Isolation of an Acid-Fast Organism from the Aqueous Humor in a Case of Sarcoidosis

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The anterior chamber fluid from the eye of this patient with sarcoidosis was found to contain microcolonies of a cell-wall-deficient organism that was propagated and identified as acid-fast by the Intensified Acid-Fast stain. The colonies were inoculated into mice and retrieved from the dead or sacrificed animals. This report suggests that the acid-fast microbe in the aqueous humor of this case of uveitis-sarcoidosis may be the same organism as that found in the blood in sarcoidosis. Thus, it may be associated not only with the primary disease, but also with the complications of Boeck’s sarcoidosis.

Introduction

Anterior uveitis is frequently a local manifestation of systemic Boeck’s sarcoidosis.1,2 In fact, an early name for sarcoidosis was uveoparotid fever. In such cases the aqueous humor appears to be sterile by routine culture methods. Similarly, in the case described here no classical microorganisms appeared in a battery of media for aerobes, anaerobes, Mycoplasma, Mycobacteria, and fungi. However, special media and staining detected an organism which resembles the isolates from the blood of nine other cases of Boeck’s sarcoidosis evaluated in a parallel study.3 Data regarding four of these patients have been published.3 This acid-fast organism remains to be identified, but with improved staining and culture methods diagnosis of sarcoidosis and its complications may be facilitated.

Case Report

A 32-year-old black man presented at the Ophthalmology Clinic with an inflamed left eye. A tissue diagnosis of sarcoid had been made following lung surgery six months before his eye difficulties started. The left eye contained many large keratitic precipitates on the cornea, an active aqueous flare, many posterior synechia (iris lens adhesions), cataract, and an elevated intraocular pressure. A diagnosis was made of granulomatous uveitis secondary to sarcoid with secondary glaucoma. In the next four months intraocular inflammation continued without relief despite the use of local, systemic and retrobulbar steroids. Treatment also required two glaucoma operations and the extraction of secondary cataract. The first tap (paracentesis) of the anterior chamber was performed four months after the onset of inflammation using the technique of Goldman and Girard.4,5 Inflammation continued for three months after the cataract was removed despite the use of steroids. Since a cell-wall-deficient infection was suspected, erythromycin (250 mg four times daily) and tri-sulfapyrimidine (500 mg three times daily) were given orally. Each was used during alternating weeks for six weeks and then discontinued. The eye remained quiet for ten months after antibiotics were stopped. At that time the anterior uveitis recurred and a second paracentesis was performed on the left eye. Cell-wall-deficient forms were isolated only from cultures of the anterior chamber fluid taken during the first paracentesis. This report concerns only those organisms propagated from the first sample of aqueous humor.
Isolation of Acid-fast Organism from Aqueous Humor

Special Laboratory Studies
Microdrops (0.03 ml) of anterior chamber fluid were treated as described in a previous report. Aliquots of growth in the Medill-O'Kane Broth were frozen and later subcultured to Kirschner's broth and Horse Muscle broth for animal inoculation. Six mice were inoculated intraperitoneally with cultures of the acid-fast organism and were simultaneously given cortisone subcutaneously. In addition to animal inoculation, confirmatory studies on isolates from these media included the Intensified Triple Acid-Fast Stain, buffered acridine orange stain, Kinyoun's Acid-Fast Stain, and rhodamine-labelled muramidase. The Intensified Acid-Fast Stain was found to be the most effective. Applied to subcultures of the aqueous humor and to preparations of animal tissue, it provided sharp distinction between acid-fast growth and the background.

Results

Leucocytes in the aqueous humor
A Leishman-stained preparation of the aqueous humor showed a predominantly mononuclear infiltrate with approximately two leucocytes per oil immersion field. A rare neutrophil and one eosinophil were seen. This finding contrasts with the cytology in the other 63 nonsarcoid uveitis cases previously studied in which no eosinophils were seen in the aqueous humor.

Organisms in direct smear of aqueous humor
A smear of the aqueous humor stained by Kinyoun's method revealed a group of slender acid-fast rods (Figure 3). A duplicate smear stained with auramine-rhodamine showed colonies of fluorescent spheres.

Microorganisms in cultures
Colonies did not appear on the surface of any medium. Growth in semi-solid agar and in pour plates was pleomorphic and acid fast in the Triple Stain (Figures 1 and 2). A control for reliability of the Triple Stain consisted of 62 blood cultures containing 14 miscellaneous species of nonmycobacterial classical bacteria. These showed no acid-fast organisms.

The organisms from the patient's cultures fluoresced when stained with auramine-rhodamine and acidine orange (Figure 4), and rhodamine-labelled muramidase (Figure 5). Acridine orange stains nucleic acids. Reaction with muramidase shows components of microbial walls, but does not require a complete classical wall. Most isolates from infection of pleomorphic organisms which fail to colonize on the surface of media retain an incomplete cell wall.

Growth occurred only at 37°C rather than at 25°C and was minimal under anaerobic conditions. Hypertonic medium containing 10% sucrose did not improve growth.

Animal inoculation
Four mice given the acid-fast culture and a cortisone dosage of 50 mg died between eight and eleven days. Two mice given 40 mg of cortisone survived for fourteen days, when they were sacrificed. Gross pathology consisted of splenic enlargement, pale plaques in the liver, and nonconsolidated nodularity in the lung. Acid-fast microcolonies were found in smears of the liver, lung (Figure 6), spleen, and blood.

When pooled fluid from the anterior and posterior ocular chambers of two mice was examined, acid-fast microcolonies were found in large numbers in both preparations (Figures 7 and 8). Histological examination of the tissues is not, as yet, completed. Current work in progress in the laboratory at Wayne State University has demonstrated propagation in the eye of an acid-fast strain from another case of sarcoidosis. As controls, six mice received cortisone alone, and six were given cortisone plus uninoculated medium. The control mice remained well for two weeks and no microcolonies were found in smears of tissue.

Discussion

It is noteworthy that the aqueous humor from the anterior chamber fluid of this patient showed organisms before antibiotic therapy, whereas they were absent after antibiotic administration. Thus, therapy may have inhibited the organisms, either reducing them below detectable numbers or altering them in a way which prevented their growth in culture media.

We have yet to determine whether the acid-fast organism which is associated with sarcoidosis is a new species or an atypical stage of the tubercle bacillus. The acid-fast growth from sarcoid cases resembles tubercle bacilli in showing some microscopic twisted strands, the so-called "cords." Mankiewicz and Kurti believe a phage is present in sarcoidosis which holds M. tuberculosis in a variant stage. Garvin, in the laboratory at Wayne State University, has electrophoretically analyzed the proteins of an organism in blood cultures of a sarcoidosis case. The pattern closely mimicked but did not exactly duplicate that of M. tuberculosis.

On the other hand, the organism seen in sarcoidosis may not be M. tuberculosis. The antibodies in the serum of sarcoidosis patients react with varied species of Mycobacteria, not with the suggestive intensity of M. tuberculosis. Acid-fast species which are difficult to propagate are gaining increasing attention. Also, while there is a difference between fastidious species and cell-wall-deficient variants,
With continued incubation in Veal Infusion Agar small colonies enlarge, still retaining their acid-fast characteristic (1000X).
Isolation of Acid-Fast Organism from Aqueous Humor

Fig. 3
Irregular acid-fast rods in direct smear of the aqueous humor (Kinyoun's stain) (1500X).

Fig. 4
Colonies from Chanock agar cultures of the aqueous humor stained bright red with acridine orange, indicating their RNA content (1500 X).
Fig. 5
Microbial nature of the colonies in cultures is indicated by fluorescence of rhodamine-labelled muramidase, which has combined with mucopeptide of the cell walls (540X).

Fig. 6
Acid-fast colony from lung of mouse given subculture from uveitis case (1000X).
Isolation of Acid-Fast Organism from Aqueous Humor

Fig. 7
Acid-fast organisms in pooled aqueous-vitreous humors of mouse (1000X).

Fig. 8
Acid-fast organisms in pooled aqueous-vitreous humors of mouse inoculated with culture from patient’s eye (1000X).
both may be difficult to propagate. Antigens of many more isolates from sarcoidosis should be analyzed by all available methods.

Another investigator has noted auramine-rhodamine staining organisms associated with sarcoidosis, again indicating the presence of Mycobacteria. Fluorescent rods were found in involved tissues of 32 patients and were absent in scalene lymph nodes of healthy persons.

Other animal models for sarcoidosis have been reported using suspensions of sarcoid tissue. Disease has been produced, but no organisms were demonstrated by the methods employed.

Many questions are pertinent: Since cortisone aids remission of both uveitis and sarcoidosis in man, how can it increase host susceptibility? It is possible that the discrepancy is related to dosage since our laboratory mice were given maximal amounts of the hormone.

Acid-fast staining of a wall-deficient mycobacterial variant results from two factors. First, the clinical wall-deficient organisms retain some mural components. Second, as Berg has shown, the tubercle bacillus has acid-fast cytoplasm as well as walls.

Wall-deficient bacteria are being isolated from a great many disease states. The animal pathogenicity of 28 microbial species in the wall-deficient stage has been described. Wall-deficient bacteria have also been found in infected ocular sites much more frequently than in noninfected eyes.

### Conclusion

In this case of uveitis, the aqueous humor contained faintly acid-fast slender rods and auramine-rhodamine staining spheres suggestive of cell-wall-deficient microorganisms. Acid-fast microcolonies in culture were inoculated into mice and retrieved from tissues of the dead or sacrificed animals. In staining reactions, growth characteristics and animal pathogenicity, the strain resembles acid-fast, cell-wall-deficient isolates from the blood of nine other sarcoidosis cases. These acid-fast colonies have not been found in over 60 control blood cultures. This is the first case of sarcoidosis in which acid-fast organisms have been found in the aqueous humor. They were found to colonize in the eyes of inoculated mice, thus suggesting an association between the organisms and the ocular disease in sarcoidosis.

### References