We report a case of chronic meningitis due to capsule-deficient Cryptococcus neoformans which could not be diagnosed by routine morphological and immunological cerebrospinal fluid (CSF) examination. Repeated CSF examination and culture did not disclose the fungal body, and the cryptococcal antigen tests were always negative. Culture of ventricular fluid showed non-encapsulated cultured cells which were stained positively by indirect immunofluorescence using diluted sera from another patient diagnosed with cryptococcal meningitis. Incubation of dispersed colonies into mice resulted in encapsulated C neoformans. It is important to suspect capsule-deficient C neoformans when the conventional diagnostic approaches fail to identify the organism or antigens.

The form of Cryptococcus neoformans which infects humans typically possesses a thick capsule and routine morphological diagnosis depends on that unique feature. However, we encountered a case of chronic meningitis caused by capsule-deficient C neoformans. Because it was so different from the classical form, this posed a diagnostic challenge since conventional approaches were not informative. Here, we report the case together with a discussion of the pitfalls in reaching a diagnosis with this particular form of C neoformans.

CASE REPORT

A 42 year old man working as a farmer was admitted to a local hospital on July 6, 1992 after having had an episode of loss of consciousness following tonic-clonic convulsion. There was a history of chronic heavy alcohol consumption. A low grade fever and general fatigue preceded the event by several weeks. His cerebrospinal fluid (CSF) count from lumbar puncture was increased to 168/mm³ (lymph:polys 1:1) and CSF protein was 116 mg/dl. Aciclovir, ceftriaxone, and sulbenicillin sodium were started intravenously. However, his symptoms did not improve and 1 month later isoniazid, rifampicin, ampicillin, and minocycline hydrochloride were started. On October 26, he had further fits and deterioration. He was transferred on December 16 to a university general hospital because of the difficulty in identifying the cause of meningitis. CSF findings were pleocytosis (74/mm³, 78% monocytes), elevated protein (221 mg/dl), and low sugar (8 mg/dl, CSF/blood glucose ratio 8.2%). Gram staining, Indian ink preparations, CSF cytology with Ziehl-Neelsen staining, cultures for fungi, and cryptococcal antigen were all negative, and CSF examinations for Mycobacterium tuberculosis by culture and the polymerase chain reaction method were also negative. Full blood count showed RBC 4.10 million/mm³, platelets 75 000/mm³, WBC 6100/mm³, lymphocytes 2257/mm³, CD4 45.4%, and CD8 23.1%. Laboratory studies revealed moderately impaired liver function (GOT 35 IU/l, GPT 16 IU/l, LDH 443 IU/l, γ-GTP 63 IU/l, cholinesterase 185 IU/l), total serum protein 7.4 g/dl, and albumin 3.9 g/dl. Fasting blood sugar was 97 mg/dl. Hepatitis B surface antigen, hepatitis C antibody, and HIV antibody were negative. CT brain scan with contrast showed a small, circumscribed enhancing area at the right side of the basal cistern, suggesting the presence of a small granuloma (fig 1A). On MRI T2 image, a circumscribed high signal intensity area was observed in the right ventral caudoputamen (fig 1B).

The patient complained of nausea. As an endoscopic examination had disclosed rather vulnerable looking oesophageal varices, endoscopic sclerosing therapy was performed on several occasions. A liver biopsy indicated advanced cirrhosis. Following generalised epileptic status which lasted intermittently for several hours, the patient became stuporous. CSF examinations, repeated several times, always disclosed lymphohypocytic pleocytosis (195/mm³) without atypical cells, increased protein (99 mg/dl), and low glucose (48 mg/dl, CSF/blood ratio 18%), but Indian ink preparations, Ziehl-Neelsen staining, cultures for fungi, and cryptococcal antigen were always negative. The differential diagnoses of cryptococcal meningitis, tuberculous meningitis, and meningeal carcinomatosis were considered. We highly suspected cryptococcal meningitis because there were no findings attributable to pulmonary tuberculosis and the antituberculous agents were not effective, and CSF cytology did not show carcinomatous cells. Therefore, an Ommaya reservoir was placed with the catheter tip inserted into the right frontal horn, both to study the ventricular fluid and to administer amphotericin B into the ventricle. However, cryptococcal antigen in the ventricular fluid via the Ommaya reservoir was reported to be equivocally positive. When the ventricular fluid was cultured on Sabouraud dextrose agar, milk-white colonies were seen after several days. The Indian ink preparation showed small round, bright spots with occasional

Abbreviations: CSF, cerebrospinal fluid
Difficulty in diagnosing chronic meningitis

Antigen was repeatedly negative by latex agglutination assay of the CSF. A diagnosis of chronic meningitis caused by capsule-deficient Cryptococcus neoformans was established by indirect immunofluorescence staining and formation of the thick capsule after inoculation into the murine peritoneal cavity. In fact, only a few cases of cryptococcal meningitis caused by the capsule-deficient form have been reported. \(^1\) \(^2\)

Capsule-deficient Cryptococcus produces sufficient capsule to inhibit phagocytosis when incubated with human lung tissue in vitro. \(^3\) In our case, the capsule-deficient type did not form thick capsules in 2 weeks when inoculated into the murine peritoneal cavity. The capsule-deficient Cryptococcus neoformans elicited an intense inflammatory response characterised by early suppuration and phagocytosis, followed by a marked histiocytic and fibroblastic reaction, but with limited spread of infection compared to the capsule-deficient yeast form. \(^4\)

Presumably, the brain MRI and CT scan showed a focus of parenchymatous inflammation and granuloma in our case. Meningitis due to capsule-deficient Cryptococcus neoformans has previously been reported in AIDS patients (isolated from CSF) and in Japanese non-AIDS patient (isolated from ventricular fluid). \(^1\) \(^2\) The difference in these cases suggests that the host immune response influences the spread of capsule-deficient Cryptococcus neoformans. Similarly, in our case repeated CSF examinations by lumbar puncture failed to detect yeast forms. The susceptibility of the capsule-deficient form to phagocytosis is probably the major reason why repeated CSF cultures failed to grow Cryptococcus, as it was quickly engulfed by macrophages on its way to the lumbar CSF space. The repeated cryptococcal antigen tests of CSF were always negative. The sensitivity of the antigen test was 76% and its specificity was 98.9% for encapsulated Cryptococcus, however capsule deficiency would make the detection of cryptococcal antigen rather difficult, since the latex agglutination test for cryptococcal antigens detects solute capsular polysaccharide antigens with the antibody fixed to latex particles. \(^5\)

It is important to consider the possibility of this capsule-deficient form when the presence of cryptococcal meningitis is strongly suspected, keeping in mind that the conventional diagnostic approaches fail to identify both the organism and the antigens.

DISCUSSION

In this case, the typical encapsulated fungal body was not observed in or cultured from the CSF. Moreover, cryptococcal antigen was repeatedly negative by latex agglutination assay of the CSF. A diagnosis of chronic meningitis caused by capsule-deficient Cryptococcus neoformans was established by indirect immunofluorescence staining and formation of the thick capsule after inoculation into the murine peritoneal cavity. In fact, only a few cases of cryptococcal meningitis caused by the capsule-deficient form have been reported. \(^1\) \(^2\)

Capsule-deficient Cryptococcus produces sufficient capsule to inhibit phagocytosis when incubated with human lung tissue in vitro. In our case, the capsule-deficient type did not form thick capsules in 2 weeks when inoculated into the murine peritoneal cavity. The capsule-deficient Cryptococcus neoformans elicited an intense inflammatory response characterised by early suppuration and phagocytosis, followed by a marked histiocytic and fibroblastic reaction, but with limited spread of infection compared to the capsule-deficient yeast form. Presumably, the brain MRI and CT scan showed a focus of parenchymatous inflammation and granuloma in our case. Meningitis due to capsule-deficient Cryptococcus neoformans has previously been reported in AIDS patients (isolated from CSF) and in Japanese non-AIDS patient (isolated from ventricular fluid). The difference in these cases suggests that the host immune response influences the spread of capsule-deficient Cryptococcus neoformans. Similarly, in our case repeated CSF examinations by lumbar puncture failed to detect yeast forms. The susceptibility of the capsule-deficient form to phagocytosis is probably the major reason why repeated CSF cultures failed to grow Cryptococcus, as it was quickly engulfed by macrophages on its way to the lumbar CSF space. The repeated cryptococcal antigen tests of CSF were always negative. The sensitivity of the antigen test was 76% and its specificity was 98.9% for encapsulated Cryptococcus, however capsule deficiency would make the detection of cryptococcal antigen rather difficult, since the latex agglutination test for cryptococcal antigens detects solute capsular polysaccharide antigens with the antibody fixed to latex particles.

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REFERENCES