Anterior Polar Cataracts in CS Rats: A Predictor of Mature Cataract Formation

Kristin J. Al-Ghoul1 and J. R. Kuszak1,2

PURPOSE. The objective of this study was to characterize the morphology of the anterior opacities formed during recovery from posterior subcapsular cataract (PSC) in Royal College of Surgeons (RCS) rats.

METHODS. Lenses from RCS rats at 8 and 12 weeks postnatal (n = 14 and 12, respectively) were examined under a dissecting microscope for the presence of anterior opacities. Lenses with anterior opacities were fixed, embedded in epoxy resin, and sectioned along the optic axis for light microscopy (LM) and transmission electron microscopy (TEM).

RESULTS. At eight weeks postnatal, 21.5% of animals (3/14) had anterior cataracts. Light microscopy of 1- to 2-μm-thick sections revealed an anomalous layer of material located at the epithelium-fiber interface, which was identified as a zone of liquefaction by TEM. Epithelial cells had minor structural defects but were not necrotic. Anterior portions of elongating and cortical fibers under the zone of liquefaction were undisturbed, whereas their posterior portions had numerous vacuoles. The anterior opacities were classified as anterior polar cataracts (APCs) based on the location and type of morphologic damage in the affected lenses. At twelve weeks postnatal, 25% of animals (3/12) had APCs that involved prominent vesiculation of the anterior cortex. Ultrastructural examination showed that large vesicles were located between and inside anterior fibers and that most extracellular spaces were abnormally widened. Posteriorly, internalization of the PSC by new fiber growth was disordered and displayed vesiculation and density variations. In the bow region, LM revealed minor structural irregularities that were identified as groups of apparently degenerating fibers by TEM.

CONCLUSIONS. APCs in RCS rats are caused by degeneration of elongating fibers in the bow region and subsequent damage in the superficial anterior cortex. The percentage of animals with APCs (25%) was consistent with the percentage of animals in which mature cataracts eventually develop. The morphologic changes, time of onset, and percentage of animals affected suggest that APC is the initial manifestation of mature cataract formation in RCS rats. (Invest Ophthalmol Vis Sci. 1999;40: 668-679)

The Royal College of Surgeons (RCS) rat is an animal model for autosomal recessive retinitis pigmentosa. The retinas of RCS rats spontaneously degenerate between 2 and 6 weeks postnatal because of an autosomal recessive gene defect that affects the phagocytic capacity of the retinal pigment epithelium.1,2 In addition, bilateral posterior subcapsular cataracts (PSCs), which appear as grainy posterior opacities by slit lamp examination, develop in these animals from 4 to 6 weeks postnatal.3,4 The rapid retinal degeneration and concomitant development of PSCs make the RCS rat an excellent model for PSC associated with autosomal recessive retinitis pigmentosa.

An interesting aspect of the RCS rat model is that subsequent to PSC formation, approximately 75% of animals "internalize" the PSCs through growth of new lens fibers. In the remaining 25% of animals, mature cataracts develop by 9 to 12 months of age.5 In a recent investigation in which RCS rat lens ultrastructure was characterized during recovery from PSC, it was determined that internalization of the plaque begins between 8 and 10 weeks postnatal.5 During the initial stages of recovery (8–12 weeks postnatal) it was noted that some lenses had developed what appeared to be anterior subcapsular cataracts (ASCs) successive to the formation of PSCs.

Typically, ASC formation is considered to be caused by metaplasia of the epithelium, producing capsular wrinkling and a fibrous plaque over the visual axis.6,7 Because metaplasia of the lens epithelium is not normally associated with retinal degenerative disease, the presence of ASCs in RCS rats is unexpected. The objective of this study was to examine the structure of the anterior opacities that were present in RCS rats during the initial internalization of the preexisting PSCs. We show that the anterior opacities were neither subcapsular nor caused by metaplasia of epithelial cells. Rather, our data show that the opacities were actually anterior polar cataracts (APCs) caused by liquefaction and structural damage in superficial lens fibers.

From the Departments of 1Pathology and 2Ophthalmology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois.

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Reprint requests: Kristin J. Al-Ghoul, Department of Pathology, Rush-Presbyterian-St. Luke's Medical Center, 1653 West Congress Parkway, Chicago, IL 60612.
Materials and Methods

Lenses

RCS rat lenses at 8 and 12 weeks postnatal were used in this investigation. Fourteen animals at 8 weeks and 12 animals at 12 weeks were sacrificed by intraperitoneal injection of sodium pentobarbital. Eyes were enucleated and placed in buffer, and the lenses were dissected from the orbit. Fresh lenses in buffer were examined under a dissecting microscope (Carl Zeiss, Thornberg, NY), and those with anterior opacities were noted and used for ultrastructural characterization of disease. Animals without anterior opacities were used for additional studies and for comparison with those lenses with anterior opacities. All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Fixation and Embedding

Immediately after enucleation and dissection, lenses were fixed for 18 to 24 hours at room temperature (2.5% glutaraldehyde in 0.07 M sodium cacodylate buffer at pH 7.2), then washed overnight in 0.2 M sodium cacodylate buffer. Lenses were examined under a dissecting microscope and photographed, and the axial dimensions were measured. Lenses were either processed whole or were sectioned along the visual axis using a vibrating knife microtome (Lancer, St. Louis, MO). Whole lenses and microtome-prepared sections were postfixed overnight in 1% aqueous osmium tetroxide at 4°C, washed in cacodylate buffer, dehydrated through a graded ethanol series to propylene oxide, then infiltrated and flat-embedded in epoxy resin for LM and transmission electron microscopy (TEM).

LM and TEM

For LM, embedded lenses were bisected along the optic axis with a jeweler's saw and 1- to 2-µm-thick sections were cut using a glass knife. Investigation of the bow region included two to three equatorial locations in each lens. Sections were mounted on glass slides and stained with a dilute mixture of methylene blue and azure II. Light micrographs were taken on
FIGURE 2. Transmission electron micrographs of 8-week-old RCS rat lenses at the central (A) and germinative (B) zones. The usual lens components, capsule (c), epithelium (e), and fibers (f), are easily distinguished. Furthermore, with the greater resolving power of electron microscopy, the anomalous layer seen in light microscopy can be identified as a zone of liquefaction (zl) that contained occasional vesicles and mitochondria (A, <; B inset, <, >). Where the zone of liquefaction borders the epithelial (A, upper inset) and fiber (A, lower inset) cells, only the plasma membranes of adjacent cells were seen, indicating that the zone of liquefaction was not membrane bound. Epithelial cells show minor structural damage including intracellular vesicles (*), enlarged extracellular spaces (A), perinuclear clear zones (↑, ↓), and condensation of nuclear chromatin. Fibers had normal ultrastructure. Because the prominent structural damage was subepithelial rather than subcapsular, the cataracts are most appropriately termed anterior polar cataracts.
FIGURE 2. (Continued).
RESULTS

Eight Weeks Postnatal

In 8-week-old RCS rats, 21.4% of animals (3/14) had an anterior opacity in one or both eyes. The cataracts were superficial, centrally located, opaque, white plaques (Fig. 1A, 1B) that varied in size and shape from lens to lens. Unfixed lenses were clear in the equatorial and nuclear regions.

Light microscopy of polar axial sections revealed that a layer of unknown material was located between the epithelial cells and the anterior ends of elongating fibers (Fig. 1C, 1D, 1E; arrowheads) that was not present in lenses without anterior opacities (data not shown). Typically, this layer was thickest over the anterior pole (Fig. 1C, 1E). Peripheral regions of the anterior surface such as the germinative zone were characterized by a thin, densely stained layer of material beneath the epithelium (Fig. 1D). Underneath the anomalous additional layer, the anterior cortical fibers were intact, and suture planes were normal (Fig. 1C, 1E; arrows). In two lenses with APC, the posterior portions of elongating fibers also showed evidence of degeneration. Specifically, vacuolization of fibers was apparent in those lenses (Fig. 1F).

Ultrastructural examination showed that in animals with anterior opacities, the epithelia of the central (Fig. 2A) and germinative (Fig. 2B) zones were structurally compromised compared with those in age-matched RCS rats without anterior opacification (data not shown). Specifically, minor defects such as enlarged extracellular spaces (Fig. 2A, 2B; arrowheads), intracellular vesiculation (asterisks), perinuclear clear zones (arrows), and nuclear chromatin condensation were apparent. In addition, TEM revealed that the additional layer between the epithelium and elongating fibers was actually a zone of liquefaction. The material in this zone was finely granular, contained small infrequent organelles such as vesicles and mitochondria (Fig. 2A, 2B inset; open arrows), and was not membrane bound. The absence of an enclosing membrane was apparent at high magnification; only the single membrane of adjacent cells was present at the interface of the zone of liquefaction with epithelial (Fig. 2A, upper inset) and fiber cells (Fig. 2A, lower inset). The anterior portions of elongating (Fig. 2A, 2B) and mature fibers underneath the liquefied zone displayed normal ultrastructure. Because the significant structural damage was located beneath the epithelium (rather than beneath the capsule), the opacities are most appropriately termed anterior pole cataracts (APCs).

Twelve Weeks Postnatal

In 12-week-old RCS rats, 25% of animals (3/12) had APCs. The cataracts were bilateral and macroscopically indistinguishable from the opacities noted in specimens at 8 weeks postnatal. However, LM of thick sections showed that the
anterior cortex was now affected (Fig. 3A, 3B). Specifically, vesiculation was apparent among the mature cortical fibers.

Ultrastructural examination of mature fibers revealed that the extracellular space was greatly distended in some places (Fig. A, asterisks), resulting in the vesiculated appearance seen in light micrographs. In general, most cell interfaces displayed widening of the extracellular space, particularly at fiber interdigitations such as edge processes (Fig. A, arrows). Slight widening of extracellular spaces was noted between elongating fibers, but large distentions or extracellular vacuoles were not seen (data not shown).

In lenses with and without APC, the posterior portions of fibers grew over the PSC plaque, internalizing the cataract. However, whereas the lenses without APC had relatively normal posterior fiber growth, as previously shown, \textsuperscript{3} lenses with APC displayed compromised growth over the PSC (Fig. 4B, 4C). In addition to vesiculation (Fig. 4B, arrowheads) and density variations seen by LM, TEM showed that the fiber organization was markedly disordered (Fig. 4C). Specifically, superficial elongating fibers were not oriented at a slight angle (20–30°) to the capsule, nor were fibers in previous growth shells arranged in ordered radial cell columns. \textsuperscript{9}

**Morphology of the Bow Region**

RCS rat lenses with and without APC were clear in the equatorial region. Light microscopy of the bow region revealed that lenses with APC (Fig. 5) exhibited minor structural abnormalities. Specifically, darkly stained elongating fibers (Fig. 5A, 5B; arrowheads) projecting toward the anterior zone of liquefaction (Fig. 5A, 5B; arrows), and clusters of nuclei (Fig. 5A, 5C; brackets) were observed in some lenses. Several lenses also displayed crescent-shaped areas of light-staining transitional cells (Fig. 5A, 5C, 5D; open arrows), adjacent to darker-stained, nascent fibers. It is important to note that the presence of these structural abnormalities was highly variable between lenses and within individual lenses. For example, Figure 5B, which shows two equatorial locations in the same lens, indicates a structurally normal bow on the left and a bow with darkly stained elongating fibers on the right. Whereas lenses with APC displayed structural variability in different equatorial locations, lenses without APC had normal bow structure in all regions examined and were comparable with that shown in Figure 5B (left image).

Ultrastructural examination of lenses with APC showed that the crescent-shaped areas seen by LM were composed of apparently normal anteriorly elongating transitional cells (Fig. 6A). However, the adjacent bidirectionally elongating fibers were structurally compromised and exhibited features consistent with degenerating cells (Fig. 6A, 6B). Specifically, the nascent fibers displayed prominent vesiculation (Fig. 6B, asterisks), perinuclear clear zones (Fig. 6B, arrowheads), and an obvious difference in cytoplasmic staining density in comparison with that of the adjacent transitional cells. The anterior ends of abnormal elongating fibers terminated adjacent to the zone of liquefaction (Fig. 6C, 2l). In addition, the material in the zone of liquefaction and the cytoplasm of abnormal fibers had similar staining densities.

**DISCUSSION**

This is the first study in which the structure of APC has been identified and characterized in RCS rats. It is apparent that RCS rat APCs are not structurally similar to most ASCs seen clinically. In humans, ASC formation has been attributed to the metaplasia of central zone epithelial cells into myofibroblast-like cells that produce excess extracellular matrix accumulations (including collagen and alpha smooth muscle actin) resulting in a fibrous subcapsular plaque. \textsuperscript{10–14} In contrast, APCs in RCS rats were characterized by a subepithelial zone of liquefaction and subsequent anterior cortical vesiculation. Epithelial cells displayed only minor structural damage; no evidence of metaplasia of epithelial cells or production of extracellular matrix components was detected. These data indicate that structural damage to fibers was responsible for the APCs in RCS rat lenses rather than metaplasia.

Eight weeks postnatal, the anterior opacification was caused by the presence of a layer of liquefied material between the epithelium and the intact fibers. The presence of abnormal, apparently degenerating fibers in the bow region suggests that the zone of liquefaction was derived from the breakdown of nascent fibers. Additionally, abnormal elongating fibers were not seen in all equatorial locations, implying that groups of degenerating fibers were distributed discontinuously around the lens equator. Because the epithelium did not display evidence of necrosis, epithelial cells did not contribute significantly to the zone of liquefaction. By 12 weeks postnatal, fibers deeper in the anterior cortex were structurally compromised, presumably contributing to the anterior opacity.

It is well established that mature cataracts eventually develop in approximately 25% of RCS rats. \textsuperscript{4} In the present investigation, subsequent to PSC formation, APCs developed in a comparable percentage of animals, clearly as a result of fiber breakdown and damage. In addition, the posterior cortical fibers of animals with APC often displayed evidence of structural damage. The morphologic changes, time of onset, and percentage of animals affected suggest that APC formation in RCS rats is an accurate predictor that a mature cataract will develop, rather than effecting a recovery by internalization of the PSC.

Although PSC is the cataract type most commonly associated with retinal degenerative disease, "ASCs" occurring in addition to the PSCs have been noted clinically. \textsuperscript{15–17} Morphologic changes, including prominent vacuolization in the epithelium and anterior fibers\textsuperscript{15–17} and areas of complete fiber breakdown and liquefaction\textsuperscript{15} were noted in "ASCs" from patients with retinitis pigmentosa. In addition, the case histories of these patients indicated that PSC developed before anterior involvement. The structural changes and the temporal sequence of cataract formation noted above indicate that the anterior opacities of patients with retinitis pigmentosa are more consistent with the APCs of the RCS rat than with ASCs of other causes. If, as our data suggest, the presence of an APC is the first manifestation of mature cataract formation, then it may be an important indicator for the appropriate clinical treatment of patients with retinal degenerative disease.
FIGURE 4. Ultrastructure of 12-week-old RCS rat lenses. (A) Transmission electron micrograph of the mature anterior cortical fibers showing that vesiculation was caused by large distensions of the extracellular space (*). Widening of the extracellular space is also apparent at fiber interdigitations (†, ‡). Light (B) and transmission electron (C) micrographs of posterior fiber growth over the preexisting posterior subcapsular cataract in the same lens as (A). Boxed area in (B) indicates location of thin-section mesa. Low-magnification overview (B) shows vesiculation (▲, ▼) and density variations between fibers, whereas higher magnification (C) reveals that fiber organization was markedly disordered.
FIGURE 4. (Continued)
Figure 5. Light micrographs of the bow region in 8-week-old (A, B, C) and 12-week-old (D) RCS rat lenses with anterior polar cataract. The micrographs in (B) depict two equatorial locations in the same lens. Although some bow regions appeared structurally normal (B, left image), most bow regions examined displayed atypical variations in staining density. These included dark-staining anterior portions of elongating fibers (•, •) projecting toward the zone of liquefaction (→) and crescent-shaped areas of light-staining transitional cells (○, ○) bordered by darker staining, nascent fibers. Occasionally, clusters of nuclei in nascent fibers were present (brackets). Magnification is identical in (A) through (D).
FIGURE 6. Transmission electron micrographs of the bow region in RCS rat lenses with anterior polar cataract. (A) Low-magnification montage of the transitional cells and nascent fibers in the bow region of an 8-week-old animal. The transitional cells (tc) appear intact and have homogeneous, light-staining cytoplasm in comparison with the nascent fibers (nf). (B) Ultrastructure of the abnormal nascent fibers in the same lens as (A). Note the prominent cisternae of the endoplasmic reticulum (※) and the perinuclear clear zones (△, △, △) indicative of degenerating fibers. (C) Anterior ends of abnormal fibers depicted in (B). The elongating fibers terminate at the zone of liquefaction, which is presumably derived from the breakdown of degenerate fibers. (Fig. 6 continued on next page.)
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References