The clinical and histopathologic features of the acute phase of experimental ocular histoplasmosis in our primate model have been reported.\textsuperscript{1,2} Organisms and inflammatory cells were present in the choroid for up to 30 days following intracarotid injection of living organisms. To determine the fate of these organisms and inflammatory cells, and to histopathologically characterize the evolution of these experimental lesions, a number of eyes were enucleated at various time points from 30 days to 3 years following injection; tissue was prepared for histopathologic, ultrastructural, and fungus culture studies. The clinical features of these resolving lesions have been described previously.

**Materials and Methods**

**Animals**

A total of 49 eyes of nonhuman primates (\textit{Macaca speciosa} and \textit{Macaca mulatta}) were studied. All experimental eyes were infected initially by the ipsilateral intracarotid injection of the fungus \textit{Histoplasma capsulatum} Campbell strain G 184B; yeast phase organisms at a dose of $5 \times 10^3$ organisms/lb were used. All inocula were prepared and administered as previously reported.\textsuperscript{1} Fundus photography and fluorescein angiography were used to document the development and resolution of the acute choroidal lesions; similar studies were performed on contralateral control eyes.

At various intervals from 1 month to 3 years after injection, animals were killed, and a total of 34 eyes were obtained for microbiologic, histopathologic, and ultrastructural analysis. At the time of enucleation, the globe was opened at the equator, under sterile conditions, and a small portion of the uveal tissue was removed, minced and plated onto blood agar and Sabouraud’s medium. The eye then was placed in Karnovsky’s fixative (2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M sodium cacodylate, pH 7.4) and fixed by immersion for at least 24 h. Using the most recent fundus photographs and fluorescein angiographs, specific lesions were identified, isolated, and submitted for transmission electron microscopy. The remaining posterior pole, including the nerve and macula, was trimmed and submitted for light microscopy.

**Light Microscopy**

Specimens were processed and embedded in either paraffin or glycol methacrylate. All paraffin blocks were serially sectioned (10 \(\mu\)m) starting 1–2 mm above and ending 1–2 mm below the nerve head, passing through...
the maculae. Every tenth section was stained with hematoxylin and eosin and analyzed by light microscopy; major vessels were mapped and specific lesions identified and correlated with the fundus photograph and fluorescein angiography. Unstained sections through lesions then were submitted for special stains, including PAS, Gomori's methenamine silver and Grocott. Selected blocks were embedded in glycol methacrylate for better preservation of cellular morphology. Glycol methacrylate blocks were sectioned serially at 2–3 μm on an MTS Sorvall microtome using a glass knife; sections were stained and analyzed in a fashion similar to paraffin-embedded tissue.

Transmission Electron Microscopy

Tissue blocks 1 mm X 1 mm containing clinically correlated lesions were postfixed in 1.0% osmium and dehydrated in graded ethanols. Blocks were embedded in Polybed 812 and sectioned on an LKB ultramicrotome. Thick sections (1 μm) were stained with toluidine blue and examined by light microscopy to locate le-

Fig. 1. Chorioretinal scar. A, Superior scar 791 days after injection.

Fig. 1. B, Histopathologic studies revealed chorioretinal adhesion with residual round cell infiltration (H & E, ×130). Insert. Choriocapillaris shows continued presence of lymphocytes and plasma cells (H & E, ×1000). Arrow = plasma cell; Ch = choroid; Open arrow = Bruch's membrane.
sions. After the lesion was identified clearly, the specimen was thin-sectioned, stained with uranyl acetate and lead citrate, and viewed on a Zeiss EM 10A electron microscope.

Results

An acute choroiditis as demonstrated by fluorescein angiography, funduscopic examination, or both occurred in over 95% of injected animals (47 of 49 injected eyes). The histopathology of acute choroiditis, followed for up to 30 days postinjection, has been reported previously. The discrete, poorly circumscribed foci of early choroiditis were characterized histopathologically by mononuclear cell infiltrates containing macrophages with phagocytized yeast phase organisms. The vitreous, retina, and anterior uveal structures were affected rarely, although damage to Bruch’s membrane was noted often on transmission electron microscopy.

By 6 weeks postinjection, organisms could be identified only rarely by routine histochemical techniques. With the disappearance of organisms, the acute histoplasmic choroiditis lesions resolved into four distinct clinical patterns: (1) atrophic scars-chorioretinal adhesions, (2) retinal pigment epithelial window defects, (3) subclinical lesions, and (4) “disappearing” lesions.

(1) Atrophic scars-chorioretinal adhesions: These posterior pole, macular lesions present clinically as discrete, circumscribed, oval, yellow-white lesions with distinct margins (Fig. 1A). Light microscopy of this “atrophic scar,” 791 days postinjection, showed complete loss of the retinal pigment epithelium (RPE) with occasional migration into the overlying retina and focal adherence of the choroid to the retina (Fig. 1B). The outer sensory segments were absent with variable disruption of the outer and inner retina associated with clumps of pigment. A round-cell infiltrate comprised of lymphocytes, macrophages, and plasma cells was present in the underlying choroid (Fig. 1B, arrow). At the margins of these lesions, the RPE exhibited proliferative changes, reduplication, and hypopigmentation.

Ultrastructural analysis of this type of scar revealed alteration of Bruch’s membrane within areas of chorioretinal adhesion. At the margins of the scar, there appeared to be a separation of the RPE and the epithelial basal lamina from the underlying Bruch’s membrane (Fig. 2A, arrow). The choriocapillaris also was absent, and there was absence of the endothelial basal lamina, an additional component of Bruch’s membrane. Within the scar, Bruch’s membrane appeared thickened, with no clear separation of the remaining layers, ie, inner collagenous, elastic, and outer collagenous layers (Fig. 2B, arrow). Areas were identified where glial cell processes extended through breaks in Bruch’s membrane (Fig. 2B, arrowhead); lymphocytes and macrophages also appeared to be migrating through these breaks (Fig. 2B, arrow). However, no new blood vessels or endothelial cells were observed to pass through the defects in Bruch’s membrane. Organisms could not be identified in tissue sections.

(2) Retinal pigment epithelial window defects: In many eyes, the acute choroiditis often resolved without atrophic scar formation but with RPE disruption characterized by alternating areas of hyperpigmentation and hypopigmentation with scattered pigment clumps. (A typical lesion, 316 days postinjection is seen in Fig. 3A). Associated histopathologic changes consisted of pigment clumping, thinning, or loss of the pigment epithelial layer, and variation in pigmentation within the RPE (Fig. 3B). There was no major disruption of the outer layers of the retina, although there was some occasional loss of neurosensory elements immediately adjacent to the changes in the RPE. As with the atrophic scars, such lesions were associated with an underlying chronic lymphocytic infiltrate in the choroid (Fig. 3B, arrow). By transmission electron microscopy (Fig. 4), areas of thinned RPE sometimes were associated with irregular thickening (arrow) and thinning (arrowhead) of the inner collagenous layer of Bruch’s membrane (Br) and atrophy of the choriocapillaris. RPE window defects also were characterized by areas of intact RPE with fewer pigment granules. Organisms could not be identified with special stains in these resolved lesions.

(3) Subclinical lesions: Many acute lesions resolved with no apparent residual abnormality on ophthalmoscopy or fundus photography. Fluorescein angiography of many of these eyes, however, showed persistence of faint, late staining in areas of prior involvement (Fig. 5A). Those eyes with persistent staining are called subclinical lesions.

The histopathology of a typical subclinical lesion 90 days after injection is seen in Fig. 5B. Note the focus of lymphocytes underlying a normal-appearing retina, RPE, and Bruch’s membrane. Serial sectioning through these lesions revealed multiple choroidal foci of small

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Fig. 2. Chorioretinal scar. A, At the margin of the scar, the RPE was separated from Bruch’s membrane (Br) as well as the epithelial basal lamina (arrow) (×9200). B, Within the scar, glial cell processes could be identified which appeared to extend through breaks in Bruch’s membrane (arrowhead). Lymphocytes and macrophages (M) within the choroid (Ch) also appeared to extend through these breaks (arrow) (×14,000).
lymphocytes and plasma cells. Other lesions had scattered plasma cells within the deeper layers of the choroid, and on occasion, large foci were seen that increased the thickness of the choroid. Lymphocytic infiltrates did not involve the choriocapillaris, leaving the choriocapillaris–Bruch’s membrane–RPE complex intact. The lack of disruption of the RPE and retina accounted for the “normal” fundus appearance despite underlying chronic choroiditis. Organisms could not be identified by GMS stain on examinations of entire lesions of over 30 routine sections.

(4) Normal fundus and fluorescein angiographic pattern—“disappearing lesions”: A large number of lesions seemed to disappear totally by clinical as well as fluorescein angiographic examinations (Fig. 6A shows a typical example 810 days after injection). Although such lesions are obviously difficult to correlate with underlying histopathology, simply because of the lack of any clinical evidence of the lesion, it was possible to make this correlation in a few selected cases by careful study of fundus maps in which the original focus of acute choroiditis was adjacent to a histologically identifiable structure (eg, vessel, optic nerve, 

![Fig. 3. Retinal pigment epithelial window defects. A, Note mottled appearance of fundus.](image1)

![Fig. 3. B, Common histopathologic changes were variations in RPE pigmentation (arrowhead) (H & E, X325) and pigment clumping (arrow) (H & E, X325). All such lesions were associated with underlying chronic inflammatory foci (asterisk).](image2)
Fig. 4. Retinal pigment epithelial window defects. A, Transmission electron microscopy of lesions correlated with RPE window defects revealed areas of irregular thickening (arrow) and thinning (arrowhead) of the inner collagenous layer of Bruch’s membrane (Br), myelin figures (open arrow), and absence of choriocapillaris (×8140). Other window defect areas showed intact RPE with fewer pigment granules.

fovea, etc. In Figure 6B, histopathology of such an area just nasal to the optic nerve shows a focus of lymphocytes in the choroid underlying a totally normal-appearing RPE, Bruch’s membrane, and retina. In fact, multiple areas of persistent choroiditis were observed in sections from various areas of the fundus. Although these scattered foci could not be correlated directly with a previously observed acute lesion, the foci were prominent and could be identified with regularity. The overlying RPE, retina, and Bruch’s membrane invariably appeared normal. No organisms could be identified in these lesions. Such areas of low-grade, multifocal choroiditis were not observed in similar histopathologic studies of four uninfected control eyes.

Long-term clinical follow-up (up to 3 years) of these “resolved” patterns of choroiditis has shown a strong tendency for clinically apparent lesions to disappear with time. The histopathologic studies of these same eyes consistently have revealed persistent nests of lymphocytes in the choroid in spite of a quiet clinical appearance. These nests could be formed under “clinically quiet” scars as old as 3 years under RPE defects, under identifiable sites of previously apparent lesions, and scattered throughout the fundus, presumably at sites of earlier clinical disease as well. The clinically obvious lesions, of course, correlated with marked histopathologic changes. The occult foci of choroiditis usually underlay an uninvolved RPE and retina. In no late lesion have subretinal neovascularization or organisms been observed.

Discussion

The ultimate goal of our study is the elucidation of the pathogenesis of macular lesions of presumed ocular histoplasmosis. Although subretinal neovascularization or clear-cut reactivation of inactive scars has not occurred spontaneously in our model to date, many clinical and histologic features of the primate lesions are similar to previously described human cases, suggesting a good correlation between our model and the human disease (nonmacular).
Four distinct healing patterns were observed: chorioretinal scars, pigment epithelial window defects, subclinical lesions identifiable only by fluorescein angiography, and lesions that totally "disappear."

Histopathologic examination of chorioretinal scars, performed years after the initial infection, revealed chorioretinal adhesions, loss of RPE or RPE pigmentation, and pigment proliferation giving the characteristic clinical appearance of an atrophic scar (Fig. 1A). Despite apparent clinical inactivity, foci of lymphocytes were associated with most such "scars," even though organisms could not be identified (Fig. 1B). The fact that such foci of lymphocytes also have been reported in human specimens is further evidence that this model is relevant to the human disease.3,5 Clinically obvious primate scars are the correlate of the typical peripheral atrophic punched-out spots of paramacular scars that characterize the human syndrome.

Other acute lesions healed with minimal residual clinical findings, usually in the form of mottling of
the RPE with variable RPE dropout. Pigment epithelial window defects were noted on clinical and angiographic examinations (Fig. 3). Histopathologically, dropout of RPE cells or loss of RPE pigmentation with an intact overlying retina and outer segments was the rule; chorioretinal adhesions were seldom seen. However, in many cases foci of lymphocytes remained in the choroid underlying such lesions. Small lesions, including RPE defects and mottling, the significance of which is still unknown, were observed in human cases in the Walkersville survey and may be important.

An unexpected healing pattern also was observed in which lesions could not be detected by clinical examination. Some of these lesions could be detected by fluorescein angiography (subclinical lesions) while others could not be found by clinical examination or fluorescein angiographic studies (disappearing lesions). The sites of these “disappearing” lesions nonetheless had significant pathologic changes, consisting of collections of lymphocytes beneath an intact and normal appearing retinal pigment epithelium—Bruch’s membrane complex and retina (Fig. 6). These lymphocytic foci were noted in eyes as long as 3 years after the initial infection. No organisms were observed in such foci.

Fig. 6. B. Insert. Histopathologic correlation of disappearing lesions (arrow) 810 days after injection revealed choroidal foci of chronic inflammation just nasal to nerve (N) (H & E, X180). Insert, Note small collection of lymphocytes (arrow) (H & E, X375).
The relevance of these findings to human disease remains unknown; it is possible, however, that such occult foci of lymphocytes may be the potential site and source for reactivation of the so-called “de novo” lesions which appear to arise from normal retina. In fact, such de novo lesions may be in areas of subclinical chronic choroiditis, as seen in the primate model. Furthermore, the appearance of “new foci” or continually changing peripheral and central presumed ocular histoplasmosis scars in humans may be the result of a chronic, low-grade choroiditis that eventually involves the retinal pigment epithelium to the extent that it begins to proliferate or lose its pigmentation, thereby allowing the lesion to become clinically obvious months or even years after initial infection.7-14 The persistence of chronic inflammatory cells (in the absence of organisms) in numerous pockets scattered throughout the choroid of apparently “healed” eyes was observed in primates. Although no organisms have been found, it is possible that residual antigen in some form persists; immunopathologic techniques are planned to resolve this issue.

Another significant feature of the disease in primates was the disappearance of organisms, as evidenced by culture and special histopathologic studies, within 6 weeks after infection, confirming earlier studies of ocular histoplasmosis in rabbits.13 This finding reinforces the clinical impression that amphotericin B has no role in the treatment of late macular disease at a time when replicating organisms are no longer present. To date, this natural history study has not demonstrated development of subretinal neovascularization by fluorescein angiography or histopathology; neither has natural, late activation of scars been observed. It is possible that naturally occurring “reactivation” of experimentally induced primate scars could take several years to develop, similar to the clinical course in the human syndrome. However, there is evidence that significant damage to Bruch’s membrane occurs in primates, thus providing an entry site into the subretinal space, as observed in several specimens by both light and electron microscopy (Fig. 2). Such damage, occurring acutely or after a more chronic choroiditis, is the anatomical prerequisite for the development of subretinal neovascularization, which could take decades to develop.

Since foci of lymphocytes and resultant low-grade choroiditis are, indeed, present in human and primate scars of inactive histoplasmosis, such areas could be susceptible to flare-ups of increased inflammation or to vascular decompensation and subretinal neovascularization from continuous low-grade damage to the Bruch’s membrane-RPE complex. The presence of such foci in human eyes,3-5 as well as in this animal model, suggests that inflammation may, indeed, be important. Hyperreactivity of cellular immunity in ocular histoplasmosis patients with macular disease16,17 suggests that such cells may be involved in late reactivation of scars. The data on HLA typing are somewhat confusing18-20 but also suggest the possibility of a genetic predisposition to this disease, possibly with an immunologic and inflammatory mechanism for the macular disease.

Correlation of existing clinical and laboratory data lends support to an etiologic role for inflammation in the macular disease of presumed ocular histoplasmosis. However, a mechanism that includes an inflammatory component in no way excludes subretinal neovascularization as a pathogenetic factor; in fact, it is quite likely that a combined mechanism of inflammation associated with the structural abnormalities resulting from the original infectious insult to the RPE-Bruch’s membrane complex is linked pathogenetically to the development of macular disease.21 Certainly damage to this complex was observed in our animal model. It is our opinion that many, if not all, active histoplasmic lesions of the macula result from the development of activity around a focus of old histoplasmic choroiditis, and that so-called new or de novo lesions develop around clinically invisible lesions. The fact that many such lesions, not identifiable clinically or on fluorescein angiography, are observed on histologic study in the primate model is indirect evidence supporting this theory. Lymphocytic foci have been demonstrated in the choroid of animal and human eyes in lesions that appear totally inactive by clinical examination.3-5 Although hemorrhage and subretinal neovascularization may provoke an inflammatory response in disciform lesions,2 it is also possible that low-grade inflammation itself plays a role in the development of subretinal neovascularization or triggers the delicate abnormal capillary fronds to leak serum and blood, resulting in the active disciform process. It seems clear that replicating fungus organisms do not play a role in the active macular process that occurs long after the original fungemia.

Although no macular disciform lesions have yet been produced in animal studies, the presence of discrete lymphocytic foci has been documented in the choroid months and years after the initial histoplasmic choroiditis. Small foci of lymphocytes in the choroid, with and without disruption of the overlying pigment epithelium, were observed at a time when lesions were either clinically inactive or invisible. These lymphocytes may be capable of reacting to specific or non-specific antigenic stimuli, although this has not been proved experimentally. It is hoped that this model will provide the system for study of such reactivation.
Key words: histoplasmic choroiditis, primate model, lymphocytic foci

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