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Unilateral sporadic retinal dysplasia: Results of histopathologic, immunohistochemical, chromosomal, genetic, and VEGF-A analyses

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Abstract

PURPOSE—To describe new findings in a case of unilateral retinal dysplasia.

METHODS—Histopathologic evaluation of an enucleated globe and analysis with immunohistochemical probes, karyotyping, and genetic analysis for the Norrie gene, and aqueous assay for vascular endothelial growth factor A (VEGF-A).

RESULTS—Histopathological examination of the globe revealed retinal dysplasia with pseudorosette formation, abnormal or absent retinal nuclear lamination, a paucity of disorganized retinal microvasculature, retinal infoldings, advanced gliosis, persistent hyperplastic vitreous, exuberant neovascularization of the vitreous, and iris neovascularization (identical to the findings observed in bilateral Norrie disease). Immunohistochemistry disclosed GFAP-positive and GLUT-1-positive gliosis and retinal and persistent hyperplastic vitreous microvessels that were CD34-positive and GLUT-1-negative. Ki-67-positive retinal cells were polarized toward the subretinal space and absent in the retinal invaginations and pseudorosettes. A normal karyotype was found, and DNA sequencing revealed no known mutation in the region of the Norrie gene (*NDP*) in sputum or retinal DNA. Aqueous obtained immediately after enucleation contained an exceptionally high concentration of VEGF-A (4.5 ng/mL).

CONCLUSIONS—Despite the failure to find an abnormal *NDP* allele, other unexplored *NDP* regions, an undetected defect restricted to retinal tissues, or an autosomal mutation coupled with

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Literature Search PubMed was searched using the following key words in all languages: retinal dysplasia, Norrie disease, persistent hyperplastic vitreous, Norrin, frizzled-4, WNT, VEGF-A, and *sonic hedgehog*. References of relevant articles were also reviewed.

disrupted signaling pathways may be responsible for the condition. High aqueous VEGF-A suggests that this cytokine may play a role in pathogenesis in conjunction with other pathways.

Noninherited, sporadic unilateral retinal dysplasia is rare¹⁻⁵ and must be distinguished clinically from retinoblastoma. It can also be encountered in disturbed ocular organogenesis.^{1,6} Because Norrie disease also manifests retinal dysplasia as a cardinal feature, associated clinical findings, X-linked inheritance, and genetic evaluation separate it from isolated retinal dysplasia.^{2,7} We performed a study that for the first time combines histopathologic evaluation with correlative immunohistochemistry, genetic investigations, and an assay to determine the level of vascular endothelial growth factor-A (VEGF-A) in the aqueous humor of a female neonate with unilateral retinal dysplasia.

Materials and Methods

Clinical records, family history, a review of systems, and diagnostic studies, including ultrasonography, were reviewed. The enucleated globe was studied in paraffin-embedded sections, stained with the hematoxylin and eosin, Masson trichrome, and Alcian blue techniques. Immunohistochemical studies were performed in the Immunopathology Diagnostic Laboratory of the Department of Pathology at the Massachusetts General Hospital. The following probes were employed: CD34 (endothelial cells), glial fibrillary acidic protein (GFAP, fibrillary astrocytes), glucose transporter protein 1 (GLUT-1, endothelial cells), CD20 (B-lymphocytes), CD3/5 (T-lymphocytes), and Ki-67 (cells in the S-phase of DNA replication). Karyotyping was done on 2 mL of peripheral blood by the G-banding method using Wright staining and trypsin in 20 metaphases. Results were recorded using the International System for Human Cytogenetic Nomenclature guidelines.⁸ Laboratory analysis was performed at the Associated Regional University Pathologists Laboratory at the University of Utah, Salt Lake City.

In the Neurogenetics DNA Diagnostic Laboratory at the Massachusetts General Hospital, sequencing analysis and amplification of exons 1-3 and their adjacent intronic sequences in the *NDP* gene in Norrie disease was conducted to identify nucleotide changes.⁹⁻¹¹ Genomic DNA extracted from the patient's sputum and DNA obtained from paraffin-embedded retinal tissue were studied by polymerase chain reaction (PCR). Other intronic and promoter regions were not assayed. The PCR amplified products were labeled bidirectionally using a fluorescent dideoxy-nucleotide chain termination method and then resolved by capillary electrophoresis on a 3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA).

Multiplex ligation-dependent probe amplification¹² was performed to determine whether gene rearrangements or gene copy number abnormalities that might be missed by PCR existed. This method employed Salsa Multiplex ligation-dependent probe amplification reagents (MRC-Holland, Amsterdam, the Netherlands) and *NDP*-specific probes designed at the Massachusetts General Hospital. The probe mix contained multiple probes targeting each of the three exons of the *NDP* gene as well as upstream and downstream regions of the *NDP* gene on the X chromosome. Probes targeting other regions of unrelated autosomal genes were used as internal control probes. The ligation and amplification products from the patient's genomic DNA were analyzed against normal and positive controls. Amplified

products from this patient and controls were run on a 3730XL DNA analyzer (Applied Biosystems) under standard fragment analysis conditions and the raw output data was analyzed using GeneMarker software (SoftGenetics, State College, PA).

Two samples of aqueous humor obtained immediately after enucleation were subjected to a quantitative sandwich enzyme immunoassay (RND Systems, Minneapolis, MN), whereby the aqueous samples were applied to a microplate coated with immobilized monoclonal VEGF-A antibody.¹³ After several washings, a polyclonal VEGF-A antibody was added to the bound sample to interact with any VEGF present in the patient's aqueous that had been adsorbed onto the microplate. A substrate reaction with a linked chromogen resulted in a color proportional to the intensity of VEGF-A in the sample. The results were compared to those derived from known amounts of control VEGF-A using a standard curve that was run simultaneously. The bioassay of VEGF levels was performed at the Vascular Biology Program at the Children's Hospital Boston.

This article complies with all local laws and the principles of the Declaration of Helsinki and was conducted in conformity with a protocol approved by the Institutional Review Board.

Results

In January 2009, a 16-day-old white girl with a normal birth and medical history was noted by her father to have a "large pupil" in the right eye. She was born at 34 weeks, and her mother was noted to have vaginal manifestations of a human papillomavirus infection. The girl was referred to one of the authors (DSW) for an evaluation. Examination revealed mild enlargement and haziness of the right cornea. The anterior chamber of the right eye was deep. The right pupil was moderately dilated, and the pupillary ruff was prominent. Inspection of the iris stroma disclosed dense neovascularization. Gonioscopy showed complete angle closure, with forward insertion of the iris to the level of Schwalbe's line. The lens was clear. Behind the lens and applied to it, a pink mass extending from the superotemporal to the inferonasal quadrants was present in the anterior vitreous. Associated with the mass was a totally detached retina. The intraocular pressure of the right eye measured approximately 45 mm Hg and 15 mm Hg in the left. A red reflex was present in the left side, but no posterior pole details could be discerned. A-scan ultrasonography demonstrated an axial length of 14 mm of the right eye and 13 mm of the left. B-scan ultrasonography of the right eye confirmed the presence of a dense mass in the anterior vitreous without any internal high reflectivity that would signify calcification; there was an associated funnel-shaped retinal detachment (Figure 1A). The left fundus was completely normal when examined under anesthesia with indirect ophthalmoscopy. There were no other systemic findings, and a detailed family history did not disclose any ocular abnormalities, including retinoblastoma. Given the presence of neovascular glaucoma, a complete retinal detachment, and a retinal mass, the prognosis for useful vision was judged to be poor. The diagnosis of a retinoblastoma or a medulloepithelioma could not be ruled out and enucleation was therefore performed. After the diagnosis of retinal dysplasia became available from the examination of the enucleated globe, genetic testing was performed and consisted of peripheral blood DNA for karyotyping and DNA extracted from cells in the patient's sputum and from the retina of the enucleated globe to detect a Norrie gene. A

pediatric evaluation failed to uncover any stigmata of Norrie disease, including deafness, seizures, and mental retardation. The patient has continued to meet developmental milestones on time during 32 months of follow-up without any emerging hearing or neurologic problems.

The enucleated globe after formalin fixation measured $21 \times 18 \times 19$ mm and the cornea measured 11.5×13 mm. The cornea did not display any gross abnormalities, but the anterior chamber was shallow due to removal of aqueous prior to enucleation. The uvea was heavily pigmented. A large, partially cystic and focally hemorrhagic mass with white strands caused thickening of the conically detached retina. This mass was plastered to the posterior surface of the lens and extended onto the posterior surface of the iris, overlapped the ciliary processes, and projected into the vitreous cavity to a height of 7 mm.

Microscopically, the cornea manifested occasional small subepithelial bullae (possibly artifactual) with an age-appropriate complement of endothelial cells; there was one focal break (paracentesis site) in Descemet's membrane. The anterior iridial surface was straightened due to involvement with a diffuse neovascular membrane, and there was a slitlike anterior chamber and approximation of the peripheral iris to the chamber angle due to the earlier withdrawal of aqueous. The lens was artifactually dislocated and displayed mild posterior subcapsular cataractous changes. There was adhesion of a richly capillarized vitreous and juxtaposed vascularized neuroglial mass to the posterior lens capsule (Figure 1B). The ciliary processes were focally elongated. The persistent hyperplastic vitreous (PHV) formed broad attachments to the surface of the retinal mass and undifferentiated peripheral primitive neuroepithelium in the region of the pars plana. This tissue stained diffusely positive with Alcian blue and focally with the Masson trichrome. There was no subretinal exudate and the choroid was not thickened, although there were scattered lymphocytes, plasma cells, and a few eosinophilic leukocytes. The pigment epithelium and choriocapillaris were intact.

The retinal mass itself displayed architectural disorganization with absent lamination, gliosis, and scattered telangiectatic vessels. Along its outer rim abutting the subretinal space were infoldings of a primordial neuroepithelium (Figure 1C). Toward the center of the mass, pseudorosette structures (Figure 1D) comprising three to five layers of neuroretinal cells were set in a glial neuropil. Extending from the retinal mass toward the periphery was a thinner strip of dysgenetic retina (but several times the thickness of normal retina) with a faint suggestion of lamination and pseudorosette formation (Figure 1E). The advitreous and subretinal portions of this retinal segment were flattened and smooth without evidence of photoreceptor differentiation throughout the entirety of the malformed retina. Luxuriant retinal-vitreous neovascularization was in intimate relationship with the anterior solid retinal mass and the persistent primitive neuroepithelium of the far periphery (Figure 1F). Drs Ralph Eagle of the Wills Eye Institute and Ramon Font of the Cullen Eye Institute reviewed the slides and concurred in the diagnosis of retinal dysplasia with accompanying PHV.

Immunostaining with CD34 highlighted the iris surface neovascularization (Figure 2A) and the new vessel formation at the retrolenticular apex of the retinal mass and adherent PHV (Figure 2B and C). There was a paucity of positively staining endothelial cells in the retinal

mass, including the dysgenetic thinner strip proceeding anteriorly (Figure 2B). Immunolabeling for the presence of GFAP demonstrated a predominance of fibrous astrocytes (actually activated Mueller cells) located mainly in the advitreal portion of the retinal mass (Figure 2D). GLUT-1 was identified in the endothelium of the iridial surface neovascularization and in the iris stromal and ciliary body vessels; it was not seen in the choroidal, intraretinal, or intravitreal vessels associated with the PHV. Ki-67 labeling revealed that cells with nuclear positivity were present only along the border of the retina facing the subretinal space (Figure 2E); they increased in number progressively toward the pars plana region. Rare pseudorosettes observed in this tissue did not display nuclear positivity (Figure 2E, inset). Invaginations with multiple layers of nuclei in their walls that commenced from the subretinal aspect of the retinal mass were also negative (Figure 2F), while the peripheral persistent primitive neuroepithelium harbored scattered Ki-67-positive cells that trailed off as the tissue became thinner anteriorly behind the iris (Figure 2F, inset). Scattered positive-staining nuclei outside of the neurotubular invagination were detected. On adjacent sections, these were found to be mostly CD3/4-positive T-lymphocytes.

The patient's karyotype was normal. Sequencing of DNA isolated from the patient's fresh sputum and diseased retina from paraffin-embedded tissue failed to show a known mutation in the *NDP* gene. In addition, multiplex ligation-dependent probe amplification revealed a normal gene copy number. The postoperative sample of the aqueous fluid evaluated with enzyme-linked immunosorbent assay revealed an exceptionally high average concentration of VEGF-A of 4.491 ± 0.239 ng/mL (two samples of aqueous fluid obtained postenucleation were measured and contained 4.322 ng/mL and 4660 ng/mL).

Discussion

Unilateral, randomly occurring retinal dysplasia (dysgenesis) is an unusual condition that has not been studied in depth for 25 years and contemporarily receives only scant and perfunctory mention in ophthalmic textbooks.^{14,15} Ultrasonography, as exemplified by the current case, discloses either diffuse or focal retinal thickening with a conically shaped retinal detachment. A mass appears to be suspended in the middle of the vitreous cavity with anechoic preretinal and subretinal spaces. As in the current case, calcification is not clinically detectable by imaging studies nor has it been found on microscopic examination of enucleated globes previously studied.^{4,5,16}

Based on prior histomorphologic studies, with which ours are in substantial agreement, retinal dysplasia has been deduced to be caused by separation of the neuroretina from the underlying retinal pigment epithelium during critical periods of cytologic retinal differentiation, lamination, and vasogenesis, or else due to partial absence of the pigment epithelium—for example, retinal dysgenesis detected overlying a choroidal coloboma or in synophthalmia.^{1,3,6,16-20} Others have suggested that abnormalities in the developmental sequence of programmed cell death (apoptosis), misdirected regenerative activity, or injuries to the retina occurring prepartum may play a role in retinal dysplasia.^{5,17} Dysplasia is not encountered as a reparative response in the adult mature retina.^{1,3-5} The histopathologic hallmark is the formation of pseudorosettes surrounded by extensive gliosis.^{1,3,16,17} The pseudorosettes are up to three cell layers thick, exhibit the coparticipation of Mueller cells,

form an external limiting membrane, and display a vestigial plexiform layer. In the Flexner-Wintersteiner rosettes of retinoblastoma, only a single layer of cuboidal cells delimits the lumen. Retinal folds in retinal dysplasia are rendered irreversible by variable degrees of ensuing gliosis caused by Mueller cell proliferation in the retina.

PHV, as exhibited in our sporadic case, is a frequent accompanying finding with retinal dysplasia that can form a retrolental membrane.^{1,21} This aspect may also play a secondary role in the formation of dysplastic rosettes by exerting traction on the still evolving retina that can induce folds. In comparison, bilateral retinal dysplasia in Norrie disease is an X-linked recessive disorder with ocular histopathologic findings indistinguishable from those encountered in nonsyndromic dysplastic cases.² Other associated clinical findings help establish the diagnosis of Norrie disease and include deafness, mental retardation, and psychosis, as well as rarer combinations of phenotypes like self-abusive behavior, microcephaly, hypotonia, seizures, cryptorchidism, growth retardation, sexualimpotence, hypogonadism, and immunodeficiency.^{2,22} None of these abnormalities was discovered in our patient.

One dimension of retinal dysplasia that has not been sufficiently emphasized is the manner of pseudorosette formation. Tubular infoldings commencing on the subretinal edge of the dysgenetic retina proceeded inward toward the center of the mass.⁴ These structures with well-delineated external limiting membranes appeared in most histopathologic sections to have arisen de novo within the retinal tissue; however, in favorable sections they could be traced to an origin from the tubular invaginations. Our immunohistochemical results pointed out another mechanism of the retinal dysplasia. Ki-67 immunostaining of the nuclei (highlighting DNA synthesis in S-phase before mitotic replication) demonstrated the presence of labeled nuclei only along the entire edge of the retinal mass facing the subretinal space, but most prominently anteriorly where the retina was the most immature. This labeling totally disappeared from the nuclei of cells that participated in the formation of the infolded neurotubules and pseudorosettes.

Over one-half of the thickening of the retinal tissue was due to florid gliosis, demonstrated by positive GFAP immunostaining. In place of the typical developing double-layered microvasculature, the haphazardly scattered intraretinal vessels (often telangiectatic) and the PHV vessels were GLUT-1-negative, whereas the vessels constituting the iris neovascularization were CD34-positive and GLUT-1-positive. This implies that the retinal and PHV-associated vessels were arrested in their metabolic development and not fully equipped to facilitate transport of glucose across the blood-retina/brain barrier.^{23,24} GLUT-1 also displayed a striking affinity for the Mueller cell-derived fibrous astrocytes responsible for the retinal gliosis. It is well known that glial cells throughout the central nervous system, and by extension those of the retina, are endowed with GLUT-1 to facilitate further glucose transfer.^{25,26}

Among the various entities with retinal dysplasias, Norrie disease has been studied in the greatest depth and can serve as a model for insights into the kinds of mechanisms that might be operative in these conditions.^{2,27-30} For example, in males, retinal dysplasia can also be encountered in congenital retinal folds and exudative vitreoretinopathy accompanied by

systemic findings.^{15,31-36} Although these diseases, like Norrie, demonstrate a germline, X-linked recessive inheritance, a retina-specific somatic mutation in the gene responsible for Norrie disease (*NDP*) has also been described.²⁷ The *NDP* gene product, norrin, is required for normal retinal neovascularization and its absence leads to incomplete and chaotic vascularization, ischemia, VEGF synthesis, and release.^{28,29} It is synthesized by many cell types, including glial cells throughout the central nervous system (and by Mueller cells and probably other cells in the retina).³⁰ In addition to disrupting vasogenesis in the retina and brain, *NDP* mutations also affect the development of the stria vascularis in the cochlea.^{29,30}

Different signaling pathways besides norrin are at least partially responsible for retinal dysplasia in autosomal forms of heritable diseases and include the WNT pathway (for which norrin is a potent agonist). The most pertinent example is familial exudative vitreoretinopathy.³⁷ Other intertwined pathways encompass the frizzled-4 (*FZD4*)³⁷ and low-density lipoprotein receptor-related protein-5 (*LRP5*) genes,³⁸ both of which localize to chromosome 11q. Norrin binds the WNT receptor FZD4, and LRP5 is an associated WNT coreceptor.³⁹ The activation of the Norrin/FZD-4/LRP5/WNT signaling pathways results in retinal vascular endothelial cell sprouting, allowing proper intraretinal capillary bed development and stabilization in the inner nuclear layer of the retina.⁴⁰ The sonic hedgehog (SHH) signaling pathway regulates retinal precursor proliferation and the normal laminar organization of the retina.⁴¹⁻⁴³ When the gene encoding the SHH receptor *Patched 1*, which lies on chromosome 7, becomes mutated, retinal dysplasia results, as has been seen in mice.⁴⁴ The SHH and WNT signaling pathways have been discovered to interact with each other. Xu and colleagues²⁹ and Ye and colleagues⁴⁵ have supplied comprehensive and detailed reviews of many aspects of these complicated pathways, which are probably impactful in the development of most retinal dysplasias.

Because the histopathologic features of unilateral sporadic retinal dysplasia are virtually identical to those of Norrie disease, we sought to discover whether the *NDP* gene could also play a role in the former despite the fact that the patient was female. Familial exudative vitreoretinopathy, Coats' disease, falciform folds with dragged maculae, PHV, and even retinopathy of prematurity have all been found to be associated with the variants of the Norrie gene, which is consequently endowed with a wide scope of influences.^{15,31-36,46,47} A putative "retinal dysplasia gene" or aberrant Norrie gene occurring as a somatic, nongermline mutation in a retinal stem cell line or mosaicism in a female patient could cause disease confined to one eye. In the current case, karyotyping of peripheral blood leukocytes and sequencing of the *NDP* gene from DNA isolated from sputum and paraffin-embedded retinal tissue sections failed to reveal any chromosomal or molecular abnormalities linked to the Norrie gene or any gene copy number abnormalities. Our failure to uncover a mutation in the *NDP* gene, however, does not completely rule out an abnormality in this gene or its functional activity in our patient. Pathogenic mutations that interfere with the function of the gene can reside in regions of the gene that were not evaluated, such as in the promoter, deep introns, or in the 3' untranslated regions. Undetected and uncharacterized changes in a segment of autosomal DNA (especially on chromosome 13, which has been noted to harbor mutations conducive to retinal dysgenesis^{18-20,43}) might be found. Additional support for

this last possibility is supplied by a unilateral autosomal-dominant hereditary form of retinal dysplasia.⁴⁸

The VEGF aqueous concentration was significantly elevated in our patient, achieving a level of 4.5 ng/mL, which is comparable with the highest values reported in the literature.^{49,50} The frequent coexistence of a richly vascularized PHV with retinal dysplasia unquestionably contributes to this finding. Another, more remote possible mechanism is the inhibition of the von Hippel-Lindau tumor suppressor protein leading to build-up of VEGF-A levels.^{51,52} With regard to VEGF, Xu and colleagues²⁹ have incisively summarized the interplay between PHV and retinal dysplasia: “In the developing eye, there may be a causal relationship between an impoverished intraretinal vasculature and the persistence of the hyaloidal vessels. For example, a primary defect in the intraretinal vasculature [due to absence of norrin] would lead to increased VEGF production by the hypoxic retina, which might promote the survival of hyaloid vessels. Conversely, a primary defect in hyaloid vascular regression could secondarily inhibit intraretinal vascularization by blunting VEGF-driven endothelial proliferation and migration.” These investigators favored the first scenario.

Any differential diagnostic value attached to the high levels of aqueous VEGF-A in retinal dysplasia in distinguishing it from retinoblastoma will depend on aqueous studies of the latter tumor, which have not yet been performed. There is a study of retinoblastoma that used RNA probes on tissue sections to demonstrate VEGF production by viable tumor cells and surrounding ischemic retina; interestingly, rosette formation reduced VEGF expression.⁵³

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FIG 1.

A, Superimposed A- and B-mode ultrasonography discloses a thickened retinal mass (arrows) with high reflectivity demonstrated on the A scan (crossed arrows). The mass appears to be suspended in midvitreous with anechoic pre- and subretinal spaces without suggestion of secondary tumor seeding. B, A microscopic section of the enucleated eyeball shows artifactitious wrinkling of the cornea (C). The ciliary processes (CP) occasionally display elongation. Peripheral primitive neuroepithelium (PN) is present next to the ciliary body and primary vitreous (V). The retinal mass (RM) comprises mostly eosinophilic glial cell proliferation and retinal cell processes (neuropil). The retinal lens (L) has been dislocated during processing. A thickened portion of disorganized retina (arrows) is present at the edge of the main mass and extends anteriorly. The subretinal space (SRS) does not contain any obvious cellular clusters and the choroid (Ch) is not expanded. C, The retinal mass manifests gliosis, ectatic vessels and an absence of nuclear lamination. The outer edges of the neuroglial mass show infoldings that penetrate as far the middle of the tissue (arrows). D, Several pseudorosettes (PR) are present in the midst of retinal nuclei and glial processes; they exhibit multiple layers of nuclei and an outer limiting membrane. E, A strip of folded retina exhibiting total disorganization shows abortive nuclear lamination with a small pseudorosette (arrow). This region corresponds to the area designated by the arrows in (B). F, In the far retinal periphery, the primitive retinal neuroepithelium (PN), which will convert into the pars plana and ciliary epithelia, circumscribes the primary vitreous with abundant neovascularization (NV). The solid-looking neovascularization on the left is at an early budding stage and has associated fresh hemorrhage. (B-F, hematoxylin-eosin: B, original magnification $\times 5$; C, $\times 25$; D, $\times 200$; E, $\times 100$; F, $\times 100$).

FIG 2.

A, CD34 stains the endothelial cells in the iris stroma as well as those comprising a surface neovascular membrane (arrows). Note there is a fine monolayer of cells covering the surface of the iris immediately beneath that is the neovascular unit. Sphincter muscle at the pupil (Sph). B, Neovascularization (NV) highlighted with CD34 immunostaining covers the surface of the retinal mass (RM) and extends toward the right of the field into the primary vitreous. C, Higher power magnification of (B) discloses small lumens in the neovascular units that have involved the surface of the retina (R) on the left and created a luxuriant intravitreal network on the right. D, The detached and disorganized retina exhibits prominent gliosis (Gl) that is GFAP-positive and dominates the inner portion of the dysgenic retina next to the vitreous (V) that harbors neovascularization. Subretinal space (SRS). E, Recurring strips of primitive retina display Ki-67 nuclear immunostaining, thereby identifying which cells are in S-phase of DNA. Note that the positive cells are restricted to the outer portions (arrows) of the tissue abutting the subretinal space (SRS). There is no exudate in the subretinal space beneath the strip of retina and the pigment epithelium (PE). The inset displays small pseudorosettes without any nuclear labeling by Ki-67, whereas the surrounding retina contains scattered positively staining cells representing inflammatory and/or endothelial cells. F, Many retinal infoldings proceeding from the subretinal space, which will eventuate in the production of “entrapped” pseudorosettes. They fail to show any nuclear staining of their primitive neuroepithelial constituent cells. The retinal tissue above contains dispersed, positively staining cells of nonretinal origin (inflammatory/or endothelial cells). On the bottom left, the thickened retina tapers toward the periphery into a multilayered stratified neuroepithelium. The inset demonstrates positive Ki-67 nuclear staining in the peripheral neuroepithelium; the staining

peters out toward the far periphery on the right. (Immunoperoxidase reaction, diaminobenzidine chromogen; A, original magnification $\times 25$; B, 10; C, $\times 200$; D, $\times 100$; E, $\times 100$; inset, $\times 200$, F, $\times 200$; inset, $\times 200$).