

# Uveitis Caused by Cytotoxic Immune Response to Cutaneous Malignant Melanoma in Swine: Destruction of Uveal Melanocytes during Tumor Regression

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In a substrain of Sinclair miniature black swine, bred for increasing incidence of cutaneous malignant melanomas, tumor regression occurs spontaneously and is accompanied by depigmentation of the skin, hair, and eyes. We conducted a 12-month longitudinal study of the ocular phenomena in 30 swine beginning at 3 weeks of age. The clinically observed sequence of depigmentation of the fundus and iris was correlated with histopathologic changes in selected enucleated eyes. Normal melanocytes of the uveal tract are destroyed between the 4th and 16th week of life. Melanocyte destruction is preceded by an invasion of the uveal tract by mononuclear cells having the ultrastructural features of lymphocytes and monocytes. Melanin and other cellular debris of ruptured melanocytes are ingested by macrophages which then migrate to the walls of blood vessels. Cataracts and band keratopathy develop secondary to the uveitis in some animals. Pilot electroretinograms show diminished electrical activity in photoreceptors of totally depigmented eyes possibly indicating ischemic or toxic damage to the retina. The retinal pigment epithelium remains essentially normal during the acute stages of uveal inflammation; later some damage and reparative hyperplasia may occur.

The death of normal uveal melanocytes that occurs during the systemic attack on the cutaneous malignant melanomas appears to be an "innocent bystander" error in the immune recognition mechanism. The antigenic basis of this immunologic cross reaction is under investigation. *Invest Ophthalmol Vis Sci* 24:1063-1069, 1983

A substrain of black miniature swine having cutaneous malignant melanoma has been inbred at the University of Missouri's Sinclair Research Farm so that 54% of animals are now born with tumors and 85% have tumors by 12 months of age.<sup>1</sup> Unlike humans with cutaneous malignant melanoma wherein mortality rates of 20-30% are common,<sup>2</sup> the swine rarely (5-10%) die from this disease. Instead, spontaneous tumor regression occurs over a period of several months, and curiously, the tumor regression is accompanied by depigmentation of hair, skin, irides,

and fundi. Some animals exhibit blind behavior after ocular depigmentation.<sup>3</sup>

We have conducted a longitudinal study of 30 of the melanomatous animals using clinical, histopathologic, and electrophysiologic techniques. We have correlated the phenomena of tumor regression and depigmentation with simultaneous invasion of the uveal tract by mononuclear cells. Also, we have found that visual impairment is caused by multiple factors including reduction in transparency of ocular tissues and decrease in photoreceptor function.

## Materials and Methods

### Clinical Examinations

Thirty Sinclair swine were examined every 2-4 weeks under general anesthesia with halothane. Pupils were dilated with 1% tropicamide. External eye examinations, slit-lamp examinations with a Kowa portable slit lamp, indirect ophthalmoscopy, and fundus photography were performed at each examination. Fluorescein angiography was performed as

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needed to illuminate the ocular vascular beds and transmission defects.

Coloration of the iris, overall fundus, and superior-temporal quadrant of each eye was graded on a scale of 0 to 4, 4 being normal pigmentation and 0 marked depigmentation. Pigs were followed as early as 3 weeks of age up to 1 year. Records of tumor development and regression were kept. Additionally, 70 other pigs were given fundus examinations to look for possible choroidal melanomas; none has been found so far.

### Histopathologic Examinations

Enucleations were done randomly and also to select certain pathological stages. In several animals one eye was enucleated at one time and the other at a later stage to follow the sequence of depigmentation. Globes were immersed immediately in aldehyde fixative (2% glutaraldehyde, 1% formaldehyde, in cacodylate buffer pH 7.2). Eyes were bisected in a superior-temporal to inferior-nasal plane. Half eyes were embedded in paraffin and sectioned at 6  $\mu$ m. Sections were stained with Wright's stain, periodic acid-Schiff (PAS), and hematoxylin-eosin (H & E). For Wright's staining the sections were deparaffinized in xylene and rehydrated, stained 3.5 min in diluted (1:1 with H<sub>2</sub>O) Wright's stain solution then destained in 0.1% acetic acid for 15 sec. The other half of selected eyes was cut into small blocks, postfixed in osmic acid, and embedded in epoxy resin for electron microscopy. One-micron thick sections were stained with toluidine blue O. Selected areas of the tissue were thin-sectioned, stained with uranyl and lead salts and photographed in a Philips 300 electron microscope.

### Electroretinography

Electroretinograms (ERG) were obtained using a PS11 Photic Simulator positioned over the dilated pupil, 27 cm above the cornea. A Burian-Allen corneal electrode was used as the positive, the lid retractor as the reference electrode, and a ground electrode was applied to a shaved ear with conducting paste.<sup>4</sup> ERGs were recorded using a Grass amplifier, Dagan averager, and Tektronix oscilloscope and camera.

After dark adaptation for 25 min, the animals were anesthetized with halothane. The pigs were placed on their side with their heads held in the same position for every ERG. Pupils were dilated with 1% tropicamide. Normal saline drops kept the electrode in good contact with the cornea. Light flashes of near maximum intensity were used at 3 Hertz (flashes/sec). Sixteen responses were averaged for each record.

## Results

### Clinical Findings

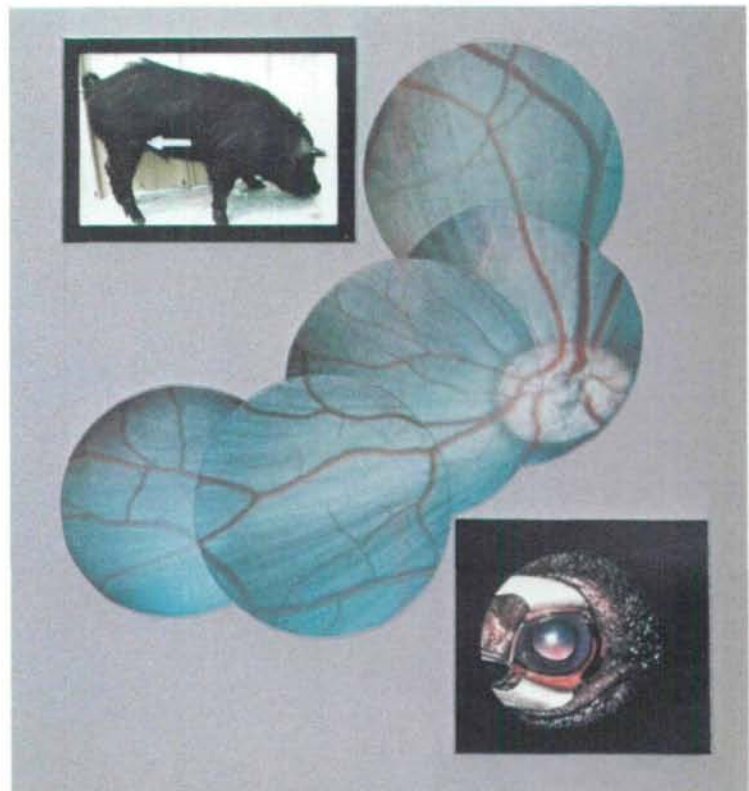
In the normal pig eye the iris is dark brown and the fundus is slate blue (Fig. 1). The superior-temporal quadrant of the fundus was always lighter in very young animals and is therefore considered to be the normal condition. The cornea is egg shaped, and the optic nerve is oval. A specific pattern of depigmentation was observed in the animals undergoing tumor regression. By 2 months of age, 50% of all irides had started to depigment, becoming less than 4+. By 16 weeks 82% of the superior-temporal quadrants and 40% of overall fundi were less than 4+. Fundus depigmentation initially was isolated to the superior-temporal quadrant. Further depigmentation then spread to the rest of the fundus; the orange choroidal vasculature became increasingly visible. Depigmentation patterns varied, being diffuse, tigroid (striped), mottled, in patches, and in fingerprint patterns. Occasionally, white specks were observed in depigmented eyes (Fig. 2); fluorescein angiography identified these star-like spots as pigment epithelial window defects (Fig. 3). The irides and fundi eventually progressed to 0/4 or lack of all normal pigmentation in four animals. In the other 26 animals, degrees of depigmentation varied from very little to almost total depigmentation. Eye and skin depigmentation progressed at varied rates.

Frequently, iris hyperemia developed during iris depigmentation. Occasionally posterior synechiae and cataracts formed. With long-standing inflammation corneal band keratopathy was seen.<sup>5</sup> Clinically, no cells or flare were ever seen in the anterior chamber with a portable slit lamp.

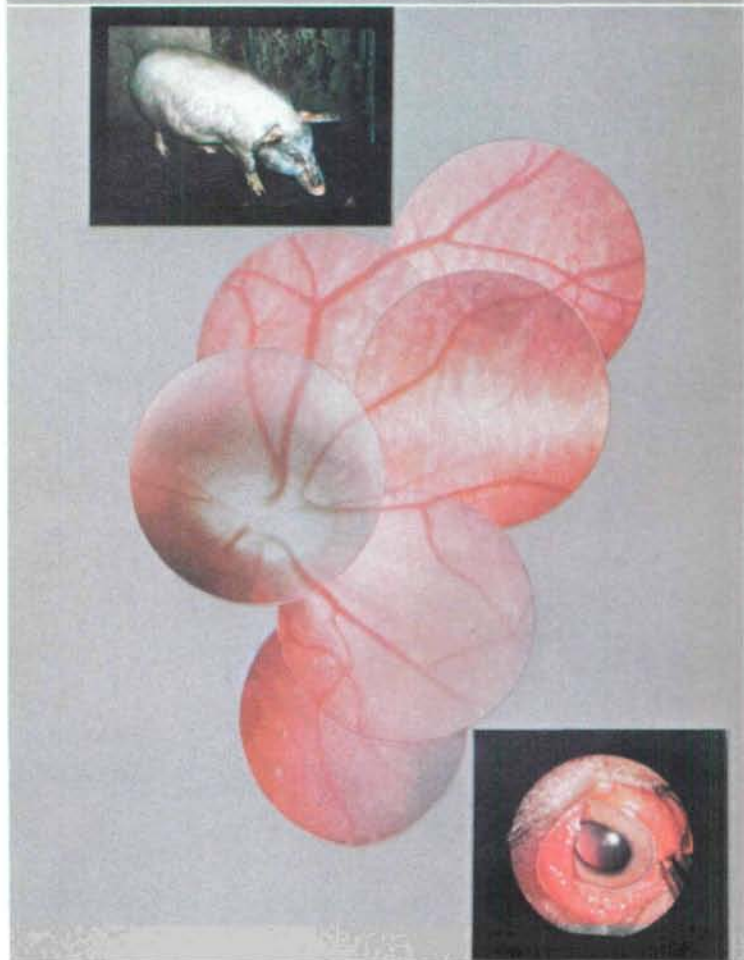
### Histopathologic Findings

The normal Sinclair swine eye is more richly pigmented than the human eye. Specifically, the conjunctiva terminates at the limbus with a ring of multilayered melanin-containing cells, and the sclera, trabecular meshwork, and lamina cribrosa are also heavily pigmented. The uveal tract contains evenly dispersed branching melanocytes throughout the choroid and iris stroma (Fig. 4). The lighter superior-temporal coloration of the normal fundus is due to fewer melanin granules in the retinal pigment epithelium (RPE) in that region. This may be an evolutionary hangover of a tapetal RPE although modern inbred swine have no tapetum lucidum. Other histologic features of the normal swine eye pertinent to this study are the unusually thin delicate Bruch's membrane and the high ratio of cone to rod photoreceptors.

**Fig. 1.** Slate-gray fundus of a young melanomatous pig shown in upper inset (arrow marks melanoma on flank); its normal pigmented iris shown in lower inset.



**Fig. 2.** Fundus of a pig following regression of multiple cutaneous melanomas. Hair and skin are depigmented (upper inset) as is the hyperemic iris (lower inset). Note punctate window defects in RPE (arrows); the fluorescein angiogram and histology of these are in Figures 5-7.



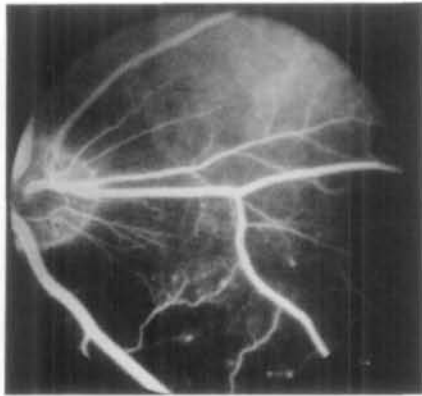


Fig. 3. Fluorescein angiogram (late venous phase) of pig fundus shown in Figure 2. Window defects (arrows) first seen during choroidal flush.

Eyes enucleated during progressive stages of cutaneous tumor regression present a sequential progression of (1) uveal invasion by mononuclear cells, (2) melanocyte destruction, (3) macrophage sequestration of melanocyte cytoplasmic components and (4) migration of engorged macrophages to the walls of choroidal vessels (Fig. 5). This redistribution of melanin from its normal dispersed location within branching melanocytes to round macrophages densely clustered near blood vessels accounts for the lightening of the fundus coloration and its tigroid pattern.

Mononuclear cells that have migrated outside the blood vessels are flattened by connective tissue so that their characteristic morphology becomes distorted, eg, nuclear shape, nuclear-cytoplasmic ratio (Fig. 6). Most of the cells are lymphocytes judging from Wright staining and ultrastructural features (paucity of organelles, monoribosomes). A small percentage of the cells that have more cytoplasm, a larger Golgi complex, and lysosome-like granules may be monocytes (eg, nonphagocytosing mononuclear cells<sup>6</sup>) or early macrophages. No plasma cells have been seen.

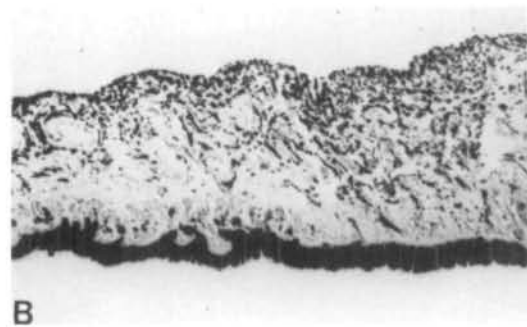
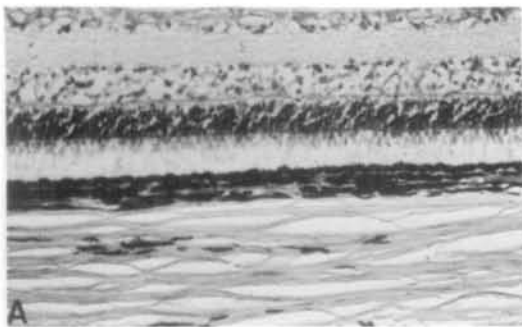


Fig. 4. A, Choroid and retina of normal pig eye. Note evenly dispersed branching melanocytes of the choroid. Paraffin section. Wright's stain,  $\times 140$ . B, Iris of normal pig. Melanocytes are evenly dispersed in anterior border layer and through stroma. Fibroblasts and few other cells are seen,  $\times 88$ .

The iris presents a similar histopathology that may precede or follow events in the choroid (Fig. 5B). Melanocyte destruction in the iris occurs simultaneously with engorgement of iris vessels. Rarely, proteinaceous fluid was found in the anterior chamber of eyes removed after extensive depigmentation of the iris, posterior synechia, and cataract development. A full blown anterior uveitis initiates numerous other secondary phenomena: anterior and posterior synechia formation, deposition of calcium phosphate in the cornea<sup>5</sup> and disruption of lens epithelium and cortical fibers (ie, cataract formation).

Occasional variations in this pattern were encountered. Three eyes with scattered star-like window defects in the fundus on clinical examination had on histopathologic examination, focal accumulations of lymphocytes that disrupted Bruch's membrane, and lifted several overlying pigment epithelial cells (Fig. 7). At these sites the RPE changed from their normal cuboidal shape to a rounded shape and progressively lost their anchorage to Bruch's membrane. RPE cells at these sites were almost amelanotic. In one extreme case the RPE duplicated itself to form a 1-mm wide placoid folded triple-layered lesion. Also, patches of retinal photoreceptors at these sites had pyknotic nuclei; however, similar patchy degeneration of the retina has been found in normal eyes, so the significance of this finding needs further evaluation.

#### Electroretinography

The normal pig electroretinogram (ERG) is very similar to the human; a- and b-waves and oscillatory potentials can be identified<sup>4</sup> (Fig. 8). In a depigmented animal, matched to the normal for age and sex, the morphology of the ERG was similar to that of the control but electrical activity was decreased. The light-adapted electroretinograms revealed a peak-to-peak amplitude of 212.5  $\mu\text{V}$  in the control eye with

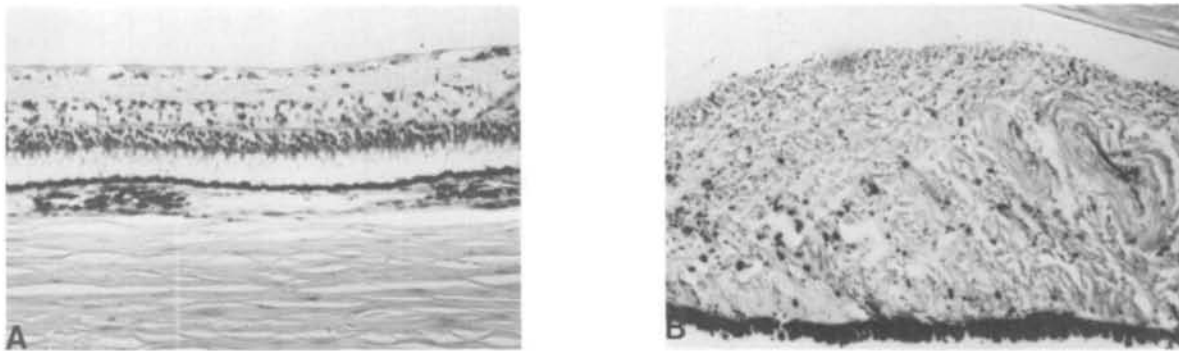


Fig. 5. A, Choroid and retina of pig during rapid phase of depigmentation and tumor regression. Melanin is concentrated within macrophages located around dilated blood vessels. Paraffin section. Wright's stain,  $\times 140$ . B, Iris of pig undergoing tumor regression and depigmentation. Melanocytes are gone. Melanin is gathered into macrophages. Stroma contains many round mononuclear cells,  $\times 88$ .

a b-wave latency of 29.7 msec. In the depigmented animal, the right eye peak-to-peak amplitude was  $85 \mu\text{V}$  while the latency remained identical to the normal. The left eye had a peak to peak amplitude of  $45 \mu\text{V}$ , while the b-wave latency was 14.35 msec. Thus, the uveitis eyes differed from each other in b-wave amplitude by 47%, but differed from the normal pig's b-wave amplitude by 60 and 79%.

### Discussion

The ocular histopathologic findings and extensive previous studies on this animal model<sup>1,7-9</sup> indicate that the ocular disease in the melanomatous swine results from a systemic immunologic process. Our investigation reveals that the destructive attack on the uveal tract is directed against normal melanocytes, not melanoma cells, since ocular melanomas have not been detected in examination of more than 100

animals. The nature of the antigen(s) and the identity of participating immunoglobulins and immune cells remains to be determined. The predictable development of the ocular disease in animals undergoing active tumor regression and the consistent reproducibility of the clinical and histopathologic findings provide an excellent animal model for further study of immunogenic uveitis.

Often the ocular changes are the first clue to tumor regression; thus, the eye provides a window to the mobilization of systemic immunologic activity. Tumor regression at cutaneous sites is difficult to assess because invasion of tumors by leukocytes and the engorgement of tissue by macrophages masks the loss of tumor mass in the early, and most significant, phases of tumor lysis.<sup>9</sup> Therefore, ocular depigmentation, occurring within the thin flat sheet of choroid or iris and being clearly visible externally, will aid in estimating the most appropriate times for sampling

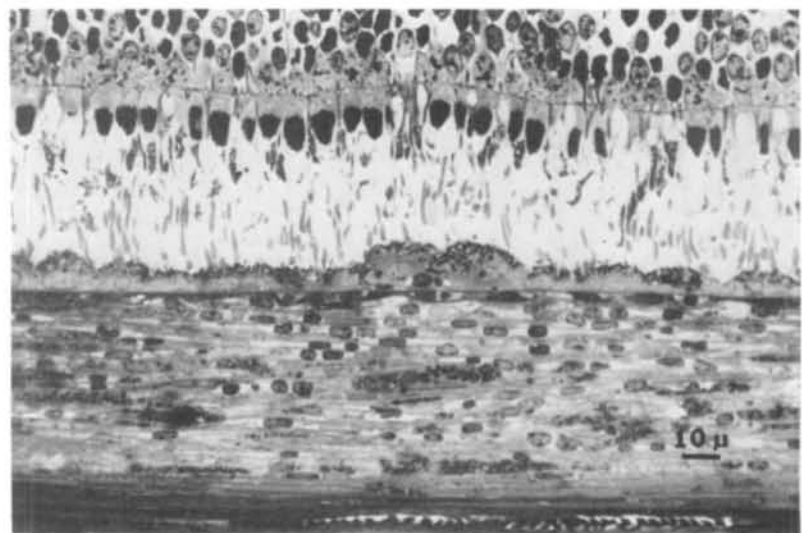


Fig. 6. Histologic section of window defect shown in Figure 5. Note lymphocytic invasion of Bruch's membrane and swelling of a few pigment epithelial cells (plastic section, Toluidine blue stain,  $\times 480$ ).

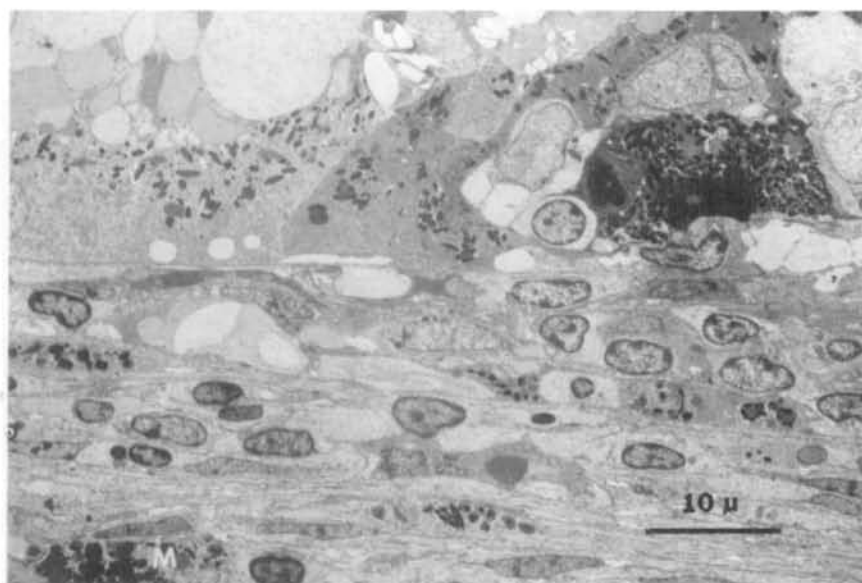


Fig. 7. Electron micrograph of choroid showing lymphocytic invasion. Macrophages (M) contain phagocytized remnants of melanocytes ( $\times 1800$ ).

blood and performing tests of the early immune response to the melanomas.

Although the eye has two types of melanin-containing cells, pigment epithelial cells, and melanocytes, only the latter cells are destroyed by the immunologic response. This indicates that melanin per se can probably be ruled out as the antigen recognized by the immune system. It seems most likely that antigens common to the surface of both the malignant skin melanocyte and the normal uveal melanocytes, cells with a common neural crest origin, are the initial basis for a cross-reacting "innocent bystander" immune response. The possibility that lysed melanocytes releases a variety of "foreign" antigens to cause a cascade of immune responses must also be considered as later epiphenomena.<sup>10</sup>

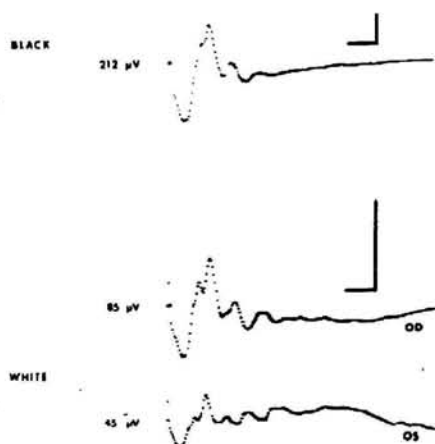


Fig. 8. Electroretinograms of normal black pig (a) and depigmenting, uveitis pig (b).

Late in the disease process when severe anterior uveitis and other secondary phenomena occur (eg, glaucoma, cataract, band keratopathy), the pigment epithelial cells of the iris, ciliary body, and retina may also be destroyed by the massive inflammatory response; this, however, is not part of the anti-tumor phenomenon. Similarly, the star-like window defects in the retinal pigment epithelium are secondary to activities of immune cell infiltrates in the choriocapillaris and Bruch's membrane. This stimulus on one occasion caused a discoid benign duplication of the RPE.

The pilot electroretinography study showed a 60–80% decrease in the amplitude of the b-wave of the ERG in the eyes of an animal with advanced depigmentation of the uveal tract. This decreased photoreceptor electrical activity undoubtedly contributes to the visual impairment in these animals. However, other disturbances in the transparency of the ocular media (ie, band keratopathy, cataract) seem to be the major cause of the blind behavior observed in the depigmented animals.

Human uveitis is divided into granulomatous and nongranulomatous types. Clinically, granulomatous uveitis is more chronic, can involve both the posterior and anterior uvea, is associated with anterior and posterior synechiae, and iris nodules. The protein content in the anterior chamber is greatly increased but not the cellularity of the aqueous humor. Clinically, the Sinclair pig's eyes have a chronic course, but few anterior and posterior synechiae, and only rarely have eyes been found with high protein content in the aqueous or vitreous. Iris nodules have never been seen. The histopathology of granulomatous

uveitis specifically includes epithelioid cell clumps surrounded by lymphocytic infiltrates, giant cells, and fibrocytic proliferation. Swine uveitis has a lymphocytic infiltrate and perivascular macrophages but does not form granulomas or have giant cells present. Therefore, it seems that the melanomatous swine uveitis cannot readily be classified as either granulomatous or nongranulomatous in type.

The cutaneous depigmentation seen in the common human disease, vitiligo,<sup>11</sup> is at times associated with uveitis<sup>12</sup> and chorioretinitis.<sup>13,14</sup> Some vitiligo patients have antibodies to melanin-producing cells<sup>15</sup> and both uveitis and vitiligo have been associated with BCG treatment of malignant melanoma.<sup>16</sup> Much remains to be learned of the relationship between uveitis and ocular tumors.<sup>17</sup> Immunology of other chorioretinal disorders such as sympathetic ophthalmia and Vogt-Koyanagi-Harada syndrome, both of which may be associated with skin depigmentation, needs further study.<sup>18,19</sup> This swine model of the immunologic destruction of melanocytes provides an opportunity to find the mechanism(s) of cell death and to develop strategy for its prevention.

**Key words:** choroidal depigmentation, cutaneous malignant melanoma, electroretinography, fundus depigmentation, iris depigmentation, melanocyte destruction, melanoma, Sinclair swine, tumor regression, uveal depigmentation, uveitis

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