Retinal Pigment Epitheliopathy and Neuroretinal Degeneration in Ascarid-Infected Eyes

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The generalized choroidal and retinal response to a focal nonreplicating infection of the eye with ascarid larvae was examined in an animal model. Intravitreal injection of Ascaris suum larvae in guinea pigs induced a diffuse eosinophilic choroiditis, retinal pigment epitheliopathy and neuroretinal degeneration, distant from focal reactions about larvae. As the choroiditis progressed, inflammatory cells separated the choriocapillaris from Bruch’s membrane, and the endothelial cells lost their fenestrations. Focal disruption of the elastic and outer collagenous layers of Bruch’s membrane occurred, but inflammatory cells rarely invaded the retina. Progressive generalized degenerative and proliferative RPE changes produced a multilayered RPE with loss of cell polarity, RPE basal infoldings and apical microvilli, formation of multiple giant cystic spaces, and proliferation of subretinal fibroblasts. Early loss of photoreceptor outer segments progressed to a generalized disruption of the outer neural retina and cystoid retinal degeneration. Eosinophil mediators and alterations of the choriocapillaris may contribute to the generalized progressive retinal degeneration distant from a parasite larva in ascarid-infected eyes. Invest Ophthalmol Vis Sci 28:1583-1598, 1987

Visual loss caused by intraocular infections with helminth parasites, eg, Toxocara canis,1–2 Baylisascaris procyonis,3–5 and Onchocerca volvulus,6 may result either from damage inflicted directly by the parasite, or from the immunopathologic response of the host to the infection. Localized inflammatory reactions to individual parasites, such as the chorioretinal granulomas of ocular toxocariasis or baylisascariasis larva migrans, or the punctate keratitis of onchocerciasis, may be followed by more generalized ocular inflammatory reactions, such as uveitis of Toxocara endophthalmitis, unilateral subacute neuroretinitis syndrome possibly due to Baylisascaris procyonis, or sclerosing keratitis of onchocerciasis.1–6

We have developed a model for ascarid endophthalmitis by intravitreal injection of T. canis or Ascaris suum second-stage larvae in guinea pigs where viable larvae actively penetrate the retina and choroid from the vitreous.7–10 Intraocular IgE antibodies were present, and uveal mast cell degranulation was maximal, by 7 days after a primary intraocular infection.7–9 Eosinophils rapidly infiltrated the uvea after 6 days, and the resulting inflammatory reaction included both focal eosinophilic granulomatous reactions around larvae, and a generalized dense uveal eosinophil infiltrate distant from the localized nonreplicating infectious agent.8 This paper describes the ultrastructure of the proliferative and degenerative changes of the retinal pigment epithelium and neural retina that are associated with the generalized eosinophilic choroiditis distant from the focal reactions about ascarid larvae.

Materials and Methods

Hartley strain female guinea pigs (500–600 g) were obtained from Skippack Farms (Skippack, PA). All animal procedures were performed following the guidelines of the ARVO Resolution on the Use of Animals in Research. Infective eggs of A. suum were suspended in sterile 0.15 M saline at 37°C and gassed with CO₂ for 30 sec. A few (20 to 30) small glass beads were added, and the preparation was rotated at 37°C for 30 min. Live second-stage larvae (L2) were separated from eggshells and other debris by the Baermann technique, where the suspension was layered over a 6 mm thick cotton pad and the active larvae migrating through the pad were collected underneath.7–10 Larvae were concentrated by sedimentation, washed repeatedly in sterile saline, incubated for 30 min in saline with penicillin (10³ U/ml), strepto-
mycin (1.3 mg/ml), and nystatin (250 U/ml), and washed two additional times in sterile saline. All preparations used in the present study contained 95% to 98% viable (motile) larvae. Freshly hatched *A. suum* L2 (300–3,000 L2 in 0.1 ml sterile 0.15 M saline) were injected through the pars plana into the vitreous under ether inhalation anesthesia, using a 30 gauge needle. Motile larvae within the vitreous were visualized with a direct ophthalmoscope. Each guinea pig eye received only one intravitreal injection. Guinea pigs were sacrificed 7, 9, 13, 20, 27 or 32 days after infection and the eyes (ten eyes for each time period) were immediately enucleated and processed. Control eyes were obtained from weight-matched female Hartley guinea pigs which were not injected, or from infected guinea pigs which had only sterile saline injected intravitreally in one eye.

Eyes were divided close to the ora serrata. The posterior segment was divided into smaller pieces, placed in 2.5% glutaraldehyde (Electron Microscopy Sciences, Fort Washington, PA), 1 mM calcium chloride, 64.5 mM sodium cacodylate buffer, pH 7.4, and

![Fig. 1. Subretinal ascarid larva and dense accumulation of eosinophils in the choroid 13 days after intravitreal infection (Methylene blue-azure II stain, magnification ×371).](image-url)
fixed on ice for 30 min and then at room temperature for 1 hr. Specimens then were placed in two changes of cacodylate buffer with 45 mg/ml sucrose for at least 1.5 hr. Tissues were postfixed in 2% cacodylate-buffered osmium tetroxide over ice for 1 hr, dehydrated with graded ethanol and propylene oxide, infiltrated in 40% propylene oxide and 60% Epon 812 or Embed 812 (Electron Microscopy Sciences) and polymerized at 60°C for 36 to 48 hr. Thick (1 μm) sections from Epon 812 or Embed 812 blocks were stained for light microscopy with methylene blue-azure II or Luna's eosinophil stain. Thin (70 nm) sections were stained with uranyl acetate and lead citrate, and examined with a JEOL 100-B electron microscope (JEOL USA Inc., Peabody, MA). Selected enucleated infected and control guinea pig eyes were fixed in 4% formaldehyde, 2.5% glutaraldehyde, pH 7.4, processed and embedded in paraffin, and 7 μm sections were stained with Luna's eosinophil stain. Ten paraffin-embedded eyes, removed 27 or
32 days after infection, were serially sectioned to enumerate the larvae remaining within the eye.

Results
A dense eosinophil infiltrate in the choroid adjacent to an ascarid larva which has penetrated the retina is shown in Figure 1. The vast majority of the intravitreally-administered larvae migrated from the eye and were found elsewhere. A maximum of seven ascarid larvae was found within a single eye on serial sectioning. Many eyes retained only a single or no subretinal larvae. A dense generalized eosinophilic chorioiditis, not restricted to regions adjacent to a parasitic larva, developed by 9–13 days (Figs. 2, 3A, 4A). Plasma cells and other lymphoid cells, but only an occasional polymorphonuclear leukocyte, were present in the generalized uveal infiltrates. There was significant variation in the magnitude of the pathologic changes at a given day post-infection in eyes receiving identical larval injections, and some tendency for the severity of the choroidal inflammatory reaction to correlate with the number of parasites.
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Fig. 3B. Degenerating and proliferating RPE forming a multilayered structure interlaced with fibroblasts and cells which may represent transitional stages between RPE cells and fibroblasts (20 days post-infection, magnification x965).

Transmission electron microscopy of choroidal eosinophils showed a large variation in granule size and shape, irregular granule core duplication, changes in the electron density of granule cores and matrices, scroll-like lamellar inclusions, marked reduction in the number of eosinophil granules, or cytoplasmic vacuolization (Figs. 5C, D), findings indicative of eosinophil activation. Some choroidal eosinophils had been phagocytosed by activated macrophages, and showed progressive degenerative changes (Fig. 6A).

retained in an eye was noted. However, severe generalized eosinophilic choroiditis occurred repeatedly in eyes retaining only a single subretinal larva, and in eyes with one or more larva in the anterior segment but none in the choroid or retina. Limited changes in the lenses occurred in some eyes but showed no correlation with the RPE and neuroretinal changes. There was a clear correlation between the magnitude of the generalized eosinophilic choroiditis and the RPE and neuroretinal changes.
Eosinophils located beneath areas of advanced retinal pigment epithelial (RPE) degeneration often were partly degranulated (Fig. 3D). Choroidal mast cells showed changes of degranulation (Fig. 6B).

As the generalized choroiditis progressed, inflammatory cells (frequently eosinophils) infiltrated between the choriocapillaris and Bruch's membrane, often widely separating these vessels from Bruch's membrane (Fig. 4A). The separated choriocapillaris frequently was widely dilated and engorged with blood, and the endothelial cells had thickened, vacuolated cytoplasm. On electron microscopy, inflammatory cells were seen to infiltrate between the elastic and endothelial basement membrane layers of Bruch's membrane, or between endothelial cells and their pericapillary basal laminae (Figs. 5B, C, D). The normal close approximation of the pericapillary basal lamina to the choriocapillaris endothelium also was distorted focally by increased periendothelial extracellular material, including collagen (Fig. 5B). In some regions, the basement membrane was reduplicated (Fig. 5B). The cytoplasm of the thickened endothelial cells showed evidence of activation, with increased polyribosomes and mitochondria. These vessels lacked the fenestrations of the normal guinea pig choriocapillaris and had a continuous type of thickened endothelial wall (Fig. 5B).

Progressive degenerative and proliferative changes were seen in the retinal pigment epithelium overlying the generalized eosinophilic choroiditis, with early cytoplasmic inclusions, micro- and macro-cystic degeneration, proliferation with the formation of multilayers, and loss of the normal RPE cellular polarity and architecture (Figs. 2, 3A, B, 4A). Transmission electron microscopy of the RPE of normal guinea pig eyes showed prominent basal infoldings. The thicker basal portion of the apical microvilli contained many electron-dense membrane-bound pigmented gran-
Guinea pig eyes infected with ascarid larvae displayed multiple giant vacuoles or cystic spaces within RPE cells by 20 days post-intravitreal infection (Figs. 5B, 7A, B). Coalescence of smaller RPE vacuoles and disruption of intercystic septi resulted in giant cystic space formation. Multiple double-membrane villous processes of varying length projected into the larger RPE cystic spaces and fine filaments were present within these vills. There were varying degrees of vacuolization of RPE mitochondria, and disruption of mitochondria leading to the formation of small cystic spaces. Double-walled ring-like membranous structures were present both in distended mitochondria and larger cystic spaces (Fig. 5B). Some RPE cells contained large intracytoplasmic lipofuscin-like granules. Organelles such as mitochondria, Golgi apparatus, rough endoplasmic reticulum and ribosomes were seen within the broader intercystic ridges. Electron-dense membrane-bound pigmented granules were present within some degenerating RPE cells.

Extensive focal or generalized loss of the RPE basal infoldings, and partial or complete loss of apical microvilli where characteristic of the degenerating RPE cells (Figs. 5B, C, D). Proliferation of the RPE, with loss of terminal bars and cell polarity, produced areas of multilayered RPE. The RPE cells became more spindle shaped and migrated into the subretinal space (Figs. 3A, B, 4A). Elongated spindle-shaped cells in the subretinal space contained electron-dense membrane-bound pigmented granules (Figs. 7A, B) similar to the apical cytoplasmic inclusions present in the normal guinea pig RPE (Fig. 5A). An extracellular electron-dense basement membrane-like fibrillar material was present about proliferating and degenerating RPE cells in the subretinal space (Figs. 7A, B). By day 20, the subretinal space of some infected eyes was filled with multiple spindle-shaped cells resembling fibroblasts or myofibroblasts, possibly of RPE origin. The myofibroblast-like cells had abundant rough endoplasmic reticulum, multiple intercellular connective.
Fig. 5. Transmission electron microscopy of normal eye, and of ascarid-infected eyes distant from a parasite larva. (A) Photoreceptor outer segments, retinal pigment epithelium (RPE) and choriocapillaris (CC) of a normal guinea pig eye. Note apical pigment granules (PG) and extensive basal infoldings (BI) of the RPE. Large RPE cytoplasmic inclusions (I) were seen occasionally in eyes from normal guinea pigs (magnification x10,800).

Changes in the neural retina were noted by 9 days, with early loss of photoreceptor outer segments overlaying areas of early cystic or proliferative RPE changes and choroid heavily infiltrated with eosinophils (Fig. 2). The outer segment destruction led to the direct apposition of the inner segments to the hyperplastic retinal pigment epithelium. The inner retina was well preserved at this time. Later, there was increasing loss of retinal structure and organization, especially affecting the outer retina. In a few eyes, there was marked cystoid degeneration of the neural retina (Fig. 4B).

Discussion

The pronounced generalized eosinophilic chorioiditis distant from focal reactions about parasites may reflect the effects of a number of diffusible eosinophilotactic agents. Intraocular IgE antibody production and choroidal mast cell degranulation have been shown to occur in these guinea pig eyes. IgE-producing plasma cells and IgE immunoglobulins have been identified in human eyes presumably infected with *Toxocara canis*. Degranulation of mast cells and basophils release a number of eosinophil cytotoxins, eg, eosinophil chemotactic factor of anaphylaxis (ECF-A), intermediate-molecular-weight...
Fig. 5 B. Cystic degeneration and loss of basal infoldings (BI) of retinal pigment epithelium (RPE), 20 days post-intravitreal infection. The underlying choriocapillaris (CC) is displaced by an inflammatory cell (IC) infiltrating within the outer collagenous zone of Bruch’s membrane between the elastic layer (EL) and the outer basement membrane layer (arrows) of the choriocapillary endothelium. The endothelium also is separated from the endothelial basement membrane layer of Bruch’s membrane by increased extracellular material and an inflammatory cell process. The displaced choriocapillaris has lost its normal fenestrations. There is discontinuity of the elastic layer of Bruch’s membrane over open phagocytic vesicles (V) in the inflammatory cell cytoplasm, which contain engulfed elastin and collagen (magnification ×16,200).

Factors and histamine. Activation of complement by antigen-antibody complexes releases other diffusible eosinophilotactic factors (eg, C3a, C5a, C567, C5b). Thymus-derived (T) lymphocytes can produce additional eosinophil chemotactic and chemokinetic lymphokines (eg, eosinophil chemotactic factor precursor, eosinophil stimulation promoter) when activated by antigen. Helminth parasites also may produce eosinophilotactic substances or may directly cause degranulation of mast cells. These chemokinetic and chemotactic factors may diffuse throughout the uvea from focal immunologic reactions occurring about individual parasites. Alternately, the migration of parasites through the eye may leave behind deposits of antigenic materials which are not readily identified on routine microscopy.

The eosinophilic choroiditis was associated with marked progressive morphologic changes of the retinal pigment epithelium. Flattening of the normal prominent basal infoldings and loss of apical microvilli were noted early. Multiple cystic formations within the RPE, resulting in a “Swiss cheese” pattern,
occurred subsequently. The presence of double-walled ring-like inclusions in some giant cystic spaces, similar to those found in swollen mitochondria, suggested that the cystic spaces may be of mitochondrial origin. However, mitochondria with relatively normal morphology were observed in some of the inter-cystic septi. These giant vacuoles could be the in vivo correlate of the vacuoles which we have observed in primary cultures of guinea pig and cat RPE during the attachment phase prior to cell division (J. J. Donnelly, L. E. Stramm, M. Khatami and J. H. Rockey, unpublished observations). RPE changes were marked in areas overlying "activated" eosinophils, suggesting that the changes were secondary to toxic diffusible mediators released by eosinophils within the choroid (eg, eosinophil granule major basic protein, eosinophil cationic protein, eosinophil-derived neurotoxin, peroxidase/superoxide anion, collagenase, leukotrienes, prostaglandins).18-29

The outer collagenous zone and endothelial basement membrane layers of Bruch's membrane were focally disrupted in later stages of the ocular ascariid infections by eosinophils and other choroidal inflammatory cells, including degranulated eosinophils, which infiltrated between the elastic layer and the choriocapillaris basement membrane. The elastic layer overlying choroidal inflammatory cells also was partially disrupted. In a guinea pig model of vernal conjunctivitis, eosinophils densely infiltrated the conjunctival epithelium and were associated with
areas of epithelial hypertrophy, thinning, or epithelial cell loss. In contrast, in the eosinophilic choroiditis of the ascarid-infected eyes, Bruch's membrane largely prevented eosinophil infiltration into the RPE and retina.

The reactive process of the RPE varies in different pathologic conditions. The RPE may show simple proliferation as in the pigmented macular lesion in myopia (Fuch's spot); proliferation with formation of cuticular masses, as in drusen; proliferation with fibrous metaplasia, as in senile macular degeneration; proliferation with calcification and bone formation; migration and phagocytosis; pseudopitheliomatosus hyperplasia; or true neoplasia. New bone formation has been noted in guinea pig eyes chronically infected with ascarid larvae. In the present model, the RPE showed diffuse simple proliferation followed by migration beneath the outer retina. The inner RPE cells became more spindle-shaped and may have transformed into the fibroblasts or myofibroblast-like cells observed subretinally. Fibrous connective tissue may arise from the RPE by a process of metaplasia, with continued basement membrane formation and persistence of intercellular junctions. Deposition of basement membrane-like material between the degenerating and proliferating RPE cells and fibroblasts was a prominent feature in the ascarid-infected guinea pig eyes.
Obliteration of the choriocapillaris may cause degeneration and hyperplasia of the RPE in areas adjacent to the occluded vessels. In the present experimental model, even in the presence of a dense eosinophilic choroiditis, the choriocapillaris and the larger choroidal vessels in general remained patent and were engorged with blood. However, the choriocapillaris frequently was separated from the inner components of Bruch's membrane by an infiltration of eosinophils and other inflammatory cells. The displaced choriocapillary endothelial cells were thickened, vacuolated and showed evidence of increased metabolic activity, and the normal fenestrae were lost. Destruction of rabbit RPE by sodium iodate may lead to choriocapillaris atrophy and loss of fenestrations, and it has been postulated that a diffusible vascular modulating factor produced by the RPE cells locally modulates choriocapillaris structure (eg, fenestrae formation). In the ascarid-infected eyes, damage to the RPE by inflammatory mediators (eg, eosinophil fac-
Fig. 6 B. Choroidal mast cell with multiformed degranulating granules and cytoplasmic filopodia (F) (9 days post-infection, magnification X25,200).

Tors) may have led to a decreased production of RPE vascular modulating factor and secondarily to the loss of choriocapillaris fenestrae. Alternately, the separation of the choriocapillaris from Bruch's membrane by inflammatory cell infiltration may have interfered with the delivery of the RPE vascular modulating factor. Interference with the delivery of nutrients, oxygen and metabolites between the displaced and abnormal choriocapillaris and the RPE may have been a significant factor in the progressive RPE degeneration.

Infiltration of the guinea pig choroid with eosinophils, and degenerative and proliferative changes in the RPE, were correlated with early photoreceptor outer segment loss followed by outer neural retinal degeneration. The inner retina usually remained fairly well preserved throughout much of the study. Since inflammatory cells penetrated the retina infrequently or not at all in the early stages when an extensive eosinophilic choroiditis was associated with a relatively normal appearing choriocapillaris but significant photoreceptor outer segment loss, toxic me-
mediators released by the choroidal eosinophils may have been responsible for the early photoreceptor outer segment loss and retinal degeneration. The guinea pig retina is nourished principally by the choroidal circulation and lacks a significant retinal vascular circulation. The anatomical structure of the guinea pig posterior segment allows the effects of inflammatory cell infiltration and vascular changes in the choroid to be isolated from the consequences of the penetration of the retina by inflammatory cells emerging from retinal vessels and retinal vascular changes. The coincidence of choroidal eosinophil infiltration and activation with RPE and photoreceptor changes suggests a causal role for the eosinophils in these processes. Interference with the transport of nutrients and oxygen from the displaced and altered choriocapillaris through the proliferating and degenerating RPE to the retina also may have contributed significantly to the later progressive neuroretinal degeneration.

The present studies show that a localized infection with a nonreplicating agent may induce pathologic retinal changes at a distance. The ability of eosinophil mediators to act at a distance to produce outer retinal changes distant from a parasite larva. (A) Degenerating and proliferating RPE bordered by two giant cystic spaces 30 days post-intravitreal infection. The cystic degenerating RPE and intervening cells which resemble subretinal fibroblast in cytoplasmic structure contain similar dark inclusions. Dense fibrillar interstitial material is present between degenerating RPE cells and fibroblast-like cells (magnification x9000).
degeneration is potentially of importance in ocular helminthiases where generalized retinal damage occurs. For example, the systemic reaction to diethylcarbamazine treatment in onchocerciasis (Mazzotti reaction), which results in release of eosinophil-derived neurotoxin and major basic protein, may also produce retinal pigmentary changes and visual field loss. The diffuse unilateral subacute neuroretinitis (DUSN) syndrome, possibly the result of ocular baylisascariasis, may also be due in part to the dissemination of toxic eosinophil mediators. Our studies emphasize the importance of the eosinophil not only in the localized reactions to the intraocular parasites but also in the generalized posterior segment disease, and form the basis for further in vivo and in vitro (ie, tissue culture) investigations of the potential role of eosinophil mediators in ocular helminthic diseases.

**Key words**: ascarid, eosinophil, choroiditis, pigment epitheliopathy, neuroretinal degeneration

**References**

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