DISCUSSION

Our electron microscopic findings are in general agreement with those of Finlay-Jones et al. and Kerr et al. The findings indicate that voluminous phagolysosomes in Hansemann macrophages form focal aggregates of microcrystalline calcium, and that the focal aggregates continue to form the electron dense central spheres of the calcospherules. The “immature” calcospherule, which is a phagolysosome with a central sphere, subsequently develops an outer calcified zone, and this outer zone gives the calcospherule its target shape. Further peripheral calcification and eventual obliteration of an entire phagolysosome result in loss of the limiting membrane of the phagolysosome with the formation of a large, target shaped calcospherule. By light microscopy the larger target shaped calcospherules are seen to have a thicker peripheral rim than the smaller ones with the target-like appearance. This thicker rim probably results from the more extensive calcification in the peripheral zone around the central calcified sphere. By electron microscopy the cytoplasm of Hansemann macrophages are found to be filled with lysosomes and phagolysosomes of varying sizes. The latter are most likely responsible for the periodic acid–Schiff positive, granular, occasionally vacuolated appearance of the macrophages evident by light microscopy.

The etiologic factors that may be involved in the lesions of malacoplakia have been reviewed by Melicow. Causes that have been suggested include nonspecific bacterial infection, tuberculosis, sarcoidosis, and fungal and bacterial infections. Malacoplakia is often associated with infections due to coliform bacteria. It has been thus postulated that the peculiar inflammatory change may represent a reaction to breakdown products of these bacteria. Histochemical reactions suggest that bacterial glycolipids are present in the calcospherules, but the bacterial bodies per se have not been identified. There is some evidence that lysosomes or phagolysosomes do not contain enzymes capable of digesting lipids, and that myelin bodies within phagolysosomes may represent the undigested membrane structures of phagocytosed materials. Myelin bodies with a fingerprint pattern were noted by us (as well as by other investigators in phagolysosomes of Hansemann macrophages. It is possible that such undigested material persisting after disintegration of bacteria within the phagolysosomes serves as a nidus for subsequent crystal precipitation of calcium apatite, thus initiating the formation of calcospherules.

Iron has been demonstrated in calcospherules by histochemical means. Such material, it is suggested, may be derived from iron containing enzymes in bacterial plasma membranes. Although ferritin grains are reported to be present in phagolysosomes of Hansemann macrophages, we were unable to demonstrate ferritin grains in either the phagolysosomes or the calcospherules. Erythroblastosis by the macrophages was not noted, thus eliminating a possible source of ferritin particles.

References

IMMUNOFLUORESCENCE AS A DIAGNOSTIC AID IN CRYPTOCOCCAL MENINGITIS

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Abstract

This paper concerns a patient who had long standing rheumatoid arthritis for which she received prednisone and chlorambucil. Steroid induced diabetes mellitus and subsequent cryptococcal meningitis caused her death. Circulating cryptococcal antigen was demonstrated in the patient’s cerebrospinal fluid and blood by the latex cryptococcal antigen test. A new rapid method utilizing immunofluorescence to identify crypto-

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Cryptococcosis is a disease that involves the lungs and the central nervous system and is caused by Cryptococcus neoformans. This organism is frequently an opportunistic secondary invader associated with malignant lymphoma, the collagen vascular diseases, and diabetes mellitus and is also found in patients receiving broad spectrum antibiotics, steroids, and chemotherapeutic drugs.

Within the last few years the diagnosis of the disease has depended on culture procedures and biochemical techniques performed with the positive cultures. An increased interest in more rapid techniques to diagnose the condition has resulted in the development of a number of serologic tests, such as tube agglutination tests, complement fixation test, immunodiffusion test, and latex cryptococcal antigen agglutination test.

Unfortunately patients ill with cryptococcosis have been considered to be immunologically inert, especially if they also had an associated malignant lymphoma or had undergone immunosuppression by the use of chemotherapeutic drugs or steroids. This report concerns a patient who had long standing rheumatoid arthritis for which she received prednisone and chlorambucil. She developed steroid induced diabetes mellitus and subsequently cryptococcal meningitis, which caused her death. Circulating cryptococcal antigen was demonstrated in the patient's cerebrospinal fluid and blood by the latex cryptococcal antigen test. A new rapid method utilizing indirect immunofluorescence to identify cryptococcal antigen and antibody in body fluids is described in this brief report.

CASE HISTORY

A 61 year old white female was admitted to the Stanford University Medical Center on March 3, 1973. She had had a long history of rheumatoid arthritis, having developed the disease at age 14. The arthritis had been treated with prednisone and for a short period with chlorambucil. She developed adult onset diabetes mellitus, which was steroid induced.

Chlorambucil therapy for arthritis was begun in early 1971. In August 1971 she developed vomiting, headache, and fever and was admitted to a community hospital complaining of these symptoms. On physical examination, she was found to have nuchal rigidity and an equivocal Kernig sign. Two lumbar punctures were performed. India ink preparations were negative, and the cerebrospinal fluid protein level was slightly increased. Culture from the first lumbar puncture grew a fungus, reported to be either Candida or Saccharomyces. No therapy was advised, and the patient was discharged.

The patient returned to the community hospital in February 1973 complaining of vomiting and diarrhea. A neurologic examination was suggestive of a resolving left cerebral lesion, possibly due to cerebral vasculitis. She was discharged and given an increased dosage of prednisone for possible adrenal insufficiency. The patient was again seen at the community hospital in March 1973 with recurrent fever. A lumbar puncture demonstrated a protein level of 285 mg. per 100 ml. The total leukocyte count of the cerebrospinal fluid was 75 per cu. mm. An India ink preparation showed a budding yeast with encapsulated forms suggesting Cryptococcus.

The patient was transferred to Stanford University Hospital on March 3, 1973. A lumbar puncture demonstrated a protein level of 250 mg. per 100 ml., a glucose level of 75 mg. per 100 ml. (simultaneous blood glucose, 300 mg. per 100 ml.), and a budding yeast on India ink preparation. She was given intravenous, intrathecal, and intracisternal doses of amphotericin B and 5-fluorocytosine. The patient had a long hospital course characterized by increasing lethargy and disorientation. She developed right upper motor neuron facial weakness and left ocular ptosis. A lumbar puncture on April 6, 1973, demonstrated a protein level of 630 mg. per 100 ml., a cerebrospinal glucose level of 25 mg. per 100 ml. (blood glucose, 175 mg. per 100 ml.), and 251 mononuclear leukocytes. No fungi were seen, and Cryptococcus was grown from early cerebrospinal fluid specimens. The latex cryptococcal antigen test on the serum was positive (1:8) and was also positive on the cerebrospinal fluid (1:128).

The indirect fluorescent antibody test for the detection of Cryptococcus was positive, with the patient's serum antibody and the patient's cryptococcal organisms as the antigen. Fluorescence of the capsule of the organisms was especially evident (Fig. 1). The major details of the test procedure follow:

1. Heat Cryptococcus cells for three hours in a 70°C water bath. Suspend killed cells in 0.9 per cent saline.
2. Place three drops of killed cell suspension in slide wells.
3. Inactivate patient's serum at 56°C for 30 minutes.
4. Dilute serum 1:20 in buffered saline, and place 0.1 ml. over Cryptococcus cells. Rotate on shaker for 30 minutes.
Figure 1. Photomicrograph demonstrating positive indirect immunofluorescence of cryptococcal organisms in case discussed. The antibody was the patient's serum antibody. Fluorescence of the capsules of the organisms is prominent.

5. Add two drops (0.1 ml) of fluorescein labeled antihuman globulin diluted 1:80. Rotate for 30 minutes.
6. Mount with glycerol mounting medium.
7. Examine slides with fluorescence microscope.

On April 21 the patient developed a temperature of 41°C and hypotension. The impression was gram negative bacteremia with septic shock, and the patient died rapidly a few hours later. Autopsy revealed diffuse cryptococcal meningitis involving the cerebral cortex and cerebellum bilaterally and the cervical and thoracic spinal cord. Diffuse ventricular enlargement was present. In addition, bilateral lobular pneumonitis was present, with autopsy cultures positive for Klebsiella and E. coli. No evidence of pulmonary cryptococcosis was found. Splenomegaly was present, with sections demonstrating acute septic splenitis. Cultures of the spleen were positive for enterococci and coagulase negative staphylococci. The liver was enlarged and fatty. Atrophy of the adrenals was present. Evidence of long standing rheumatoid arthritis was present, with contracture and deformity of the joints of the hands and feet. The cause of death was cryptococcal meningitis and bilateral lobular pneumonia, with septicemia due to gram negative organisms.

DISCUSSION

Cryptococcal meningitis and pneumonitis are major problems, especially in patients with altered resistance to infection such as those suffering from malignant lymphoma, the collagen vascular diseases, and those receiving corticosteroids and cytotoxic agents. In the patient described, the predisposing factors that may have been important included rheumatoid arthritis, treatment with prednisone and chlorambucil, and development of steroid induced diabetes mellitus.

The major problem in the management of cryptococcal infection is to effect rapid and specific diagnosis of the disease. The indirect immunofluorescence test described in this report is a more specific and more sensitive diagnostic test than the serologic tests, such as tube agglutination, complement fixation, immunodiffusion, and latex cryptococcal antigen agglutination tests, currently available.5 False positive cryptococcal latex agglutination tests may result from the presence of rheumatoid factor.6 Both circulating cryptococcal antigen and antibody were detected in the patient's serum. The fluorescence procedure utilized the patient's serum antibody to detect the patient's cryptococcal cerebrospinal fluid antigen.

The high mortality rate of the untreated disease and the high degree of toxicity of amphotericin B and 5-flucytosine stress the importance of rapid and accurate diagnostic procedures in this disease. We thus believe that this indirect immunofluorescence procedure may be a useful diagnostic test to identify cryptococcal infection.

References