Early Morphogenesis of Persistent Hyperplastic Tunica Vasculosa Lentis and Primary Vitreous

A Transmission Electron Microscopic Study

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This report provides transmission electron microscopic observations on the early pathogenesis of persistent hyperplastic tunica vasculosa lentis/persistent hyperplastic primary vitreous (PHTVL/PHPV) in affected canine fetuses at days 28–44 postcoitum.

The retrothal tissue by which this anomaly is characterized consists of loosely arranged fibroblasts in a randomly oriented meshwork of collagenous fibrils. Some of these cells contain melanosomes at day 44. In one day-44 eye, cells of neuroectodermal origin (Müller cells; fibrous astrocytes) were observed. From day 37 onward, the posterior subcapsular part of the lens contains rounded, increased intercellular spaces, resembling vacuoles, which deform the shape of the lens fibers. The posterior lens capsule develops normally until day 30. From day 35 onward the capsule has an amorphous ultrastructure, as opposed to the clearly laminated ultrastructure in reference eyes at day 35. In addition, the capsule's thickness increases until day 35, and, instead of growing thicker, decreases thereafter.

Based on these results, it is hypothesized that a primary metabolic disorder in the lens fibers, subsequently leading to the formation of an abnormal posterior lens capsule, constitutes the primary defect in the sequence of events leading to PHTVL/PHPV. Invest Ophthalmol Vis Sci 31:1886–1894, 1990

Persistent hyperplastic tunica vasculosa lentis/persistent hyperplastic primary vitreous (PHTVL/PHPV) is a congenital eye anomaly known to occur in humans and animals. The main feature of the anterior form of this pathologic entity is the proliferation and persistence of retrothal fibrovascular tissue. The loss of vision in severely affected individuals is due to the development of a cataract.1–6

In a previous light microscopic study on the early morphogenesis of this anomaly in Doberman pinschers,6 in which the entity is known to be a hereditary disease,7 it appeared that PHTVL/PHPV is a developmental anomaly starting between days 30–37 of gestational age in the dog, which roughly corresponds to days 43–66 of ophthalmic development in humans. In a transmission electron microscopic reference study we investigated the development of the lens capsule, TVL, and anterior vitreous of nonaffected beagle fetuses of comparable gestational ages.8

The current study, providing transmission electron microscopic observations of the fine structure of the lens and retrothal elements of affected Doberman pinscher fetuses, was undertaken to elucidate the pathologic process leading to PHTVL/PHPV in these animals. The results indicate that a disturbed development of the posterior capsule is an initiating factor for the retrothal proliferation.

Materials and Methods

From matings of Doberman pinscher dogs with severe PHTVL/PHPV, fetuses were collected at 28, 30, 35, 37, and 44 days postcoitum. Gravidity of the bitch and collection of the fetuses were accomplished using previously described standard methods.9 Animal care and treatment in this study were in compliance with the ARVO Resolution on the Use of Animals in Research.

Nineteen eyes were used in the current study. Immediately after collection and death of the fetuses, the eyes were removed and prefixed for 3 hr in 5% glutaraldehyde buffered with 0.1 M phosphate (pH 7.2). The specimens were stored in a 0.1 M phosphate

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buffer containing 0.2 M sucrose and then rinsed in 0.1 M sodium cacodylate-HCl (pH 7.2 to 7.4) for 1 min. Postfixation was done in 2% osmium (VIII) oxide, buffered with 0.1 M sodium cacodylate HCl. The eyes were then dehydrated in acetone sequentially from 70–100%. After treatment with propylene oxide and araldite (Durcupan, Fluke Chemie AG, CH-9470, Basel, Switzerland), they were embedded in araldite. Grid staining was done with uranyl acetate (2%) and lead citrate.

The posterior parts of the lens (vesicle), its capsule, the TVL posterior, and some of the adjacent vitreous were examined in 1-μm sections stained with toluidine blue and selected for ultrathin sectioning. Ultrathin sections (silver to pale gold) were used also selected for transmission electron microscopy (Philips EM201G, Philips, Eindhoven, The Netherlands).

In sections of two eyes at day 28, three at day 30, three at day 35, six at day 37, and five at day 44, the thickness of the central part of the posterior lens capsule was measured in duplicate. The final magnification of the micrographs was calculated using a calibrated grating replica. The mean, median, range, standard deviation, and standard error of the mean were calculated for the thickness of the central part of the posterior lens capsule at each gestational age.

**Results**

**Lens**

By days 28 and 30 the posterior pole of the lens vesicle still consisted of undifferentiated epithelial cells, which are known to develop into the primary embryonic lens fibers. These lenticular cells contained a nucleus and (especially their capsular parts) numerous mitochondria, a well-developed network of granular endoplasmic reticulum, a small number of lysosomes, and many polyribosomes (Figs. 1, 2). By day 35 the posterior cells had already partially differentiated to lens fibers (Figs. 3–5). However, they still contained numerous cytoplasmic elements, of which the clear, swollen mitochondria were the most obvious (Fig. 5). Polyribosomes were still numerous, and lysosomes were still present. By day 37 the posterior lens fibers still contained numerous and clear mitochondria. By days 37 and 44 the most prominent feature was the presence of large (2.5–4.5 μm), clear vacuoles, bordered by membranes. Since the membranes were continuous with the cell membrane (Fig. 6), the vacuoles must be considered to be intercellular vacuoles. In scanning electron micrographs of lenses of 8-week-old (postnatal) PHTVL/PHPV-affected dogs, large vacuolar invaginations of the fiber membranes were found (Figs. 7, 8), supporting this interpretation.

**Lens Capsule**

The posterior lens capsule was continuous at all stages. It had a laminated ultrastructure by days 28 and 30 (Figs. 1, 2). From day 35 onward the regular and laminated appearance of the capsule had changed into a more or less amorphous to fine granular structure, with only a faint indication of lamination (Figs. 3, 4, 9, 10), making discrimination of separate lamellae impossible.

The average thickness of the central part of the posterior lens capsule increased from 0.38 μm at day 28 to 1.78 μm at day 35, but it decreased to 1.2 μm at day 44 (Table 1).

Mainly in the early stages (days 28 and 30), electron-dense fibrillar material behind the lens was positioned against, and partially attached to, the posterior lens capsule (Figs. 1, 2).

**Retrolental Elements**

At all gestational stages studied, the retrolental plaque consisted of fibrovascular tissue, i.e., capillaries of the TVL, fibroblasts and fibroblast-like cells, and collagenous fibrillar material. The capillaries at all stages were completely covered by a basal lamina, were unfenestrated, and had circumferentially incomplete pericyte coverage.

By days 28 and 30 only a few TVL capillaries were observed. Numerous loosely arranged cells were present in the region just posterior to the lens vesicle. These cells contained large, slightly irregular, pale nuclei, the standard cytoplasmic organelles, a prominent granular endoplasmic reticulum, and in some cases, a lipid droplet. The space between these cells and that between the cells and the lens capsule contained fibrillar material similar to that attached to the lens capsule.

By day 35 the retrolental tissue consisted of rounded or flattened capillaries and fibroblasts. Some of the capillary endothelial cells contained some round, electron-dense cytoplasmic organelles, probably secondary lysosomes (Figs. 3, 4). Their basal side was covered by a continuous basal lamina. The endothelial cells were interconnected by tight junctions with numerous marginal folds extending into the capillary lumen (Fig. 3). A relatively small number of pericytes was observed, containing a prominent granular endoplasmic reticulum. The fibroblasts of the plaque were positioned directly against the lens capsule close to each other, without junctions or other connections. They were rich in mitochondria and granular endoplasmic reticulum. The intercellular space contained randomly oriented fibrils, predominantly in proximity to both the endothelial
Figs. 1–6. Fig. 1. Electron micrograph showing the posterior polar portion of the lens and anterior vireous of a PHTVL/PHPV-affected Dobermann dog fetus at D28. The lens capsule (LC) consists of six layers. A capillary belonging to the TVL posterior is present. Loose, finely fibrillar and electron-dense material (FM) surrounds the capillary. (EN = endothelial cell, GER = granular endoplasmic reticulum, Ly = lysosome, Mi = mitochondriun, Nu = nucleus, PLF = primary lens fibers). Fig. 2. Electron micrograph of the posterior polar portion of the lens and anterior vireous (PHTVL/PHPV-affected canine fetus at D30). The lens capsule (LC) consists of seven layers. A TVL capillary is present. Loose, finely fibrillar material is located between the capillary and the lens capsule. (arrows point to polyribosomes, EN = endothelial cell, Mi = mitochondriun, Nu = nucleus, PLF = primary lens fibers). Figs. 3, 4. PHTVL/PHPV affected canine eye at D35. Electron micrograph of the posterior capsule’s central portion (LC) and the posterior part of primary lens fibers (PLF). The PLF contain numerous clear mitochondria (Mi). The laminated ultrastructure of the capsule at the earlier stages has changed into an amorphous structure. Part of a large, flattened capillary, belonging to the tunica vasculosa lentis posterior, is present posteriorly against the capsule. Intercellular tight junctions are detectable (arrows). A pericyte (Pe) lies next to an endothelial cell (EN). Marginal folds (MF) are present at the luminal surface of the endothelium. Loose, electron-dense fibrillar material (FM) is present between the capillary endothelium and the capsule and surrounds the basal side of the capillary endothelium. (Lu = capillary lumen). Fig. 5. Canine PHTVL/PHPV-affected fetal eye at D35, showing the posterior part of a few primary lens fibers, just subcapsular. Numerous clear and swollen mitochondria (Mi) and many polyribosomes (arrows) are present. Some lysosomes (Ly) are recognizable. (CM = cell margins). Fig. 6. Posterior part of the lens of a PHTVL/PHPV-affected dog at D37. Between the lens fibers there are increased spaces resembling vacuoles (Vac) but intercellular in location. These represent a precataract. (LC = posterior lens capsule, Lu = (TVL) capillary lumen, Mi = mitochondriun).

Table 1. Values (in μm) regarding the posterior polar part of the lens capsule of PHTVL/PHPV-affected Dobermann fetuses at different ages

<table>
<thead>
<tr>
<th></th>
<th>D28</th>
<th>D30</th>
<th>D35</th>
<th>D37</th>
<th>D44</th>
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<tr>
<td>Mean</td>
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<td>0.51</td>
<td>1.64</td>
<td>1.59</td>
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<td>Median</td>
<td>0.50</td>
<td>1.77</td>
<td>1.60</td>
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<td>Min.</td>
<td>0.38</td>
<td>0.46</td>
<td>1.45</td>
<td>1.52</td>
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<td>Max.</td>
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<td>0.56</td>
<td>1.80</td>
<td>1.65</td>
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<td>SD</td>
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<td>0.05</td>
<td>0.18</td>
<td>0.05</td>
<td>0.06</td>
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<tr>
<td>SE</td>
<td>0.06</td>
<td>0.03</td>
<td>0.69</td>
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Discussion

Lens

Until day 35 the morphology of the posterior lens fibers was comparable to that of the reference material. By days 37 and 44 posterior subcapsular vacuoles appeared in the lenticular tissue (Figs. 6, 14). These vacuoles were located extracellularly and protruded into the outlines of the lens fibers, thus deforming their shape, as supported by scanning electron microscopy of lens fibers of PHTVL/PHPV-affected dogs at 8 weeks of (postnatal) age (Figs. 7, 8). Although similar vacuoles have been described for the development of hereditary congenital cataract in the miniature Schnauzer dog, their development is yet unexplained. They probably develop due to osmotic disturbances which may arise from an inherent lenticular metabolic disorder or a nutritional disorder, resulting from the presence of the retrolental plaque or the different organization of the lens capsule. However, it cannot be excluded that the observed vacuoles constitute extruded intracellular vacuoles. Intracellular vacuoles may be associated with metabolic disorders in subjects with (lysosomal) enzyme defects. Metabolites from the affected metabolic pathway are accumulated in such vacuoles. The described vacuoles seem to represent the initiation of

basal lamina and the lens capsule. Sometimes these fibers had periodic cross striations (Fig. 11).

By day 37 the retrolental plaque had morphologic features similar to those on day 35 (Figs. 9, 12, 13). By day 44, the cells of the retrolental plaque were arranged more regularly than in the previous stages (Figs. 14–16). The most posterior cells were disc shaped and had a spindle-shaped-to-elongated cross section with similarly shaped, large, regular nuclei. Apart from the standard cytoplasmic organelles, these cells had a more prominent Golgi apparatus than previously observed and frequently contained stage 3 and 4 melanosomes (Fig. 17). The intercapsular contained a large amount of fibrillar material which was more electron dense than in the previous stages.

The vitreous cells in proximity to the plaque were rounded and generally had an irregular, vacuolated appearance (Figs. 16, 18), as in mononuclear phagocytes. Of these cells, the ones in close proximity to the plaque had a more regular, oval-to-elongated appearance (Fig. 16).

Retinal cells, with morphologic features of Müller cells of the inner retinal layer, were present in the retrolental plaque of one eye and were characterized by large, irregular nuclei with multiple indentations and irregular cell margins having numerous recesses and pseudopodia. Groups of these cells were surrounded by electron-dense fibrillar material (Fig. 19).

Also in one day-44 eye, cells with the characteristics of fibrous astrocytes were present in the posterior portion of the retrolental plaque. Although the quality of fixation was not always optimal, the thin basal lamina surrounding the complexes and the large number of filaments suggested fibrous astrocytes, and the ultrastructure of the perikarya, although less specifically, did not contradict this (Figs. 20, 21).
These "vacuoles" represent the cataract that was present in this animal (see also Fig. 8). Fig. 8. Magnified image of fibers and "vacuoles" similar to those described in Figure 7. Fig. 9. Posterior lens capsule and TVL-capillary of the eye of a PHTVL/PHPV-affected canine fetus at D37. The capsule has an amorphous to fine granular ultrastructure. (Lu = capillary lumen, EN = endothelial cell, Mi = mitochondrion, Nu = nucleus). Fig. 10. Posterior lens capsule and capillary of the tunic vasculosa lentis at D37. The ultrastructure of the capsule is faintly laminated to amorphous. (B = blood cell, GER = granular endoplasmic reticulum, Lu = capillary lumen, EN = endothelial cell, Nu = nucleus). Fig. 11. Canine PHTVL/PHPV-affected fetal eye at D35. The collagen-like fibrillar material (FM) in the vitreous, near the plaque, shows a faint periodic cross striation. Fig. 12. Retrolental fibrovascular plaque of the eye of a PHTVL/PHPV-affected canine fetus at D37. Fibroblasts (F) are situated within a fibrillar matrix.

cataract, as has been reported for postnatal subjects with PHTVL/PHPV.

**Lens Capsule**

The lens capsule is a protective barrier surrounding the lenticular tissue. It is considered to be the thickest basal lamina in vertebrates, and it has a layered ultrastructure in all mammalian species investigated. The lens capsules of the unaffected beagle fetuses had a layered substructure at all fetal stages studied, but in the affected fetuses, the ultrastructure of the capsule was different from day 35 onward. From day 37 the thickness of the posterior polar part of the lens capsule decreased (Table 1). To elaborate the significance of these differences, larger numbers of reference and affected fetal eyes and subsequent statistical analysis would be required.

Capsular anomalies have been reported for postnatal human and canine cases of PHTVL/PHPV. Because the lens capsule is primarily or solely formed by the underlying lens fibers, the capsular abnormalities we observed are probably caused by a primary defect located in the lens fibers. These capsular changes may be related to changes in the permeability of the capsule, thus giving rise to the flow of morphogenetic active substances from the lens toward the vitreous. On the other hand, the capsule's ultrastructure may become abnormal secondary to the proliferation of retrolental tissue in close proximity, which could interfere with essential metabolite exchange by the capsule.

**Retrolental Elements**

**Cellular components:** Light microscopic studies showed the presence of retrolental fibrovascular tissue (plaque) in PHTVL/PHPV-affected fetal canine eyes as early as day 33, together with the presence of elements of neuroectodermal origin by day 37 and pigmented cells by day 44.

The capillaries in the plaque, like those of the TVL in the reference eyes, can be classified as A-1-α capillaries according to Bennett et al. and they were not abnormal compared with reference eyes. The main cell type of the plaques had the morphologic characteristics of fibroblasts.

By day 44 plaque cells contained clusters of melanosomes.

As in some of the eyes in the light microscopic study, cells of neuroectodermal origin were observed in one day-44 eye. The cells had an ultrastructure comparable to that of the Müller cells of the inner retinal layer and that of fibrous astrocytes.

More vitreous cells were present in close proximity to the plaque on day 44 than at the preceding stages. Because of their irregular outlines, their numerous and large cytoplasmic vacuoles and the large, irregular nucleus, they resemble mononuclear phagocytes. These cells are considered to be closely related to mesenchymal cells and hyalocytes. When such cells were observed in close proximity to the retrolental plaque, they had an oval, more regular shape. They had a somewhat flattened shape if positioned close to the plaque. These observations suggest that the retrolental plaque is, at least partly, formed by the accretion of vitreous cells migrating to the lens.

**Fibrillar contents:** From day 35 onward the fibrils between the cells of the plaque had a faint, but recognizable, periodic, striated banding pattern, resembling collagen fibrils (Figs. 11, 13). These fibrils are probably produced by the fibroblasts.

Developing vitreous collagen fibers of human fetuses at 8 and 10 weeks of pregnancy have been described by Akiya and co-workers and Uemura. These gestational ages correspond to about days 40 and 50 of canine ophthalmic development. Vitreous fibrils in the retrolental region were 10–20 nm in diameter and shorter than in adults. A distinct periodicity of these fibrils was not observed. Electron-dense material was associated with the fibrils. On the other hand, the collagen-like fibrils in postnatal human eyes with PHPV had diameters of 40–50 nm and banding patterns with a periodicity of about 65 nm. Besides this type of fibril, other nonstriated types of various diameters were observed.
Fig. 13-18. Fig. 13. Tight junctions (Ju) between two capillary endothelial cells at D37. Collagenous fibrils (Col) are present in proximity to the capillary's basal lamina (arrows). Fig. 14. Survey micrograph of the retrolental plaque at D44. A TVL-capillary is present against the lens capsule (LC). Subcapsular “vacuoles” (Vac) are a sign of cataract. (Lu = capillary lumen, F = fibroblast). Fig. 15. At D44, the retrolental plaque consists of flat, slender fibroblasts (F) and a randomly oriented, electron-dense fibrillar matrix. Some cells contain clusters of melanin granules (arrows). Fig. 16. Survey micrograph of the posterior part of the retrolental plaque at D44. The cells of which the plaque consists are situated closely together. The vitreous cell located furthest from the plaque (arrow) has the characteristics of a mononuclear phagocyte. The vitreous cell lying closest to the plaque has an ovoid shape (see also Fig. 18). Fig. 17. The plaque at D44. Clusters of melanin granules (arrows) are present in the cytoplasm. Fig. 18. Higher magnification of one of the vitreous cells of Figure 16. The cytoplasm contains numerous vacuoles (Vac), indicating phagocytic properties (see also Fig. 16).

Although the described differences between normal fetal and abnormal postnatal vitreous fibrils may have been caused by developmental differences, the faintly periodic fibrils on days 35 and 37 we describe had diameters of about 30 nm, which is considerably thicker than described for normal fetal human vitreous. There have been no reports that may serve as reference for evaluating canine vitreous collagen fibrils.

Fig. 19–21. Fig. 19. Plaque at D44, showing a cell resembling a Müller cell of the inner retinal layer and having a large, irregular and multiple indented nucleus (Nu) and irregular cell margins with numerous recesses and pseudopodia (arrows). Fig. 20. Neuroectodermal elements in the retrolental plaque of a PHTVL/PHPV-affected canine fetus at D44. Numerous bundles of filaments (Fi) are present. (Nu = nucleus). Fig. 21. Part of a conglomerate of fibrous astrocytes. A thin basal lamina (arrows) surrounds the complexes. A capillary of the TVL and fibrillar material (FM) are present in the vitreous. (Nu = nucleus).
brils to our knowledge. It may be that PHTVL/PHPV is associated with the production of abnormal collagen fibrils, as has been suggested previously.25

In addition to confirming the results of previous studies on the development of PHTVL/PHPV, our findings contribute new elements to the knowledge of the pathogenesis of this anomaly. The ultrastructural changes of the lens capsule and the posterior subcapsular vacuoles of the lenticular tissue constitute the two most notable findings of our study. In addition, the participation of retinal elements (Müller cells and fibrous astrocytes), and probably also vitreous cells, to the formation of the retrolental plaque was established.

Key words: PHTVL. PHPV. cataract. dog. animal model

References