CLINICAL AND EXPERIMENTAL OCULAR HISTOPLASMOSIS

M. E. Woolsey, Marcel Binstock, and M. J. McDonald

Life Science Department, University of Tulsa, Tulsa, Oklahoma

Evidence linking *Histoplasma capsulatum* to ocular disease is circumstantial. Clinical and experimental studies were undertaken to obtain further evidence which could implicate this fungus as a cause of ocular disease. Eye examinations of patients with active histoplasmosis revealed presumed "histo spots" in 42% of such patients, as compared to 5% of patients without active disease. Ocular lesions were induced in 90% of Dutch Belted rabbits which were injected intravenously with live yeast-phase *H. capsulatum*. Both the human and animal studies supported the hypothesis that *H. capsulatum* causes primary choroidal lesions as a result of dissemination of the organism to the eye during active infection.

Presumed ocular histoplasmosis is a disease of the eye which often involves the macular region and frequently results in blindness. This disease can be diagnosed by a typical clinical picture characterized by multiple, small, chorioretinal, atrophic lesions (1, 2). The disease is endemic in eastern Oklahoma. Approximately one new case per month is treated in the Eye Clinic at Hillcrest Medical Center, Tulsa, Oklahoma.

Histoplasma capsulatum has been implicated as the causative agent in such cases by an extensive accumulation of circumstantial evidence (2, 3). A high correlation exists between the ocular disease and positive histoplasmin skin tests or calcified pulmonary lesions which are suggestive of past clinical or subclinical histoplasma infection. In addition, most of the patients come from areas where *H. capsulatum* is endemic.

Presumed ocular histoplasmosis was first described by Woods and Wahlen in 1959 (4). They theorized that the peripheral choroidal lesions, the most characteristic feature of the disease, represent an ocular manifestation of subclinical dissemination of *H. histoplasma* which may cause focal lesions in many body organs. Since *H. capsulatum* sensitizes the tissues for delayed hypersensitivity reactions, they further reasoned that a later encounter with *H. capsulatum* could trigger a reaction, which, if it involves the macular region, may result in marked loss of visual acuity. Today this is still the favored hypothesis.

Experimental ocular histoplasmosis has been studied in a variety of animals (5, 8). The most popular method of experimental infection has been direct intraocular injection of living *H. capsulatum*. Though this approach has produced clinical chorioretinal disease, it is probably not the natural route of infection. Wong and Green reported production of focal histoplasmic choroiditis in rabbits by intravenous injections of viable yeast-phase *H. capsulatum* (9). Smith and his associates also produced choroidal lesions in rabbits by intracarotid injection (10).

MATERIALS AND METHODS

Clinical study

Patients, in the Tulsa area and at the Missouri State Chest Hospital, who were skin test-positive for histoplasmosis or were known to have histoplasmosis by isolation of the organism in cultures, were examined for chorioretinal lesions by indirect ophthalmoscopy. The patient's eyes were dilated with 1% Mydriacyl (Alcon Laboratories, Inc., Ft. Worth, Texas) and then examined by an ophthalmologist, a specialist in retinal disease who had no prior knowledge of the patient's medical history. Photographs, using a Kowa RC-2 fundus camera, were taken of characteristic and presumptive "histo" lesions for future reference.

Experimental study

Animals. Young New Zealand white rabbits (purchased locally) and Dutch Belted rabbits (Ancare Corp., Manhasset, N.Y.) of mixed sexes were used in the experimental work. The rabbits were given water and Purina Chow ad libitum.

Organism. The Scritchfield strain of *H. capsulatum*, kindly furnished by Dr. Howard Larsh, University of Oklahoma, was

maintained in the yeast phase by periodic subculture at 37 C on brain-heart infusion agar (Difco) containing 10% sheep blood, the medium used throughout the study.

Inoculum. To prepare the inoculum, yeast-phase *H. capsulatum* was grown on blood brain-heart infusion agar at 37 C for 72 hr. Growth was washed from the agar surface with a sterile solution of 0.85% NaCl for the living cell inoculum or of 0.5% formalin in normal saline for the killed cell inoculum. The yeast cells were held in formalin-saline until all organisms were dead, as determined by no growth in subcultures. Both viable and killed cells were washed three times and resuspended in sterile normal saline. A 1:100 dilution of the suspension was counted in a standard Neubauer hemacytometer. An aliquot of the diluted live cell suspension was then cultured to determine the viable count and to check purity. The average viable count was 60% of the total cell count.

The size of the inoculum injected into the marginal ear vein depended upon the experiment, and is reported as the total cell count determined by using the hemacytometer. When killed yeast cells were used, 1 ml of the suspension containing 1 x 10^8 organisms was injected at weekly intervals for four weeks. The first group of rabbits injected with live yeast cells was given a single injection containing 2.4 x 10^8 organisms per kilogram body weight; the second group received 4.6 x 10^8 organisms per kilogram body weight in a single injection.

Hypersensitivity test. Skin tests were performed by intradermal injection of 0.1 ml of either a commercial preparation of histoplasmin (CDC Lot No. H-42-68) skin test antigen diluted 1:25 or a 1:100 dilution of the Scritchfield skin test antigen (prepared in the laboratory of Dr. Howard Larsh, University of Oklahoma). In most cases skin tests were performed prior to injection of *H. capsulatum* and at various intervals following inoculation.

Serologic techniques. The presence of precipitating antibodies against *H. capsulatum* was detected by immunodiffusion tests. Sera obtained by periodic bleedings from the marginal ear vein were tested against H-42 histoplasmin as outlined by NCDC (11).

Examination of animals. Fundus examinations were performed with an indirect ophthalmoscope (American Optical Co.) prior to injection and all abnormalities were recorded. Post-infection examinations were made at varying intervals. Fundus pictures were taken of selected animals prior to injection, and post-infection chorioretinal lesions were also photographed using the Kowa RC-2 fundus camera.

RESULTS

Clinical study

Of the 219 patients examined for ocular lesions: 33 had active histoplasmosis; 79 were histoplasmin skin-test positive, but had no active disease; 107 were skin-test negative and had no record of histoplas-

TABLE 1. *Survey of presumed ocular histoplasmosis in patients.*

Patients	Number of patients	Number of patients with presumptive "histo" lesions				Patients with lesions (%)	Significance
		4+[a]	3+	2+	1+		
Cases of clinical histoplasmosis	33	1	2	5	7	45	<.01
Skin test +; no clinical histoplasmosis	79	2	0	1	1	5	N.S.
Skin test —; no clinical histoplasmosis	107	0	0	2	2	4	

[a] 4+ = Typical lesions in both eyes.
3+ = Typical lesions, one eye; questionable lesions in second eye.
2+ = Typical lesions, one eye only.
1+ = Questionable lesions, one or both eyes.

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TABLE 2. *Ocular lesions in rabbits injected intravenously with yeast-phase* H. capsulatum.

Number of rabbits injected	Nature of inoculum	Number of organisms injected	Number of injections	Number of rabbits with lesions	
				One eye only	Both eyes
12	Killed yeast	1×10^9	4a	0	0
10	Live yeast	$2.6 \times 10^6/kg$	1	1	2
13b	Live yeast	$4 \times 10^9/kg$	1	1	9

a Weekly injections.
b Severe systemic infection in this group resulted in death of one rabbit.

mosis. Table 1 summarizes the frequency of characteristic and presumptive ocular lesions in each of the three groups. Of the patients with active histoplasmosis, 45% had ocular lesions, as compared to 5% with ocular lesions among the patients who had positive skin tests but no record of active histoplasmosis. Four per cent (4%) of the patients who were skin test-negative had ocular lesions, an incidence which does not differ significantly from the number found in the histoplasmin-positive group.

Experimental histoplasmosis

Results of experiments designed to induce ocular histoplasmosis in rabbits are summarized in Table 2. Frequent eye examinations of the first group of animals, 12 New Zealand white rabbits which were injected once a week for four weeks with killed yeast cells, failed to reveal the development of lesions. Most of the 10 animals in the second group, a combination of New Zealand white and Dutch Belted rabbits, which were injected with 2.6×10^6 live organisms per kilogram body weight, demonstrated symptoms of only a mild systemic infection. Frequent fundus examinations disclosed the presence of chorioretinal lesions in three of these 10 rabbits.

These results led us to belive that more lesions could be obtained by use of a larger inoculum. Also at this point it was found that, because their eye pigmentation permitted clearer distinction of chorioretinal lesions, the Dutch Belted rabbit was superior to the New Zealand white for testing purposes. Therefore, the third group consisted of 13 Dutch Belted rabbits which were injected with 5×10^8 living organisms per kilogram body weight. Symptoms of systemic infection were much more pro-

nounced in this group and resulted in the death of one rabbit, in which instance viable yeast-phase *H. capsulatum* was recovered from serous discharges of the nose and eyes. Of the 12 rabbits remaining, 11 formed ocular lesions in 7 to 10 days following the single injection. Continued eye examinations revealed that in approximately 30 days the lesions healed with only vestigial scars remaining.

Two rabbits, not shown in Table 2, which were inoculated with 5×10^8 live yeast-phase organisms also developed lesions and were enucleated for cultural histological studies. Viable *H. capsulatum* was recovered from the lesions; histological studies are now in progress.

None of the rabbits tested prior to inoculation had either a positive skin test or precipitating antibodies against H-42 histoplasmin. All rabbits developed positive skin tests following the injection of live yeast cells; only a small percentage developed detectable precipitating antibodies during the period. of observation.

DISCUSSION

The results of the clinical survey and experimental studies in rabbits support the hypothesis that the inactive peripheral scars seen in patients with presumed ocular histoplasmosis represent previous foci of infection due to *H. capsulatum*. These studies also suggest that such foci result from seeding of the eye during the disseminated phase of histoplasmosis. Forty-five percent (45%) of the patients with a history of clinical histoplasmosis showed ocular lesions. This is a significantly higher percentage than the 5% incidence of ocular lesions found in patients with a positive-

histoplasmin skin test and no record of the disease and the 4% incidence in patients with a negative-histoplasmin skin test. Previous subclinical histoplasmosis cannot be ruled out as the possible cause of lesions found in the skin test-negative group, since it is well known that the skin test can reverse from positive to negative. Furthermore, chest x-ray of one of the patients in this group, who also demonstrated anergy to the tuberculin skin test, showed calcified lesions characteristic of past infection with histoplasmosis.

The experimental studies confirm the ability of H. *capsulatum* to cause ocular lesions during active disease and suggest that inoculum size is important in determining the number and severity of the eye lesions produced. These lesions healed approximately 30 days post-infection leaving minimal residual lesions which probably represent scar tissue.

Atrophic macular scars appear to have clinical significance in the human disease as there is evidence that they sometimes develop into the active hemorrhagic lesions. It is tempting to speculate that these scars also originate from primary foci of active infection by disseminated histoplasmosis. Further clinical and experimental studies are in progress to test this hypothesis.

ACKNOWLEDGMENTS

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