Veterinary Ophthalmology (2007) **10**, Supplement 1, 117–124

Retinal pigment epithelial damage, breakdown of the blood–retinal barrier, and retinal inflammation in dogs with primary glaucoma

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Abstract

Objective This paper aims to determine if abnormalities of the retinal pigment epithelium (RPE) and retinal inflammation occur in primary glaucoma.

Procedure Twenty-three canine globes with primary glaucoma, goniodysgenesis, and elevated intraocular pressure were evaluated. Sections from 6 control and 23 glaucomatous canine globes were stained with hematoxylin and eosin, *Griffonia simplicifolia* isolectin B4, or immunohistochemically stained for CD3 or albumin. The retinal sections were evaluated with light microscopy for morphological and immunohistochemical evidence of pigmentary changes and inflammation.

Results Abnormal pigmented cells including displaced RPE cells and macrophages (identified by lectin binding) were found in the neuroretinas and vitreous bodies of glaucomatous eyes. Other abnormalities included hypertrophy of RPE cells and loss of RPE continuity. Regions of neuroretina with more displaced pigment had fewer remaining neurons. Signs of retinal inflammation found in glaucomatous eyes included infiltration with leukocytes, retinal swelling, and albumin leakage from vessels. Accumulation of perivascular CD3-positive T lymphocytes also occurred in glaucomatous retinas. Chronic glaucomatous retinas had increased pigmentary changes, fewer neutrophils, and less swelling than acute glaucomatous retinas.

Conclusions Disruption of the RPE, increased permeability of the vascular endothelium, accumulation of inflammatory cells, and retinal swelling or thinning occur in canine primary glaucoma. The displacement of pigment and accumulation of inflammatory cells in the neuroretina suggests that inflammation may be an important contributor to retinal damage.

Key Words: dog, goniodysgenesis, primary glaucoma, retinal damage, retinal pigment epithelium

INTRODUCTION

Glaucoma is characterized by a progressive loss of retinal ganglion cells, and is often associated with damage to the anterior segment and increased intraocular pressure.[1,2] Primary glaucoma (PG) in dogs is a neurodegenerative disease that can involve all layers of the neuroretina and not just the retinal ganglion cells.[3,4] Pigment dispersion and inflammation in the anterior segment have been reported in a form of canine primary angle-closure glaucoma (PACG) and in the DBA/2J murine model of pigmentary glaucoma.[5–7]

Uveitis occurs in association with some types of canine PACG,[8–10] and it is an important cause of secondary glaucoma in cats[11–13] and horses.[14–17] In both canine PACG[18] and DBA/2J mouse glaucoma,[7] inflammation and pigment dispersion occur in the anterior segment. Inflammation of the anterior uveal tract in canine PACG and DBA/2J glaucoma may contribute to iridocorneal angle and trabecular meshwork alterations, and a subsequent increase in intraocular pressure.[6,18] Pigmentary uveitis in Golden Retrievers is associated with pigment dispersion in the anterior chamber, and in one study a 46% incidence of glaucoma was reported.[19] Another glaucoma syndrome has been associated with ocular pigment deposition and the accumulation of putative melanophages in the canine choroid and retina.[20]

It is possible that abnormalities of pigment epithelium and pigment dispersion contribute to inflammatory responses in the anterior and posterior segment. Loss of pigment

epithelium from the posterior iris, pigment epithelial clumping, accumulation of pigmented cells in the anterior and posterior chambers and dependent regions of the iridocorneal angle, and anterior uveitis have been described in association with pigment dispersion in canine PACG.[18] Disruption of pigment epithelial cells presumably results in a loss of cellular tight junctions and breakdown of the blood–aqueous barrier. Breakdown of the blood–retinal barrier occurs when tight junctions between retinal vascular endothelial cells or retinal pigment epithelium (RPE) cells are compromised.[21] In an experimental model of posterior uveitis, intraocular accumulation of albumin occurred in association with retinal vascular damage and increased permeability.[22] It is possible that further inflammation and retinal damage may occur as naïve intraocular tissues are exposed to cells of the immune system.

The anterior chamber, vitreous body, and retina are immune privileged sites, and breakdown of the blood–ocular barriers can be associated with an autoimmune response. A number of proteins have been isolated from the retina[23–25] that act as auto-antigens in rodents[24,26–28] and horses[26,29–31] when exposed to the immune system. For example, an eye-specific glycosylated type I collagen isolated with melanin from RPE induces an autoimmune anterior uveitis in rodents.[23] Other type I collagens with different forms of glycosylation are not able to generate this inflammatory response.[23]

No studies have specifically examined whether disruption of the RPE and inflammation of the retina occur in dogs with PG. In one study, hypertrophy of the RPE not associated with retinal detachment was identified in dogs with PG.[32] These canine retinas also contained inflammatory cells including polymorphonuclear cells and macrophages.[32] We hypothesize that pigmentary changes and inflammation are important factors associated with retinal degeneration in canine PG.

MATERIALS AND METHODS

The archived, paraffin-embedded canine globes used in this study were obtained from one of the investigators (R.R.D.), and were from 6 control dogs, 11 dogs with acute glaucoma (acquired within 5 days after the onset of clinical signs), and 12 dogs with chronic glaucoma (acquired not earlier than 5 days after the onset of clinical signs). Dogs in the control group included a 2-year-old Golden Retriever, a 6-year-old Samoyed, a 1.7-year-old Sheltie (both eyes), a 12-year-old Scottish Terrier, and a 7-year-old Staffordshire Terrier. There were 19 Cocker Spaniels (5–13 years old), 3 Basset Hounds (4–6 years old), and a 10-year-old Dachshund that represented in the glaucoma group, and all had elevated intraocular pressure. All dogs in the glaucoma group were diagnosed with goniodysgenesis and PG.

Treatment of glaucoma was described for six dogs, and no response to treatment was indicated for two dogs; however, treatment was not specified. One dog was treated with transscleral laser cyclophotocoagulation, one dog was treated with latanoprost and pilocarpine, and a another dog was treated with latanoprost. The other three dogs were treated with various combinations of mannitol, methazolamide, timolol, and oral and topical steroids.

Saggital, 5-μm sections were obtained from each eye and stained with hematoxylin and eosin (H&E) to evaluate pigment dispersion and structural changes. Other sections from the same blocks were immunohistochemically stained for albumin or CD3 using an avidin-biotin method. The sections were dewaxed in xylene and graded ethanol. Sections were then autoclaved for 15 min in a pH 6.0 citrate buffer for antigen retrieval. After endogenous peroxidase was inactivated in 3% peroxide for 15 min, sections were incubated in primary rabbit antisera to albumin (1 : 400 in phosphate-buffered saline) or CD3 (1 : 100 in phosphate-buffered saline; Dako, Carpinteria, CA, USA) overnight. A Vectastain elite kit for rabbit antisera (Vector Laboratories, Burlingame, CA, USA) was then used to visualize the primary antibody using peroxidase and diaminobenzidine as chromogen. A negative control for specificity of the antibodies was performed by eliminating the primary antibodies, which resulted in greatly reduced staining. As an additional negative control for albumin immunostaining, the primary antibody was preincubated with bovine serum albumin (1 mg/mL in phosphate-buffered saline; Sigma-Aldrich, St. Louis, MO, USA), which resulted in decreased staining. All retinas were examined to ensure that the antibodies were indeed labeling known positive structures. This included labeling of the lumens of blood vessels by albumin immunostaining in both control and glaucomatous retinas, and labeling of putative T lymphocytes within the lumens of blood vessels by CD3 immunostaining.

Other sections were stained with biotinylated *Griffonia simplicifolia* isolectin B4 (GSL) (Vector Laboratories). Sections were processed as described for albumin immunohistochemistry with biotinylated lectin diluted 1 : 100 substituting for the primary antibody. The biotinylated lectin was visualized with the ABC component of the Vectastain kit with metal-enhanced diaminobenzidine (Sigma-Aldrich) as chromogen. All slides were dehydrated through graded ethanol, cleared in xylene, and coverslipped. A negative control for lectin staining was performed by eliminating the lectin, which resulted in greatly reduced staining. A positive control to ensure that the lectin was indeed labeling known positive structures was the strong labeling of blood vessels in all retinas, including control retinas that did not have macrophage infiltrates.

Chronic glaucomatous retinas were evaluated for thinning by comparing regions with abnormal pigmentary changes to adjacent regions within 1000 μm that lacked pigmentary changes. The retinas were considered thinned if the inner nuclear and outer nuclear layers were < 67% of the thickness of adjacent regions that lacked pigmentary changes. Retinal thickness was evaluated in the tapetal regions of chronic glaucomatous retinas for comparison to nontapetal regions with increased pigmentary changes and thinning.

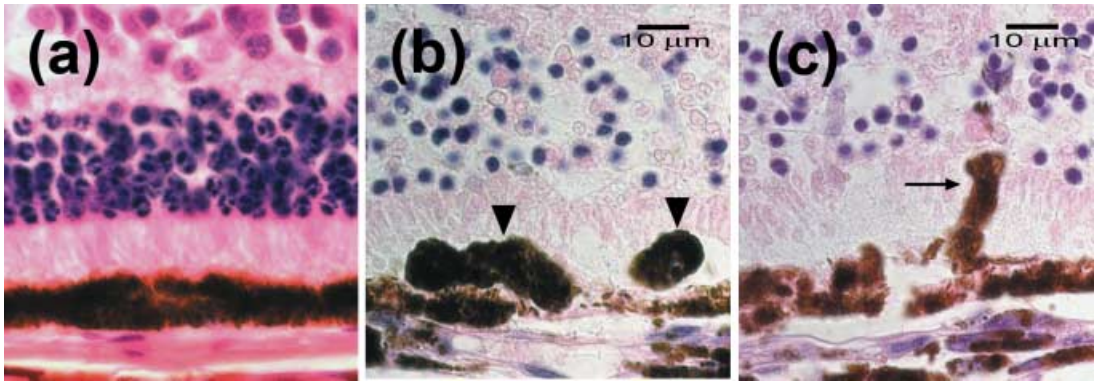

Figure 1. The retinal pigment epithelium (RPE) was abnormal in glaucomatous eyes. (a) Control RPE (H&E stain). (b) Hypertrophied and disrupted RPE (arrowheads) with a rounded appearance in primary glaucoma (PG) retina (H&E stain). (c) Putative RPE cells (arrow) displaced into the neuroretina in PG (H&E stain).

Acute glaucomatous retinas were evaluated for swelling by comparing regions with significant neutrophilic infiltrates to adjacent regions within 1000 μm that contained few or no neutrophils. Control retinas were also evaluated for average thickness (137 ± 12.4 microns). Photomicrographs (Zeis Axioplan/2Axiovision, Carl Zeis Microimaging, Thornwood, NY, USA) were obtained, and measurements from the outer limiting membrane to the inner limiting membrane were acquired using an image analysis program (ImageJ 1.37, National Institutes of Health, Bethesda, MD, USA). Comparison of retinal thickness was evaluated with statistical software (GraphPad InStat 3.06, San Diego, CA, USA) by applying values of swollen and not swollen adjacent regions in a paired *t*-test. The values were expressed as a percentage of the mean ± SEM, and significance was designated as values of *P* less than or equal to 0.05.

RESULTS

Abnormalities of RPE and pigment distribution may occur in PG

The nontapetal RPE displayed focal to multifocal abnormalities in most glaucomatous eyes. Hypertrophy and rounding of RPE cells with separation from the neuroretina consistent with retinal detachments were identified in two eyes. In 9 of 23 (39%) glaucomatous eyes, some of the RPE cells were hypertrophied and had a rounded appearance without retinal detachment (Fig. 1b). In 12 of 23 (52%) acute and chronic glaucomatous eyes there were isolated RPE cells in the outer layers of the neuroretina (Fig. 1c), and 6 of 23 (26%) eyes showed apparent large disruptions in the RPE in areas where there were no obvious sectioning artifacts. Changes were more severe and common in chronic glaucoma. In sections from six control eyes stained with H&E, no disruption or invasion of RPE cells into the underlying neuroretina was observed.

Abnormal distribution of pigment granules and pigmented cells was also seen in the neuroretinas of glaucomatous eyes. In contrast to control eyes, 20 of 23 glaucomatous eyes were found to have free pigment granules and/or pigmented cells in the neuroretinas greater than 1.0 mm from the ora ciliaris retinae. Pigmented cells in glaucomatous neuroretinas greater than 1.0 mm from the ora ciliaris retinae included both putative macrophages (Fig. 2a) and putative displaced RPE cells in H&E-stained sections. To confirm that some of these pigmented cells were macrophages, staining with GSL was performed. Lectin staining of adjacent sections confirmed the presence of macrophages in these regions (Fig. 2b), and indicated that many of the pigmented cells were most likely macrophages. Numerous lanceolate pigment granules that did not appear to be contained within cells were also seen in glaucomatous neuroretinas (Fig. 2a). There were four pigment-containing cells (one cell in two different retinas, and two cells in one retina) in the periphery of control neuroretinas, and in more central regions they were absent. No associated inflammation or retinal damage was identified.

Increased numbers of pigmented cells and free pigment granules were observed in the vitreous bodies of glaucomatous eyes compared to controls. The pigment granules were round (Fig. 3a), and possibly associated with pigment loss from the peripheral retina or pigment epithelium of the anterior segment. Pigmented cells were found in the vitreous bodies within 0.5 mm of the inner limiting membrane in 19 of 23 glaucomatous eyes. These pigment changes occurred more frequently and to a greater extent in chronic glaucoma as compared to acute glaucoma. Most of these cells had the morphologic characteristics of macrophages that had phagocytized pigment granules (Fig. 3a). Of the nucleated cells in the vitreous body, almost all stained with GSL (Fig. 3b), confirming that many of the cells containing pigment were macrophages. Other pigmented cells in the vitreous body included putative pigment epithelial cells from the anterior segment. These cells were larger than macrophages, usually contained a very high density of pigment granules, and did not stain with lectin. A small number of free pigment

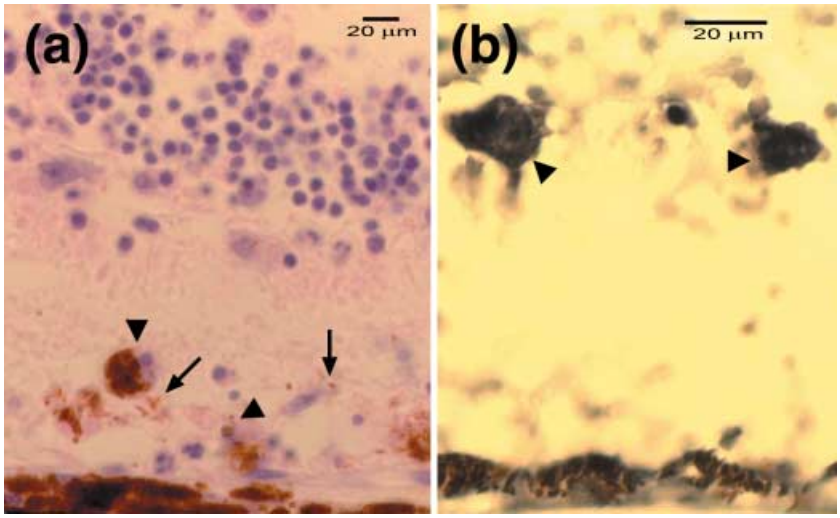

Figure 2. Putative macrophage/microglia and pigment granules accumulate in the neuroretina of glaucomatous eyes. (a) Putative macrophages (arrowheads) containing pigment granules and free pigment granules (arrows) in the neuroretina in primary glaucoma (PG) (H&E stain). (b) Lectin-labeled macrophages (arrowheads) in the neuroretina in PG (GSL stain).

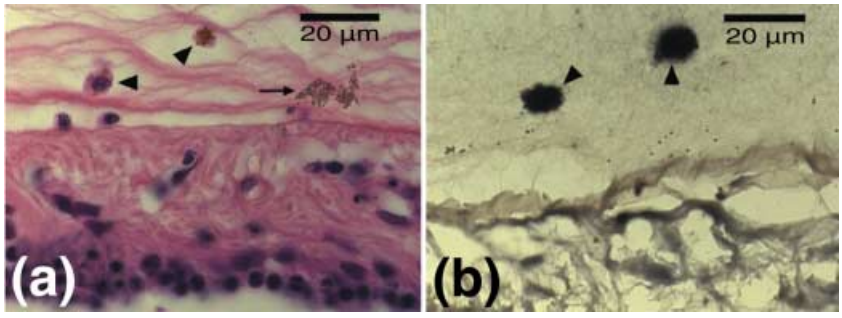

Figure 3. Pigmented cells and pigment granules accumulated in the vitreous body of glaucomatous eyes. (a) Pigment-containing cells with the typical appearance of macrophages (arrowheads) and free pigment granules (arrow) found in the vitreous body of an eye with primary glaucoma (PG) (H&E stain). (b) Lectin-positive cells (arrowheads) in the vitreous consistent with macrophages (GSL stain).

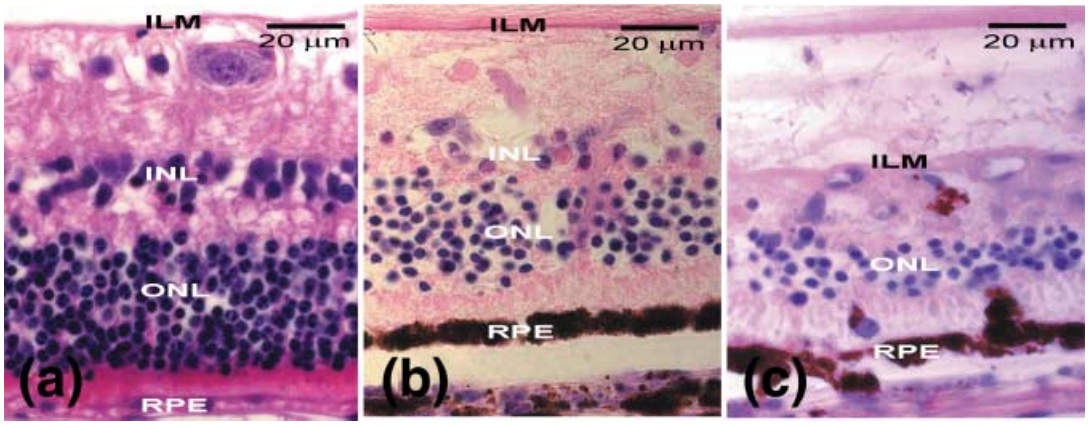

Figure 4. Regions of neuroretina with pigment accumulations were more severely damaged than other regions. (a) Tapetal region showing no thinning or abnormal pigmentation (H&E stain). (b) Nontapetal region without pigmentary changes (H&E stain). (c) Loss of the inner nuclear layer (INL) and attenuation of the outer nuclear layer (ONL) in a region with an accumulation of pigmented cells and pigment granules (H&E stain). ILM, internal limiting membrane; RPE, retinal pigment epithelium.

granules and only two pigmented cells were seen in the vitreous bodies within 0.5 mm of the retinas in the control eyes.

Regions of retina with more pigment showed a more severe thinning of the inner and outer nuclear layers

Regions of glaucomatous retinas that contained pigmented cells or free pigment granules were examined for signs of neuronal damage and death. There was less retinal swelling in chronic glaucoma, and damaged regions with obvious neuronal loss and thinning of the nuclear and plexiform layers were identified. In the tapetal regions there were fewer pathologic pigmentary changes and disruptions of the RPE (Fig. 4a). Neuronal damage was generally less severe in the nonpigmented tapetal regions than in the pigmented nontapetal

regions of the same retinas. Thinning of the neuroretina occurred in 14 of 23 heavily pigmented regions (Fig. 4c).

Inflammatory changes were seen in glaucomatous retinas

The magnitude of inflammatory cell infiltrates and alterations in retinal thickness appeared to be associated with the duration of glaucoma in most cases. Neutrophilic inflammation and retinal thickening were associated more commonly with acute glaucoma. Chronic glaucoma tended to have a greater association with retinal thinning, and more significant pigment accumulation. Lymphoplasmacytic inflammation was less specific in terms of duration of glaucoma, but CD3-positive T lymphocytes were identified in the vitreous and perivascularly in the neuroretinas of dogs with PG. In addition, the observation of albumin leakage from blood vessels indicated breakdown of the blood–retinal barrier.

Neutrophilic inflammation was seen in the vitreous and retinas of eyes with acute glaucoma. Large numbers of cells with morphologic characteristics consistent with neutrophils were seen in the neuroretinas of all acute glaucoma eyes (Fig. 5b,c). The distribution of neutrophils varied from focal to multifocal, and in one eye retinal detachment appeared to be associated with the accumulation of neutrophils and macrophages in the subretinal space. In the central retinal regions containing many neutrophils, retinal thickness from the outer limiting membrane to the inner limiting membrane was increased to 122 ± 13.8% ($P < 0.004$) of adjacent nonswollen regions. Neutrophils were not observed in the neuroretinas of control dogs. The mean thickness in control retinas was 137 ± 12.4 μm, and there was no significant difference when compared to nonswollen regions ($P = 0.7$).

Cells consistent with lymphoplasmacytic inflammation were seen in the vitreous bodies and neuroretinas of glaucomatous eyes, but not in control eyes. Perivascular inflammatory cells were observed in the inner layers of the neuroretina (Fig. 6a). Presumptive T lymphocytes with CD3 immunoreactivity were found in both acute and chronic glaucomatous retinas. Furthermore, many of the inflammatory cells observed in the perivascular regions of H&E-stained sections were CD3-positive T lymphocytes (Fig. 6b). Control retinas contained a population of cell bodies confined to the inner nuclear layer that were labeled by immunohistochemical staining for CD3 (Fig. 6c). These presumptive amacrine cells often had visible processes projecting into the inner plexiform layer, and did not appear to have any association with blood vessels. In contrast to control retinas, very few cells of the inner nuclear layer of glaucomatous retinas were labeled by immunohistochemical staining for CD3.

Other signs of inflammation, including breakdown of the blood–retinal barrier occurred in glaucomatous retinas. Following immunohistochemical staining, albumin accumulation was observed around retinal blood vessels, which indicated an abnormal increase in vascular permeability. Detection of albumin outside the vessel lumina was seen in 15 of 23 glaucomatous neuroretinas, but not in control retinas (Fig. 7a,b).

DISCUSSION

In a form of canine PACG, pigment dispersion and inflammatory changes were found in the anterior segment.[18] The accumulation of pigment-containing macrophages in the retina has also been associated with glaucoma and ocular pigment deposition in dogs.[20] In the present study, we determined if abnormalities of the RPE and abnormal pigment distribution were associated with inflammatory changes in the retinas of dogs with PACG.

Morphologic changes in the RPE and abnormal pigment distribution in the neuroretina and vitreous body occur in PG.[20,32] In contrast to control retinas, hypertrophy of the RPE occurred in many regions of glaucomatous retinas. Extensive RPE hypertrophy without retinal detachment, which was repeatedly observed in numerous glaucomatous eyes, appears to be associated with inflammation. In regions of two eyes, RPE hypertrophy with separation of the neuroretina consistent with retinal detachemnt was seen. However, it should be noted that these changes may have been due to artifacts associated with the processing of severely damaged retinas. In addition, substantial numbers of pigmented cells and free pigment granules were found in the neuroretinas and vitreous bodies of glaucomatous eyes, but not in control eyes. The majority of these pigmented cells were macrophages identified by their appearance with H&E and confirmed with GSL staining. Retinal necrosis occurs in glaucoma,[3,4,32] and the presence of macrophages would be consistent with the phagocytic role of these cells in damaged and inflamed tissues. Melanophages have been described in the uveal tract and retina in association with pigment dispersion.[18,20] The infiltration of the neuroretina by clumps of cells contiguous with the RPE also suggest that many of the infiltrating pigmented cells may be from the RPE. Pigment granules contained within pigmented cells or free in the neuroretina were lanceolate, which is typical of pigment granules contained within the RPE of dogs.[33] In a process termed phagocytic metaplasia, RPE may transform into macrophages in response to an inflammatory episode.[34] This could explain the observation of migration of RPE cells and the presence of macrophages in the neuroretina.

Pigment in the neuroretina may be associated with more severe damage. In most areas of the neuroretina where pigmented cells and free pigment accumulated, retinal damage was more severe. Pigmentary changes appeared to be more prominent in the chronic cases; hence, some of the neuronal loss in these regions may be due simply to a longer duration of the disease. To eliminate differences in time of onset, neighboring regions of the same retina were compared. Retinal thinning was more severe in regions with pigmentary changes within the same retina, which is consistent with these regions being associated with more local damage. In contrast to nontapetal regions, tapetal regions had fewer pigmentary changes and there were no significant changes in retinal thickness. Other studies have reported that the

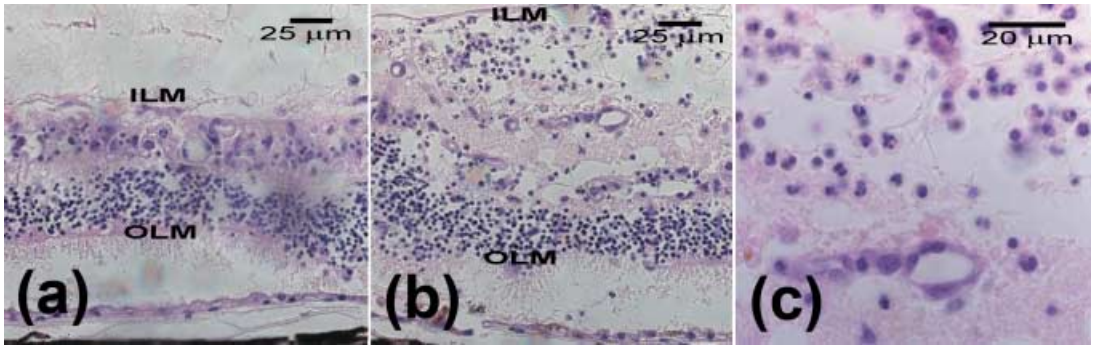

Figure 5. Adjacent regions of nonswollen and swollen regions exhibiting neutrophilic inflammation in acute PG. (a) Nonswollen region (H&E stain). (b) Neutrophilic inflammation in the inner layers of the retina (H&E stain). (c) Neutrophils in the neuroretina (H&E stain).

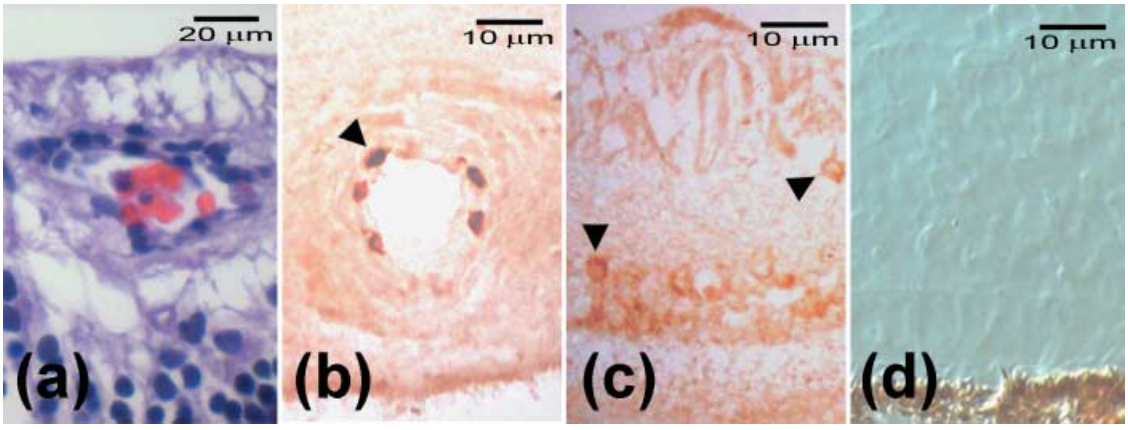

Figure 6. Perivascular inflammation in the neuroretinas of glaucomatous eyes. (a) Perivascular inflammatory cells in the neuroretina in PG (H&E stain). (b) CD3 positive cells (arrowhead) associated with a retinal blood vessel in PG (Immunohistochemical CD3 stain). (c) Presumptive amacrine cells with cross-reactivity to CD3 in the inner retina of a control eye (Immunohistochemical CD3 stain). (d) A negative control showing absence of staining when the primary antibody is not applied (Immunohistochemical CD3 stain).

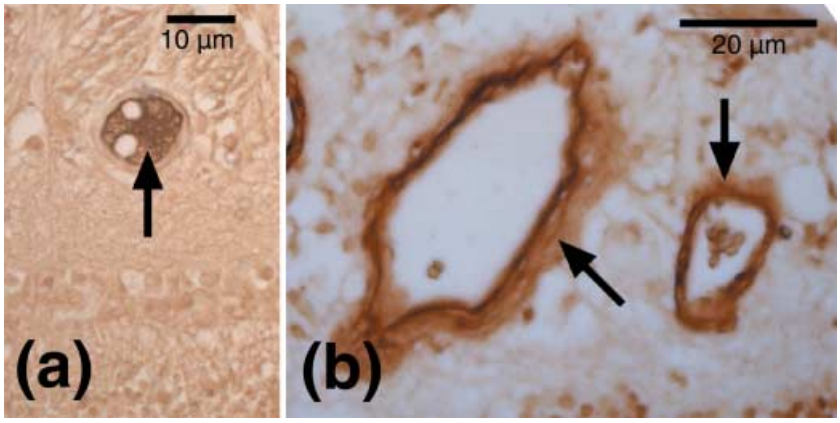

Figure 7. Albumin leakage from retinal blood vessels. (a) Control retina showing albumin within the blood vessel lumen (arrow) (Immunohistochemical albumin stain). (b) Albumin leakage (arrows) from a vessel in the outer layers of the retina in primary glaucoma (immunohistochemical albumin stain).

tapetal regions of neuroretinas tend to have less severe damage than the nontapetal regions.[4,32]

Inflammatory cells, including neutrophils, lymphocytes, and plasma cells, have been reported in the anterior segment of dogs with PACG.[18] The presence of inflammatory cells has also been reported in the neuroretinas of dogs with PG.[32] In this study macrophages were present in the neuroretinas and vitreous bodies of dogs with both acute and chronic glaucoma. Neutrophilic infiltrates were seen primarily in acute glaucoma and were associated with retinal swelling. Cells with the morphologic characteristics of lymphocytes and plasma cells were also found in the neuroretinas of dogs with acute and chronic glaucoma. Immunohistochemical staining for CD3 indicated that T

lymphocytes accumulated around some blood vessels of the inner layers of the retina. To our knowledge, there is no precedent for the observation of CD3-positive cells in the inner nuclear layer of normal controls or for the absence of these cells in glaucomatous neuroretinas. Based on location and morphology, it is possible the CD3-positive cells in control eyes were amacrine cells, which expressed reactivity to the CD3 immunohistochemical stain. Furthermore, these cells were negative for CD3 immunoreactivity when the primary antibody was withheld (Fig. 6d). The lack of CD3-positive cells in the inner nuclear layer in glaucomatous eyes may reflect damage to these cells; however, the significance of this staining profile is unknown. The presence of perivascular CD3-positive cells in neuroretinas of glaucomatous eyes, but not in control eyes, provides evidence of an inflammatory disease process.

Both disruption of the RPE and increased vascular permeability may lead to exposure of retinal auto-antigens to the immune system, perhaps initiating or contributing to neuronal damage. The observations of perivascular inflammatory cells and albumin leakage from retinal blood vessels indicate inflammation and a breakdown of the blood–retinal barrier, respectively. In rodents[24,26–28] and horses[26,29–31] purified or synthesized antigens from the RPE have been used to induce both uveitis and uveoretinitis. The histopathologic changes seen in some types of uveoretinitis have several similarities to those seen in PG in the dog. These changes include eventual loss of most types of retinal neurons including photoreceptors, damage to the RPE, vasculitis, accumulation of pigment in macrophages, and regional differences in the severity of damage.[3,4,20,32] Thus, the inflammation associated with PG could lead to a cascade of events resulting in further neuronal damage.

It remains unclear if the retinal inflammation and RPE abnormalities observed this study are initiating factors in certain forms of PG or just a response to other causes of retinal damage and necrosis. The pigmentary changes, and association between duration of glaucoma and inflammatory cell morphology, parallel changes seen in the anterior segment in dogs with PACG.[18] As stated in the study by Reilly *et al.*, a cause-and-effect relationship cannot be discerned through histopathology, and there are inherent limitations to classifying and comparing samples based on a presumptive chronology of the disease process.[18] Other factors, including previous medical or surgical intervention, may also affect histopathologic analysis. One dog in this series was treated with laser cyclophotocoagulation, which could have contributed to more severe inflammation and pigmentary changes. Latanoprost and/or pilocarpine were used in two dogs, and these procedures can cause anterior uveitis. In humans, latanoprost has been associated with macular edema and increased pigmentation of the iris.[35–38] The pigmentary changes and inflammation, however, did not appear to be more severe when compared to cases that did not list treatments in the history. Similarly, we cannot make any conclusions about the effects topical or oral steroids may have had on slowing the progression of retinal damage in several cases. It should also be noted that these results represent a small sample size, and are limited to one form of canine PG. The results do, however, warrant a retrospective and prospective assessment for signs of pigmentary and inflammatory changes in the neuroretinas of dogs with various forms of PG.

The findings of RPE abnormalities and inflammation in the neuroretina in canine PG may have implications for further studies of pathogenesis and treatment. Damage to the RPE and leakage of albumin from blood vessels indicates a disruption of the blood–retinal barrier. We speculate that disruption of the blood–retinal barrier could make these eyes more susceptible to immune-mediated processes, which could cause further retinal degeneration. However, it is also possible that the pigmentary changes and inflammation are simply a response to severe retinal damage caused by other factors, such as elevated intraocular pressure and/or vascular damage. Regardless of whether or not pigmentary changes and inflammation are key risk factors in canine PG, they may contribute substantially to its progression. If true, anti-inflammatory treatment may prove effective in slowing the progression of neurodegeneration in canine PG.

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