglia were found in the ridges. Hypercellularity in the ridge vanguard may arise by cell division or by focal accumulation of astrocyte precursors whose radial migration is interrupted at the border of vascularized and avascular retina. The first possibility is supported by enhanced proliferation of retinal astrocyte precursors in low oxygen, while the second is supported by reduced astrocyte migration in hypoxia. Modest and variable proliferation among ridge cells and the scarcity of astrocyte precursors anterior to the ridge are consistent with both mechanisms. Resolution of this issue requires analysis of proliferation in retinai with late stage 1 and early stage 2 ROP, when...
Figure 2. Immunohistochemical staining of the ridge rearguard. Asterisk indicates artifactual separation. Scale bar, 200 µm. A and B, CD31 (A) and CD34 (B) labeled endothelial cells in rearguard vascular structures. C, Several CD68+ cells scattered in the rearguard. D, Very few glial fibrillary acidic protein (GFAP)+ positive cells (arrows). E, Several PAX2+ cells mostly surrounding the vascular structures. F, Rare Ki67 cells in the rearguard (arrows).

Figure 3. Cellular proliferation in ridge spindle cells. A and B, Ki-67 immunoreactivity in ridge cells (arrows) in cases 2 (A) (original magnification ×30) and 1 (B) (original magnification ×20). C and D, Ki67 labeled occasional cells in a tuft of neovascularization and in the inner retina (C) (arrows) (original magnification ×10) and basal corneal epithelium (D) (arrows) (original magnification ×20).
the ridge begins to form. Animal models of ROP are of limited value, as they do not exhibit a ridge.

Astrocyte precursors that lie ahead of developing retinal vessels secrete vascular endothelial growth factor and other cytokines that appear to stimulate and guide peripheral extension of retinal vessels. It seems likely, then, that vascular endothelial growth factor secretion from ridge cells may stimulate neovascularization, which typically appears just posterior to the ridge. If this is the case, laser ablation of the ridge might be beneficial in cases where peripheral avascular retinal laser therapy is insufficient to cause regression of neovascular tissue. Interestingly, the exuberant dilated vascular tissue at the rearguard exhibited little or no cell proliferation, suggesting that either proliferation had given rise to these vessels earlier but had ceased by the time of death or that vessels there respond to vasoactive cytokines with dilation rather than frank neovascularization. In summary, ridge spindle cells consist mainly of astrocyte precursor cells and, to a lesser extent, mature astrocytes. Cell proliferation contributes at least partly to ridge formation.

AUTHOR AFFILIATIONS: Department of Ophthalmology, Stanford University School of Medicine, Palo Alto, California.

CORRESPONDENCE: Dr Gariano, Department of Ophthalmology, Room A-157, 300 Pasteur Dr, Stanford University School of Medicine, Palo Alto, CA 94305 (rgariano@stanford.edu).

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