

# Immunocytochemical Characterization of Cysts in the Peripheral Retina and Pars Plana of the Adult Primate

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**PURPOSE.** To better characterize the cellular constituents of cysts in the peripheral retina and pars plana of the adult monkey.

**METHODS.** Frozen sections of the peripheral retinal margin and pars plana from monkeys (*Macaca nemestrina*) between 1 and 15 years of age were stained with toluidine blue or immunolabeled with a variety of glia- and neuron-specific antibodies.

**RESULTS.** In animals 1 to 2 years of age, the nonpigmented inner layer of the pars plana is a pseudostratified columnar epithelium. In these young animals, the peripheral retina had distinct layers and did not contain cysts. In animals 6 years of age or older, there were numerous cysts in the pars plana and in the peripheral retina. In the peripheral retina, neurons were randomly distributed and did not have a laminar organization. Cells surrounding cysts were immunoreactive for different types of markers for retinal neurons. Some of the cells surrounding cysts in the pars plana were also unexpectedly immunoreactive for antigens normally expressed only in retinal neurons and glia.

**CONCLUSIONS.** Cysts form in the peripheral retina and pars plana in adult monkeys. The peripheral retinal cysts disrupt the normal lamination of the cells, but all types of retinal neurons are still present in the cysts. In an unexpected finding, cysts in the pars plana also contained cells immunoreactive for a few of the markers of retinal cells, suggesting that neurogenesis may occur in the pars plana of the adult primate. (*Invest Ophthalmol Vis Sci.* 2001;42:3256-3263)

Cystoid degeneration in the peripheral retina, ora serrata, and the pars plana is common in the eyes of primates. Typical cystoid degeneration in the peripheral retina occurs more frequently in superior and temporal regions,<sup>1</sup> is common in the eyes of all humans over the age of 8 years, and increases in frequency with increasing age.<sup>2,3</sup> Most humans older than 50 years also have pars plana cysts that seem to have no known pathologic consequence.<sup>3</sup> The current view is that pars plana cysts are formed by a separation of the pigmented epithelium from the inner nonpigmented epithelium, analogous to retinal

detachment, whereas retinal cysts form between retinal layers.<sup>3</sup> Although it remains uncertain why these cysts form, traction by the vitreous and zonules, vascular sclerosis, secretory phenomena, or inflammation have been suggested as possible causes.<sup>4-7</sup> The pars plana cysts are more common with increasing age<sup>4</sup> and tend to be confined to the temporal side of the pars plana.<sup>4,6</sup> Although there have been studies of the ultrastructure of cysts in the pars plana and peripheral retina,<sup>8</sup> and it is presumed that the fluid-filled cavities contain hyaluronic acid,<sup>3</sup> there has been no systematic immunocytochemical study of the peripheral retinal or pars plana cysts.

Therefore, the purpose of this study was to better characterize the cells that form cysts in peripheral retina and the pars plana in the primate eye. The eyes of *Macaca* monkeys were used, because the retina of these animals is widely accepted as a model for that of humans (reviewed by Dacey<sup>9</sup>). We applied well-characterized immunocytochemical markers for retinal neurons and glia to identify cells in the cysts of the far peripheral retina and pars plana of the adult monkey eye.

## MATERIALS AND METHODS

### Animals

Eyes of *Macaca nemestrina* or *Macaca fascicularis* were obtained through the Tissue Distribution Program at the Regional Primate Research Center at the University of Washington. The use of animals in these experiments was in accordance with the guidelines established by the National Institutes of Health, the University of Washington Animal Care Committee, and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Tissues were obtained from 10 animals: three between 1 and 1.2 years of age, four between 3 and 7 years of age, and three between 12 and 15 years of age. One monkey year is equal to 3 to 4 human years,<sup>10</sup> and the youngest group of monkeys was therefore equivalent to a 4-year-old human and the oldest to a 48- to 68-year-old human.

### Fixation and Sectioning

Eyes were hemisected equatorially and the gel-vitreous trimmed away. The entire anterior hemisegment (including peripheral retina, pars plana, and ora serrata) was fixed for 30 minutes at 20°C in 4% paraformaldehyde plus 3% sucrose in 0.1 M phosphate buffer (pH 7.4), washed three times in PBS (0.05 M phosphate buffer, 195 mM NaCl [pH 7.4]), cryoprotected in PBS plus 30% sucrose, soaked in optimal cutting temperature (OCT) embedding medium (Tissue-Tek; Miles Laboratories, Elkhart, IN) for 10 minutes, and freeze-mounted onto aluminum sectioning blocks. Transverse sections 14  $\mu$ m thick were thaw mounted onto glass slides (Super-Frost; Fisher Scientific, Fairlawn, NJ), air dried, and stored at -20°C until use.

### Immunocytochemistry

Standard immunocytochemical techniques were used as described elsewhere.<sup>11,12</sup> Because antigens were not available for preabsorption controls, we evaluated specificity mainly by comparison with the results of previous studies using these antibodies and, where possible, by known homologies between the immunizing proteins and the primate counterparts.

Working dilutions and sources of antibodies used in this study included: rabbit anti-calbindin at 1:1000 (Swant Immunochemicals,

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From the Department of Biological Structure, University of Washington, Seattle.

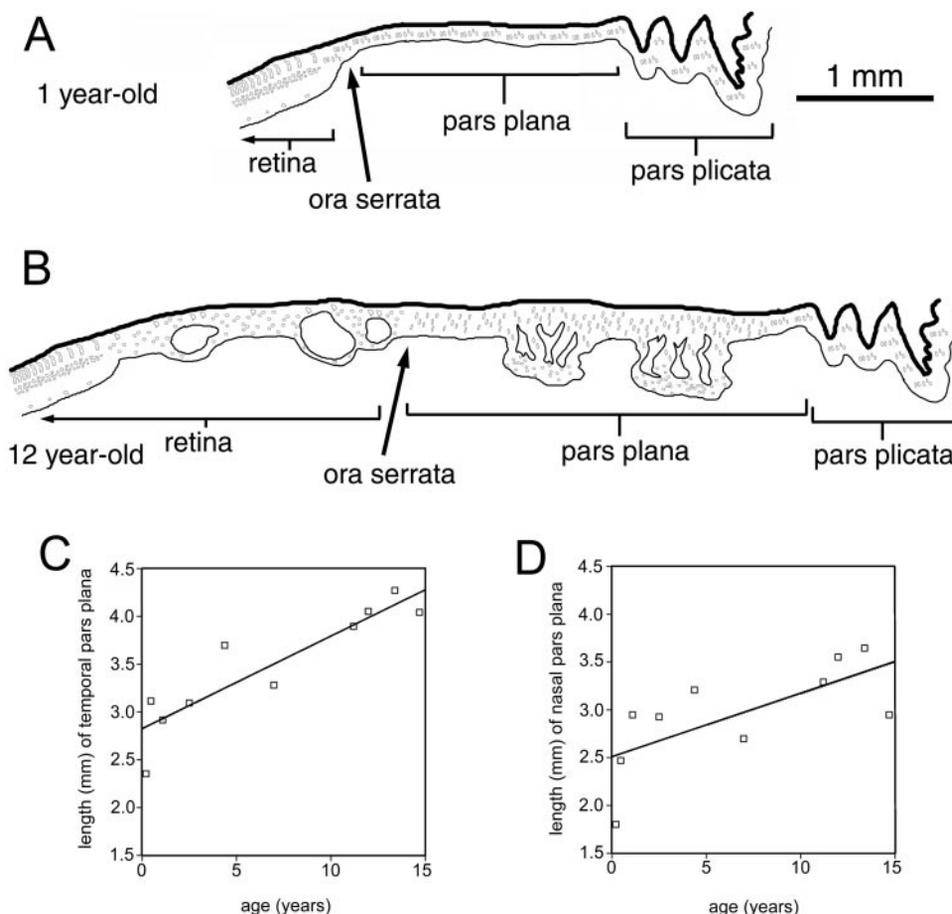
Supported by fellowships from the Alberta Heritage Foundation for Medical Research and the Canadian Institutes of Health Research (AJF); by grants from The Foundation Fighting Blindness (TAR); and by National Institutes of Health Grants NS28308 (TAR) and EY04536 (AH). The Islet-1 and neurofilament antibodies developed by Thomas Jessell and J. Wood, respectively, were obtained from the Developmental Studies Hybridoma Bank developed under auspices of the National Institute of Child Health and Human Development and maintained by The University of Iowa, Department of Biological Sciences, Iowa City.

Submitted for publication February 6, 2001; revised June 22, 2001; accepted July 30, 2001.

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**FIGURE 1.** Schematic diagrams of the peripheral retina and pars plana that represent a radial section from a (A) 1-year-old and (B) 12-year-old monkey. Scatter plot demonstrating the increasing length of the (C) temporal and (D) nasal pars plana with increasing age of the animal. Each data point represents the mean length (in millimeters) of at least three measurements from one subject. The slopes of the best-fit lines are 0.098 mm/year for the temporal pars plana and 0.066 mm/year for the nasal pars plana.

Bellinzona, Switzerland), rabbit anti-calretinin at 1:1000 (Swant Immunochemicals), rabbit anti-GABA at 1:1000 and rat anti-glycine at 1:1000 (both from David V. Pow, University of Queensland, Australia), mouse anti-NeuN at 1:1000 (Chemicon, Temecula, CA), mouse anti-Islet-1 at 1:50 (39.4D5; Developmental Studies Hybridoma Bank, University of Iowa, Iowa City), rabbit anti-Prox1 at 1:1000 (Stanislav Tomarev, National Eye Institute), mouse anti-neurofilament at 1:100 (RT97; Developmental Studies Hybridoma Bank), mouse anti-β 3-tubulin at 1:500 (Covance, Princeton, NJ), mouse anti-rhodopsin at 1:800 (4D2; Robert Molday, University of British Columbia, Canada), rabbit anti-GCAP2 at 1:2000 (Krzysztof Palczewski, University of Washington), rabbit anti-cellular retinaldehyde-binding protein (CRALBP) at 1:5000 (John Saari, University of Washington, Seattle), and rabbit anti-recoverin at 1:1000 (James Hurley, University of Washington).

**Histology**

Toluidine blue staining was performed as described elsewhere.<sup>12</sup> In short, sections were washed three times in PBS and incubated with 0.1% toluidine blue plus 0.2% Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> in distilled H<sub>2</sub>O (pH 11.4) for 60 seconds. This was followed by three washes in PBS, dehydration through graded ethanol, and mounting with coverslips (Permount; Fisher Scientific, Fairlawn, NJ) for observation under a compound microscope.

**Microscopy and Photography**

All microscopy was performed on a compound microscope (Axioplan 2; Carl Zeiss, Thornwood, NY) and micrographs were obtained with a digital camera (Spot slider-RT camera; Diagnostic Instruments, Inc., Sterling Heights, MI). Digital images were optimized for contrast and brightness with image-management software (Photoshop 5.5; Adobe, Mountain View, CA).

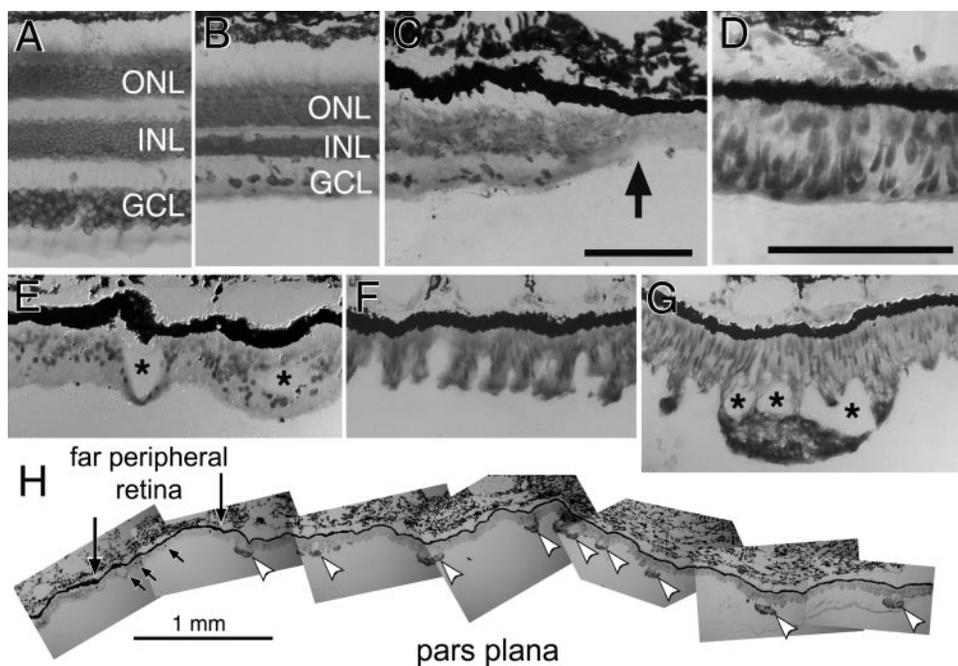
**RESULTS**

**Postnatal Growth and Cysts of the Retina and Pars Plana**

There was a large increase in the radial length of the pars plana with increasing age. In young animals between 1 and 2 years of age, the pars plana measured 2.5 to 3.5 mm from the peripheral edge of the retina to the pars plicata of the ciliary body (Fig. 1). In the eyes of older monkeys between 6 and 15 years of age the temporal pars plana was 4 to 5 mm in length (Fig. 1). There was some variability between animals, but in any given eye the length of the temporal pars plana was longer than that of the nasal pars plana. In the 10 eyes surveyed, there appeared to be a greater increase in length of the temporal pars plana, compared with that of the nasal pars plana (Figs. 1C, 1D).

Figures 2A through 2D demonstrate the central, peripheral, margin of the retina, and pars plana of a young monkey as seen in toluidine blue-stained frozen sections. Whereas central regions of retina contained multiple layers of cells in the outer nuclear layer (ONL), inner nuclear layer (INL), and ganglion cell layer (GCL; Fig. 2A), the thickness of cell layers and the plexiform layers was greatly reduced in peripheral regions of the retina (Fig. 2B). In all animals examined, the retinal layers became tapered and fused together at the peripheral retinal margin or ora serrata (arrow; Fig. 2C). In these young animals, the peripheral edge of the retina was clearly defined where the retinal layers became narrowed and joined the inner nonpigmented epithelium of the pars plana (Fig. 2C). The inner epithelial layer was a uniform, uninterrupted monolayer of cells forming a pseudostratified columnar epithelium (Fig. 2D).

Figures 2E through 2H demonstrate the far peripheral retina and pars plana in an older monkey. Based only on morphologic



**FIGURE 2.** Toluidine blue-stained sections of the (A) central retinal, (B) peripheral retina, (C) peripheral retinal margin, and (D) pars plana from monkeys less than 1.5 years old. (E-H) Sections from the eye of a 12-year-old monkey from the far peripheral retina (E, H) and pars plana (F-H). Type I cysts are demonstrated in (E), and a type-II cyst is demonstrated in (G). (\*) Cysts; (arrow, D) peripheral edge of the retina. Scale bar, 50  $\mu$ m; bar in (D) applies to (D) alone.

features, it was possible to determine the peripheral retinal margin in older animals. The pars plana consisted of columnar cells immediately adjacent to the pigmented epithelium, whereas the peripheral retina was composed of cells that were similar to more central regions of retina, but without the clear laminar organization. There was always a clear transition between noncolumnar cells in the peripheral retina and columnar cells in the pars plana. Therefore, with careful inspection, we could distinguish peripheral retina from pars plana. The considerable disorganization of the far peripheral retina and absence of lamination of the retinal layers spanned as much as 3 mm of radial length (Fig. 2E). In older animals, numerous cysts were found in the far peripheral retina and in the pars plana (Figs. 2E-H). These cysts were predominantly in the temporal sector of the eye, and therefore all the data used in the study were obtained from this quadrant.

In the eyes of monkeys more than 6 years old, we identified two morphologically distinct types of cysts, which we have designated type I and type II. Type I cysts were located up to 3 mm within the edge of the retina, where lamination of the retinal layers was not obvious. These cysts were relatively small (30–100  $\mu$ m in diameter), usually had a round lumen, and were surrounded by cells that were indistinguishable from the retinal cells (Fig. 2E). The cysts in the pars plana were morphologically quite different from the cysts in the peripheral retina. In the pars plana, what had been a pseudostratified epithelium of nonpigmented cells in young animals, now formed multiple layers with convolutions (Fig. 2F). Pigmented cells were found in the lumen and walls of type I cysts (Fig. 5G) and scattered among the nonpigmented columnar cells in the pars plana (data not shown). However, many of these cells were not as densely pigmented as cells remaining in the pigmented epithelium.

Type II cysts were located past the peripheral retinal edge and were scattered randomly in the nonpigmented ciliary epithelium up to the pars plicata (Figs. 2G, 2H). These cysts were more common in animals older than 10 years, were relatively large (80–200  $\mu$ m in diameter), had an irregularly shaped lumen, and were surrounded by cells with differential staining affinity for toluidine blue (Fig. 2G). The scleral layers of type II cysts contained columnar cells that were weakly stained, whereas the majority of cells near the vitread surface of these

cysts were round or squamous and were intensely stained with toluidine blue (Fig. 2G).

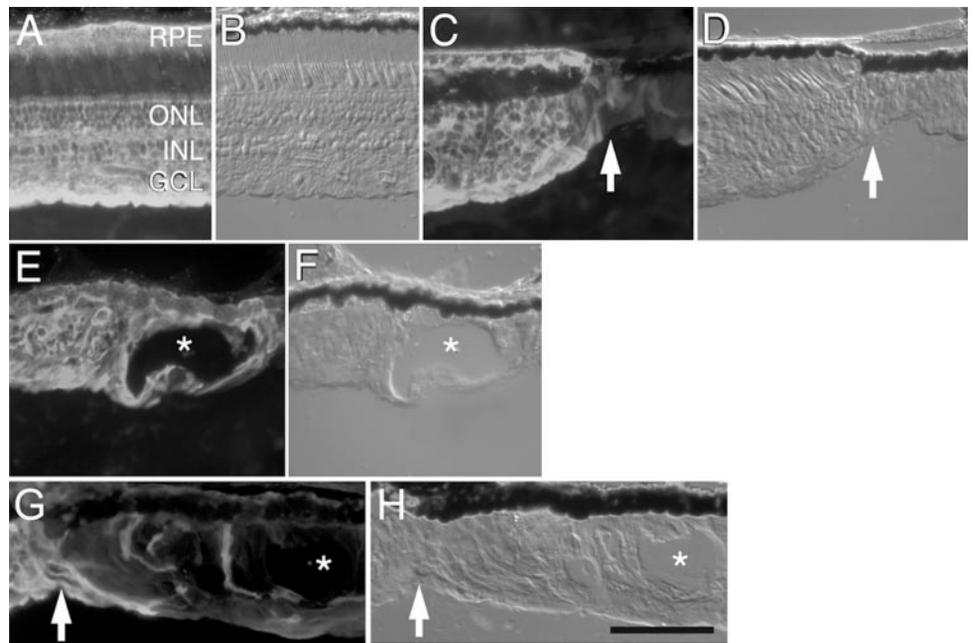
### Identification of the Retinal Margin with CRALBP Immunolabeling

In older animals, immunohistochemical studies allowed us to clearly identify the peripheral edge of the retina as the region where Müller cells were no longer present. Müller glia were identified by immunolabeling for CRALBP. In central retina, CRALBP immunoreactivity was detected in Müller glia and in the pigmented epithelium (Figs. 3A, 3B). Müller glia had CRALBP-immunoreactive processes that extended from the inner limiting membrane to the outer limiting membrane (Fig. 3A). In the peripheral retina of young animals, immunolabeling for CRALBP ended abruptly at the posterior border of the pars plana (Figs. 3C, 3D). CRALBP immunoreactivity was not detected in the pigmented or nonpigmented layers of the pars plana.

In animals more than 7 years of age, the far peripheral retina was disorganized, had no lamination, and contained cysts (described earlier). CRALBP immunoreactivity was detected in Müller glia and pigmented epithelial cells in far peripheral retina, even where clear lamination was no longer present around type I cysts (Figs. 3E, 3F). The continuous labeling for CRALBP was present in the peripheral retina of the older monkeys, and the disruption of this continuity at the retinal margin coincided with a loss of CRALBP expression in the pigmented epithelium (Figs. 3G, 3H). Although most of the cells of the pars plana did not express CRALBP, a few scattered cells surrounding type II cysts were immunoreactive for CRALBP (Figs. 3G, 3H, and see Figs. 8C, 8D). It was clear that these cells were in the pars plana, because the underlying pigmented epithelium did not express CRALBP.

### Immunocytochemical Characterization of Peripheral Retinal Cysts

In animals more than 6 years of age, all classes of retinal neurons were present in the peripheral retina; however, their lamination was severely disrupted by the overall thinning of the epithelium and by the formation of cysts. Figure 4 shows sections of peripheral retina in labeled for two cytoskeletal

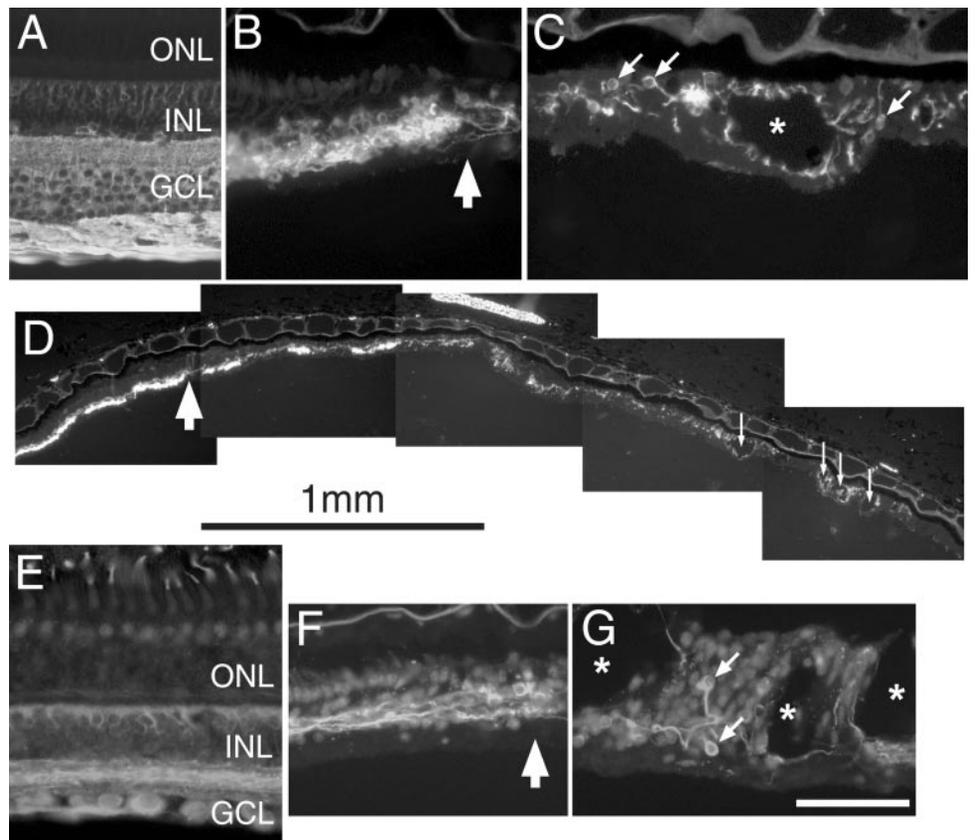


**FIGURE 3.** (A–D) Vertical sections of (A, B) retina and (C, D) retinal margin of a 1.5-year-old monkey that were labeled for CRALBP immunoreactivity. (E–H) Vertical section of the far peripheral retina and pars plana of a 14-year-old monkey that was labeled for CRALBP immunoreactivity. (B, D, F, H) Bright-field Nomarski images of the panels immediately to their left. (C, D, G, H; *large arrows*) Peripheral retinal margin; (E–H, \*) cysts. Scale bar, 50  $\mu$ m.

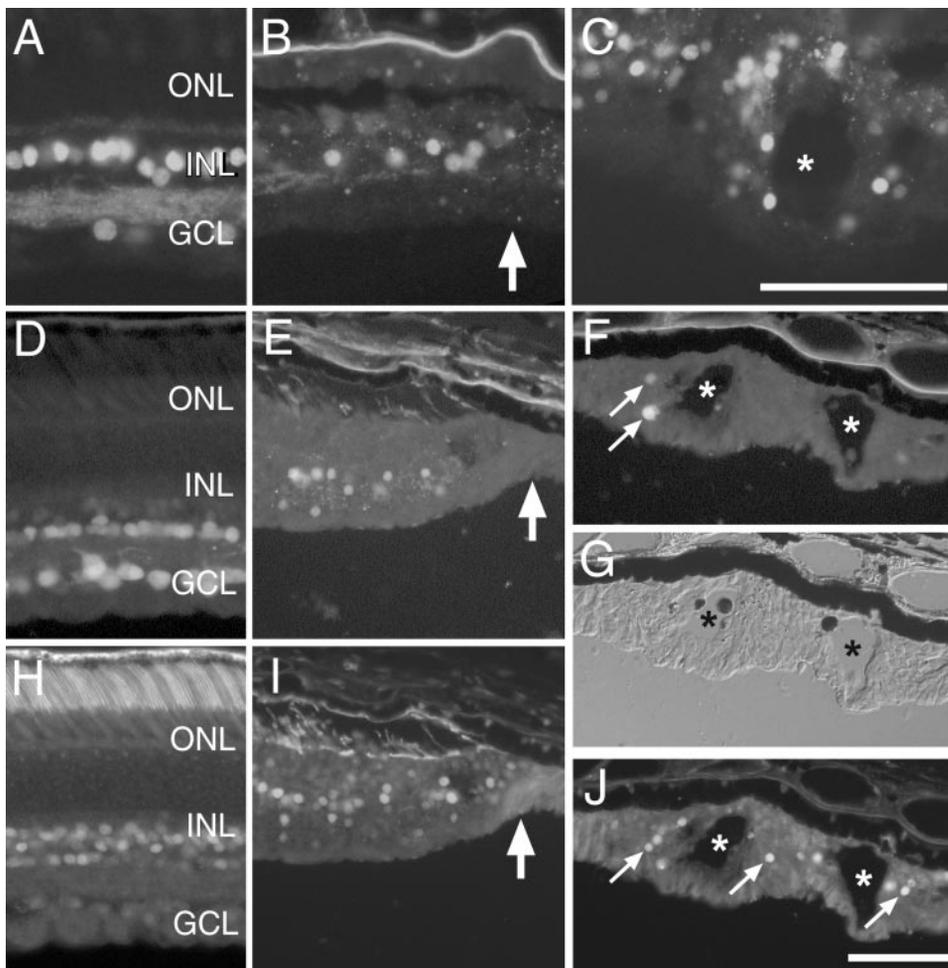
proteins,  $\beta$ 3-tubulin (Figs. 4A–D) and neurofilament (Figs. 4E–G). These proteins are expressed in many inner retinal neurons (amacrine and ganglion cells) in the central retina. In the far peripheral retina of older monkeys, both  $\beta$ 3-tubulin- and neurofilament-immunoreactive cells were present in the cells surrounding the cysts (Figs. 4C, 4G, respectively). In addition, neurofilament-immunoreactive processes projected longitudinally within the inner plexiform layer (IPL) in the far peripheral retina (Fig. 4F). The cells that were immunoreactive for  $\beta$ 3-tubulin and neurofilament had neuronal morphology but were

smaller than ganglion cells and usually had only a few processes.

To further characterize cysts in the pars plana, sections were labeled with antibodies to transcription factors that are expressed by subsets of differentiated neurons. Islet-1 is a homeodomain transcription factor that is known to be expressed by ganglion, bipolar, and cholinergic amacrine cells in the rodent retina.<sup>13,14</sup> In central regions of the primate retina, Islet-1 immunoreactivity was detected in the nuclei of bipolar and ganglion cells (Fig. 5A), as judged by the known location



**FIGURE 4.** Vertical sections of the (A, E) central retina, (B, D, F) retinal margin where lamination of layers ceases, and (C, D, F) the far peripheral retina from monkeys more than 7 years old. These sections have been labeled with antibodies to (A–D)  $\beta$ 3-tubulin or (E–G) neurofilament. (D) Low-magnification montage of images demonstrating  $\beta$ 3-tubulin immunolabeling in the peripheral retina. (B, D, F; *large arrows*) Peripheral edge of lamination within the retina and (C, G, \*) indicate type-I cysts. (D, *small arrows*) type I cysts. Scale bars, (A–C) 50  $\mu$ m; (D) 1 mm.



**FIGURE 5.** Vertical sections of the central retina (**A, D, H**); the peripheral retina, where lamination of retinal layers ceases (**B, E, I**); and the far peripheral retina (**C, F, G, J**) from monkeys more than 7 years old. Sections were labeled with antibodies to (**A–C**) Islet-1, (**D–F**) NeuN, or (**H–J**) Prox1. (**G**) Bright-field Nomarski image of a section through type I cysts that was doubly labeled for (**F**) NeuN and (**J**) Prox1 immunoreactivity. (**B, E, I, large arrows**) Retinal margin; (**F, J–L, small arrows**) labeled cells in the walls of cysts; (**C, F, J, \***) type I cysts. Scale bar, 50  $\mu$ m; bar in (**C**) applies to (**A–C**) and bar in (**J**) applies to (**D–J**).

and distribution of these cell types. At the retinal margin, a few Islet-1-immunoreactive cells were detected (Fig. 5B), but many Islet-1-positive cells were scattered throughout the far peripheral retina, including around cysts (Fig. 5C).

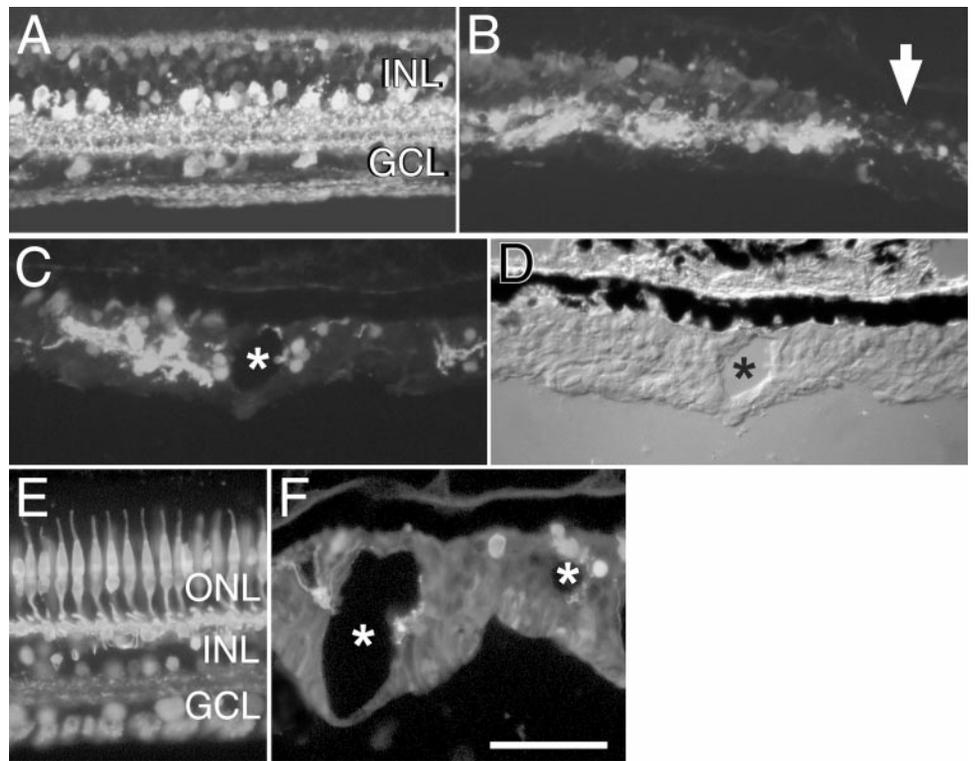
NeuN is a nuclear protein that is present in many neurons in the central nervous system of mammals.<sup>15</sup> In central retina, NeuN immunoreactivity was detected in the nuclei of cells in the amacrine cell layer of the INL and cells in the GCL (Fig. 5D). At the retinal margin NeuN immunoreactivity was present in the nuclei of a few cells in the INL, but these decreased in abundance with increasing proximity to the peripheral edge of the retina (Fig. 5E). NeuN-positive cells reappeared around cysts in the far peripheral retina (Fig. 5F).

Prox1 is a homeodomain transcription factor that is expressed by postmitotic neurons in many regions of the central nervous system.<sup>16</sup> In the primate retina, Prox1 immunoreactivity was detected in the nuclei of many neurons in the INL and a few in the GCL (Fig. 5H). The antibody to Prox1 also cross-reacted with a Prox1-like antigen in rod outer segments (Fig. 5H). At the peripheral retinal margin, the number of Prox1-positive neurons was diminished (Fig. 5I), but in the far peripheral retina many Prox1-immunoreactive nuclei were present, including around cysts (Fig. 5J). Many Prox1-positive cells were found around cysts (Fig. 5J), but few NeuN-positive cells were observed (Fig. 5F). This suggests that horizontal and bipolar cells are more abundant than amacrine and ganglion cells in the far peripheral retina.

Calretinin and calbindin are calcium-binding proteins known to be expressed by different types of neurons in the retina of a variety of species.<sup>17,18</sup> In central retina, immunoreactivity for calretinin was observed in a subtype of amacrine

cells (Fig. 6A), consistent with previous reports that all amacrine cells in the primate retina contain calretinin.<sup>19</sup> In addition, calretinin immunoreactivity was detected in the IPL, outer plexiform layer (OPL), nerve fiber layer (NFL), in a few cells in the GCL, and at low levels in cells in the distal INL, possibly indicating horizontal cells (Fig. 6A). The continuous laminae of calretinin-immunoreactive cells was disrupted in the far peripheral retina, and only scattered cells or small clusters of cells were present around the cysts (Figs. 6B–D). In the central retina, calbindin immunoreactivity was detected in cone photoreceptors, some bipolar and amacrine cells, and many ganglion cells (Fig. 6E), consistent with previous reports.<sup>17,20</sup> Many calbindin-positive cells were found around cysts in the peripheral retina (Fig. 6F). Other markers for inner retinal neurons, including  $\gamma$ -aminobutyric acid (GABA) and glycine, also were detected in cells that surrounded type I cysts in the far peripheral retina (results not shown).

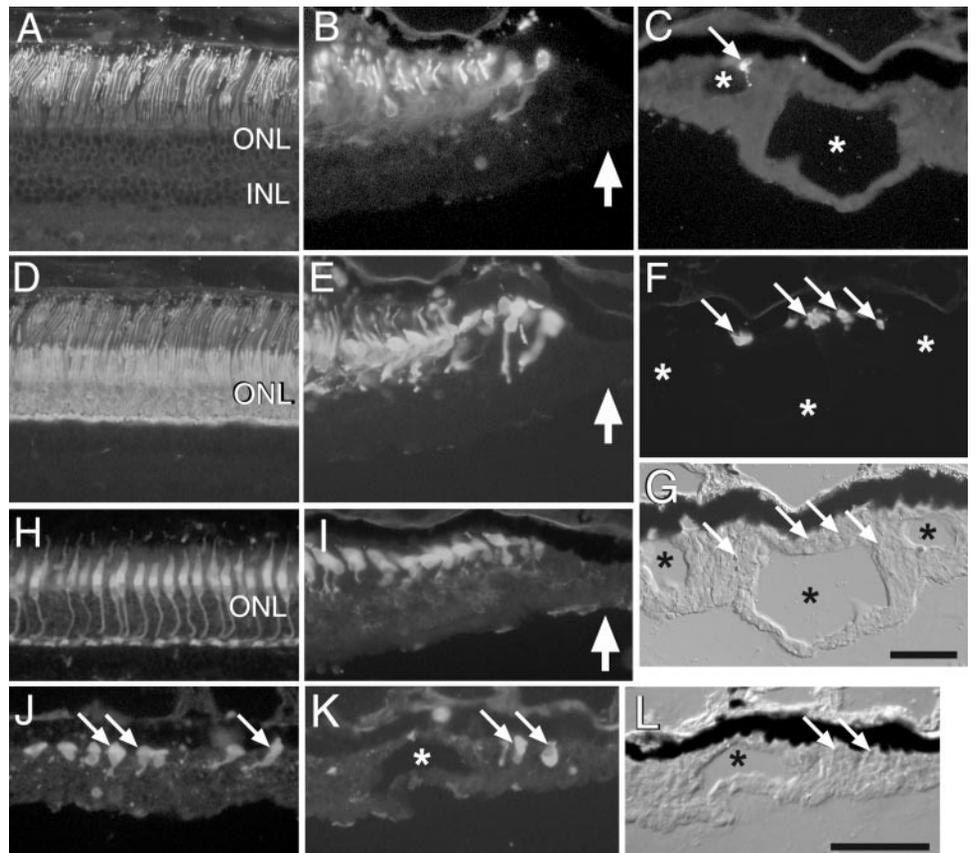
We also analyzed the peripheral retina for the expression of photoreceptor markers. In central (Fig. 7A) and peripheral retina (Fig. 7B), rhodopsin immunoreactivity was strong in the outer segments of rod photoreceptors, whereas weak immunoreactivity was detected in the inner segments. A few rhodopsin-immunoreactive cells were seen around cysts in the far peripheral retina, but these cells did not have well-developed outer segments, and the rhodopsin immunoreactivity was distributed evenly throughout the cell bodies (Fig. 7C). Recoverin is a protein that is expressed by all photoreceptors.<sup>21,22</sup> Recoverin immunoreactivity was detected throughout central rod and cone photoreceptors (Fig. 7D) and persisted up to the peripheral edge of the retina (Fig. 7E). In addition, many recoverin-positive cells were present in the far peripheral ret-



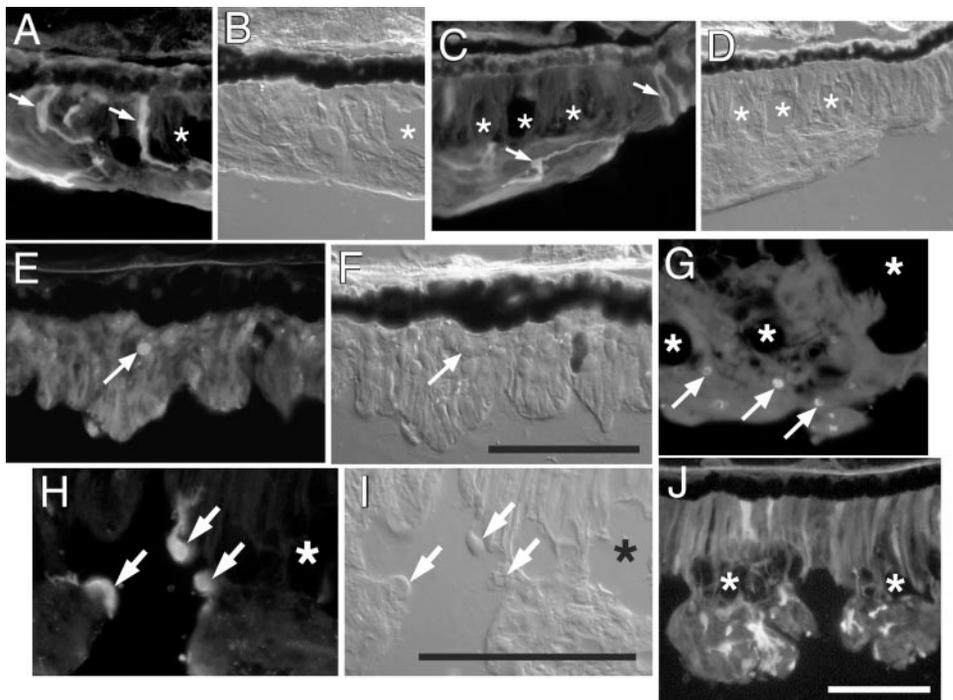
**FIGURE 6.** Vertical sections of the central retina (A, E); the peripheral retina, where lamination of layers ceases (B), and the far peripheral retina (C, D, F) of monkeys more than 5 years old. Sections were labeled with antibodies to (A–C) calretinin or (E, F) calbindin. (D) Bright-field Nomarski micrograph of section in (C). (\*) Type I cysts; (B, *large arrow*) peripheral edge of the retina. Scale bar, 50  $\mu$ m.

ina, particularly around cysts (Figs. 7F, 7G). These cells did not have well-defined outer segments or axon terminals. In central retina, antibodies to guanylate cyclase-activating protein (GCAP)-2 labeled cone photoreceptors (Fig. 7H), consistent with previous reports.<sup>23,24</sup> Near the peripheral margin of the

retina, GCAP2-immunoreactive photoreceptors were present at reduced frequency (Fig. 7I). Many GCAP2-immunoreactive cells were found unexpectedly in the far peripheral retina (Fig. 7J), especially around cysts (Figs. 7K, 7L). These GCAP2-immunoreactive cells did not have a well-developed outer segment



**FIGURE 7.** Vertical sections of central retina (A, D, H); peripheral retina, where lamination of layers ceases (B, D, I); and the far peripheral retina (C, F, G, J–L) of monkeys more than 7 years old. Sections were labeled with antibodies to (A–C) rhodopsin, (D–F) recoverin, or (H–K) GCAP2. (G) Bright-field Nomarski image of section in (F); (L) Nomarski image of (K). (J) GCAP2-immunoreactive cells in the far peripheral retina that are not associated with cysts. (B, E, I, M, *large arrows*) Retinal margin; (C, F, G, J–M, *small arrows*) labeled cells; (C, F, G, K, L, \*) cysts. Scale bar (50  $\mu$ m) in (G) applies to (A–G); scale bar (1 mm) in (L) applies to (J–L).



**FIGURE 8.** Vertical sections of pars plana labeled with antibodies to (A–D) CRALBP, (E–G) Prox1, (H, I) calretinin, or (J) calbindin. (A, B) CRALBP-positive cells in the pars plana, in a region devoid of type II cysts. (E, F) Prox1-immunoreactive nucleus in a region of the pars plana that lies between type II cysts. (C, D, G, H–J) Labeling around type II cysts in the pars plana. (B, D, F, I) Bright-field Nomarski images of the panels directly to their left. Arrows: cells that have been immunolabeled; (\*) lumen of type II cysts. Scale bars, 50  $\mu\text{m}$ ; bar in (F) applies to (A–G); bar in (H) applies to (H, I); bar in (J) applies to (J) alone.

or axon terminal and their morphology was similar to that of immature photoreceptors in the human and monkey retina.<sup>25,26</sup> Similar to cells labeled with other antibodies to neural proteins, GCAP2-positive cells were found within the far peripheral retina, but these cells did not have the morphology of mature photoreceptors (Figs. 7J, 7K). These findings are consistent with those of Chen et al.,<sup>27</sup> demonstrating that opsin-immunoreactive photoreceptors in the retinal margin of the adult monkey (*Macaca mulatta*) appear to be immature.

### Characterization of Cells in the Pars Plana

In animals less than 1.5 years of age, cells labeled for neuronal markers were confined to the retina and stopped abruptly at its peripheral edge. The pars plana did not contain cells that were immunoreactive for the neuron-specific markers calretinin,  $\beta$ -tubulin, GCAP2, or Islet-1 (results not shown).

In older monkeys, some of the markers described above also were found in cells in the pars plana, surrounding type II cysts. In the eyes of monkeys that were older than 10 years, numerous CRALBP-immunoreactive cells were detected in the pars plana (Fig. 8), including the sclerad and vitread layers of type II cysts (Figs. 8C, 8D). These cells were relatively large and usually had vertical processes that extended through the nonpigmented epithelium of the pars plana. High levels of CRALBP immunoreactivity were not detected in other cell types or regions of the eye.

In addition to the expression of a putative Müller glial marker in the pars plana cysts, we also found evidence for cells immunoreactive for some of the retinal neuronal markers. Cells immunoreactive for Prox1 were found both in regions of the pars plana that did not contain cysts (Figs. 8E, 8F) and in the vitread layers of pars plana cysts (Fig. 8G). The type II pars plana cysts also contained cells that were immunoreactive for calretinin. Figures 8H and 8I show examples of three of these cells lining the wall of a cyst. In some of the labeled cells, small processes can be seen extending from the cell body. In addition to the calretinin-immunoreactive cells, we also found examples of calbindin-immunoreactive cells in the type II pars plana cysts. Many of these cells had fine processes extending from the cell bodies, and were located in the vitread layers of the cysts. Calbindin immunoreactivity was also detected in

cells in sclerad layers of cysts, but these cells had a columnar morphology (Fig. 8J).

### DISCUSSION

In the eyes of primates less than 2 years of age there is a sharp border between retina and pars plana, there are no cysts in the far peripheral retina or the pars plana, and there are no cells expressing glial or neuronal markers in the pars plana. By contrast, the border between the far peripheral retina and pars plana of monkeys older than 10 years of age is not as obvious. The clear lamination of the retina is disrupted in the periphery both by the extreme thinning of the layers, as well as by the appearance of cysts. In the normal monkey, the type I retinal cysts were relatively small, and all the cell types present in the central retina were present around these cysts. By contrast, in the pars plana we found a very different type of cyst, which was designated type II. These were relatively large and contained morphologically distinct cell types: columnar cells in basal layers and noncolumnar cells in apical layers. An unexpected finding was that type II cysts contained cells that expressed antigens normally found in retinal Müller glia and in certain types of retinal neurons: calbindin, calretinin, and Prox1. These findings suggest that at some time between 2 and 10 years of age, cells with some characteristics of Müller glia and neurons accumulate around cysts in the pars plana. It remains uncertain how these cysts in the pars plana arise from a monolayer epithelium that does not contain cells immunoreactive for glial or neural markers.

The significant amount of growth that occurs in the pars plana of the monkey may involve both cellular proliferation and stretching. After birth and into adolescence, it is known that the eye continues to grow and expand, and the retina becomes stretched and thinned.<sup>28,29</sup> However, as the eye expands with increasing age, the pars plana increases in radial length, increases in thickness, and multiple-layered cysts develop. This growth must be accompanied by proliferation of cells, for a multilayered epithelium to emerge from a monolayer as the eye expands. Recently, we demonstrated that nonpigmented cells in the ciliary epithelium of the avian pars plana continue to proliferate during juvenile development.<sup>11</sup> In

addition, we have found proliferating cells at the retinal margin and in the nonpigmented layer of the pars plana of the juvenile primate eye (Fischer, Hendrickson, and Reh, unpublished observation, 2001). It is possible that some cells in the far peripheral retina and pars plana may be newly generated in the adult eye.

In the far peripheral retina, with increasing age, the layers became narrowed and cease to be obvious. It remains uncertain whether the disorganization of retinal layers occurred because of the formation of cysts or whether the formation of cysts is secondary to retinal stretch, leading to disorganization of the retinal layers. Because we observed pigmented cells in and around cysts in the far peripheral retina, it may also be that cells from the pigmented layer proliferate, lose pigmentation, invade the peripheral retina, and contribute to the formation of cysts.

Little research has been focused on the pars plana of the vertebrate eye. However, several recent studies have indicated that this region of the eye may hold the potential for neural regeneration. In postnatal chickens, for example, newly formed neurons are added to the peripheral retina, and these neurons are generated by a population of stem cells that are found directly at the retinal margin and may extend into the adjacent pars plana.<sup>11</sup> This zone of proliferating neural stem cells at the retinal margin of chicks is similar to that described in the eyes of teleost fish and amphibians (for reviews, see Refs. 30–32). In addition, Tropepe et al.<sup>33</sup> and Ahmad et al.<sup>34</sup> have demonstrated that the ciliary body of the adult rodent eye contains pigmented cells that are capable of producing new neurons in vitro. Although retinal stem cells have not been demonstrated in primates, these recent reports and the results presented here warrant closer investigation into this region of the eye.

### Acknowledgments

The authors thank Ann Milam, Melanie Roberts, and Ryo Kubota, for helpful comments during the preparation of the manuscript and Andra Erikson and Blair Dierks for expert technical assistance.

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