Amebic Meningoencephalitis Caused by *Balamuthia mandrillaris*: Report and Review

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*Balamuthia mandrillaris*, formerly referred to as a leptomyxid amoeba, is a free-living amoeba that has recently been identified as a cause of meningoencephalitis. Previously, only two genera, *Naegleria* and *Acanthamoeba*, were recognized as causes of central nervous system (CNS) infections in humans. In contrast to *Naegleria*, *Balamuthia* causes a subacute-to-chronic infection of the CNS. Distinct from *Acanthamoeba*, which appears to favor the immunocompromised host, *Balamuthia* is capable of infecting both healthy and immunosuppressed hosts. Retrospective analyses as well as an accumulation of newly identified cases have demonstrated that this amoeba is an increasingly important pathogen to recognize. We report the isolation, histopathologic features, and confirmation by indirect immunofluorescence of *B. mandrillaris* in a case of fatal amebic meningoencephalitis.

Small, free-living aerobic amebae belonging to the genera *Naegleria*, *Acanthamoeba*, and *Balamuthia* are known to cause CNS infection [1, 2]. Classically, two distinct entities are described: (1) primary amebic meningoencephalitis, an acute, fulminant, necrotizing meningoencephalitis caused by *Naegleria fowleri* that usually results in death within 5–10 days; and (2) granulomatous amebic encephalitis (GAE), a subacute-to-chronic disease that leads to death within 1 week to several months. Most cases of GAE in the past have been ascribed to *Acanthamoeba* species. Recently, however, *Balamuthia mandrillaris*, a newly described free-living amoeba, has become increasingly recognized as an important etiologic agent of amebic meningoencephalitis [2, 3]. *B. mandrillaris*, previously referred to as a leptomyxid ameba and formerly regarded as an innocuous soil organism incapable of infecting mammals, has been implicated retrospectively in several cases of undiagnosed encephalitis or GAE previously attributed to *Acanthamoeba* species. Recently, however, *Balamuthia mandrillaris*, a newly described free-living amoeba, has become increasingly recognized as an important etiologic agent of amebic meningoencephalitis [2, 3]. *B. mandrillaris*, previously referred to as a leptomyxid ameba and formerly regarded as an innocuous soil organism incapable of infecting mammals, has been implicated retrospectively in several cases of undiagnosed encephalitis or GAE previously attributed to *Acanthamoeba* species. *Balamuthia mandrillaris*, formerly referred to as a leptomyxid ameba, is a free-living ameba that has recently been identified as a cause of meningoencephalitis. Previously, only two genera, *Naegleria* and *Acanthamoeba*, were recognized as causes of central nervous system (CNS) infections in humans. In contrast to *Naegleria*, *Balamuthia* causes a subacute-to-chronic infection of the CNS. Distinct from *Acanthamoeba*, which appears to favor the immunocompromised host, *Balamuthia* is capable of infecting both healthy and immunosuppressed hosts. Retrospective analyses as well as an accumulation of newly identified cases have demonstrated that this amoeba is an increasingly important pathogen to recognize. We report the isolation, histopathologic features, and confirmation by indirect immunofluorescence of *B. mandrillaris* in a case of fatal amebic meningoencephalitis.

At present, all reported balamuthia infections have been confirmed only after death. Increased awareness of this clinical entity, in concert with the new developments in culture techniques and identification, might allow earlier diagnosis and effect execution of therapeutic trials. We report a case of fatal *B. mandrillaris* meningoencephalitis and review the previously reported cases of infections due to this increasingly important and newly recognized pathogenic species of ameba.

Case Report

A 32-year-old Mexican man with a history of intravenous drug and alcohol abuse who had been a resident of the United States for 2 years was admitted to a local hospital because of 1-week history of nausea, vomiting, and severe headache. His temperature was 39.8°C, and his neurological findings were unremarkable. A complete blood count and results of a blood chemistry analysis were normal; HIV serology was negative. Examination of CSF demonstrated 220 cells/mm³ (44% neutrophils and 56% monocytes), 200 mg of protein/dL, and 45 mg of glucose/dL. The patient had antibody to hepatitis C virus and a positive PPD reaction measuring 11 mm. Findings on an echocardiogram and abdominal CT scan were unremarkable. Blood and CSF cultures for bacteria, fungi, and mycobacteria were negative, as were tests for serum cryptococcal antigen and cysterceriosis.

A CT scan of the brain without administration of contrast medium showed an area of low attenuation in the left temporal lobe. Empirical antibacterial therapy with ceftriaxone, nafcillin, and metronidazole was started. MRI of the head, performed 2 days later, demonstrated lesions in the left temporal lobe and both cerebral hemispheres, which enhanced with gadolinium contrast medium. Because MRI performed 1 week later demonstrated an increased number of enhancing lesions (figure 1), the patient was transferred to our institution for evaluation of worsening neurological status and radiological findings despite antibacterial therapy.

Physical examination disclosed an alert, well-nourished man who was oriented only to person. The cranial nerves were intact, with the exception of gaze-evoked nystagmus in all directions. Bilateral dysmetria (right greater than left) was accompanied by a mild bilateral intention tremor. His gait was unsteady with truncal ataxia and poor postural control. There was no meningismus. The leukocyte count was 5,600/mm³, with a normal T lymphocyte count. The hematocrit was 37.4%, and the platelet count was 273,000/mm³. Lumbar puncture was repeated, and CSF analysis revealed a cell count of 40/mm³ (86% lymphocytes and 14% monocytes), 160 mg of protein/dL, and 37 mg of glucose/dL.
Amebic Meningoencephalitis Due to *B. mandrillaris*

Over the ensuing days, he became progressively obtunded and agitated and developed increasing hyperreflexia with bilateral ankle clonus (more severe on the left). A CT scan of the brain on the fourth hospital day showed multiple ring-enhancing lesions, the largest of which measured 2.5 cm in diameter in the left posterior temporal lobe; additional lesions were noted in the brain stem and both cerebral hemispheres.

A stereotactic brain biopsy of the left temporoparietal lobe was performed. Examination of hematoxylin-eosin (HE)-stained tissue demonstrated cores of gray and white matter with prominent coagulation necrosis, chronic perivascular inflammation, and microglial nodules in the white matter. At that time, a diagnosis of toxoplasma infection was entertained, and serum IgG antibodies to *Toxoplasma gondii* were detected. However, direct fluorescent antibody and immunohistochemical testing of the biopsy specimen were negative. No bacterial, fungal, parasitic, or viral elements were identified. Routine bacterial, mycobacterial, and fungal cultures yielded no organisms except for *Staphylococcus aureus*, which was isolated only from cultures with Schaedler’s broth.

A four-drug antituberculous regimen was started, and the patient continued to receive antistaphylococcal treatment and antibiotic therapy for coverage of anaerobes throughout his hospital course. However, he developed progressively increasing cerebral edema with increased intracranial pressure and required endotracheal intubation, hyperventilatory support, and steroid and mannitol therapy. His neurological status further deteriorated, and his fever persisted (temperature to 41.6°C); he developed recurrent seizures and signs of progressive transtentorial herniation. Despite aggressive measures, he died on the 35th hospital day.

At autopsy, the most significant findings were confined to the CNS. Gross examination of the brain revealed diffuse cerebral edema and hemorrhagic necrotic lesions within the cortical ribbon, lobar white matter, brain stem, and cerebellar deep white matter. Lesions were seen in the right gyrus rectus, right inferior temporal gyrus, right hippocampus, bilateral occipitotemporal gyri, left parahippocampal gyrus, left midbrain, and bilateral cerebellar deep white matter. Examination of HE-stained brain sections revealed purulent meningeal exudate, with large numbers of neutrophils and mononuclear inflammatory cells in a subpial and perivascular distribution overlying diffuse cortical necrosis. Many amebic trophozoites with prominent central karyosomes were identified in areas of inflammation and were especially prominent in Virchow-Robin spaces (figure 2). Similar inflammatory changes and amebae were identified within cerebral white matter; occasional microglial nodules were identified within cerebral white matter. No granulomas were seen.

Deparaffinized sections failed to react by indirect immunofluorescence [3] with polyclonal or monoclonal antisera to *Acanthamoeba castellanii*, *N. fowleri* [9], or *Hartmannella vermiformis*, but the trophozoites fluoresced brightly following incubation with rabbit antiserum to *B. mandrillaris* and fluorescein-labeled goat antibody to rabbit IgG (figure 3). Motile trophozoites were detected in Schaedler’s broth inoculated with brain tissue. Subcultures on monkey kidney and monkey lung tissues showed a readily observable foamy cytopathic effect, but the amebae were not evident microscopically.

**Discussion**

Previously known as a leptomyxid ameba, *B. mandrillaris* was first isolated from the brain of a mandril baboon at the San Diego Wild Animal Park (San Diego) [3]. Fundamental differences in morphology and antigenic characteristics mandated creation of a new genus, named in honor of William Balamuth [10]. Following the development of an immunofluorescence assay with use of rabbit antiserum to the washed trophozoites and cysts from cultures of *B. mandrillaris*, a number of human cases of meningoencephalitis were diagnosed retrospectively [3, 11]. Several of these infections had
been initially attributed to *Acanthamoeba* [4–7, 12]. As of January 1997, 63 infections from around the world (30 from the United States) had been reported to the Centers for Disease Control and Prevention; at least seven had occurred in patients with AIDS and eight had occurred in animals [13, 14]. Thus far, this organism has been isolated from humans and other animals only after death and has not been isolated from the environment [15].

Similar to *Acanthamoeba* species, *B. mandrillaris* causes subacute or chronic granulomatous meningoencephalitis eventuating in death within 1 week to several months after the onset of symptoms. The disease presents typically with focal neurological signs (cranial nerve palsies or hemiparesis) and fever and is frequently associated with seizures, headaches, and occasional nausea, vomiting, or diarrhea.

Examination of CSF demonstrates mononuclear pleocytosis (with cell counts ranging from zero to 1,790/mm$^3$ but predominately from 10/mm$^3$ to 500/mm$^3$), an elevated protein level, and normal to low glucose concentrations. For most patients, imaging studies reveal multifocal abnormalities; lesions are typically hypodense, and enhancement is frequently demonstrated [16–18]. CT and MRI often demonstrate mass lesions and therefore lend serious consideration (as in the present case) to other etiologies such as tuberculosis, septic emboli from bacterial endocarditis, and toxoplasmosis.

The patient described herein presented with the typical subacute course, prominent mononuclear pleocytosis suggestive of granulomatous disease, and multiple enhancing intracranial mass lesions. His subacute course contrasts with the much more fulminant course characteristic of meningoencephalitis caused by *Naegleria*. Similar to most pediatric and several adult infections (which occurred in previously healthy patients without overt defects in immune status), this patient also had no under-
lying illness and was HIV seronegative with a normal T lymphocyte count. Hence, *Balamuthia* is clearly capable of infecting the CNS of the immunocompetent host, distinguishing it from *Acanthamoeba* (which favors the immunocompromised host). As the pathogenesis of and the immune response to infection with *Balamuthia* have not yet been elucidated, it is not clear what role this patient’s drug and alcohol use may have played in the development of his infection. Several other cases of *B. mandrillaris* infection in the setting of chronic alcoholism have been reported [3, 19, 20], thus suggesting that alcohol abuse may be a predisposing factor for this infection.

The histopathology of *B. mandrillaris* infection consists of a predominantly chronic inflammatory process, consisting mostly of lymphocytes, monocytes, plasma cells, and rare giant cells [21]. Although true granuloma formation has been described [6, 8, 12, 22], it has also been absent in immunocompetent patients with this disease. The trophozoites and cysts have frequently been noted to be angiotropic, tending to cluster around blood vessels with resultant hemorrhagic necrosis of the meninges and brain [5, 21].

HE staining is sufficient to identify the trophozoites of *B. mandrillaris*, which range in size from 12 μm to 60 μm (mean size, 30 μm). They are typically uninucleated with a prominent dark karyosome, but nuclei with two or three nucleoli have been observed [8, 9, 13]. Cysts may be more readily visualized with either Gomori methenamine–silver or periodic acid–Schiff stains [8, 23]. Cysts range in size from ~6 μm to 30 μm (mean diameter, 15 μm). By light microscopy, cysts appear to be double-walled, with a wavy and irregular outer wall. Electron microscopy has confirmed three layers: a thick, electron-dense innermost layer or ectocyst, a mesocyst, and a thin, irregular outer layer or ectocyst [10, 24].

On HE-stained preparations, *Balamuthia* is very difficult to distinguish from *Acanthamoeba*; thus, further differentiation via immunofluorescence studies is required [25–27]. Although *Entamoeba histolytica* infection would be considered in the differential diagnosis of a brain lesion, brain involvement is very unusual [28], and *E. histolytica* is quite distinct morphologically from *B. mandrillaris*. *B. mandrillaris* is characterized by the presence of a large, densely staining nucleus as well as the presence of two or three nuclei in some trophozoites. In contrast, *E. histolytica* has a single, small karyosome and densely staining chromatin granules lining the nuclear membrane.

*B. mandrillaris* is a fastidious organism and was previously grown only in living tissue culture cells [22], including monkey kidney tissue monolayers [3]. Recently, a cell-free growth medium has been developed, and the successful axenization of three isolates of *Balamuthia* has been reported [29]. With the availability of an easily prepared cell-free growth medium, isolation of the organism may prove more successful in the future and provide the means by which to screen isolates more readily for susceptibility to antimicrobial agents. Moreover, an animal model for infection with this ameba also has been developed recently, and accordingly, the first studies to assess the virulence of *B. mandrillaris* and the evaluation of potential therapeutic regimens to treat GAE have been initiated [30]. In one study [30], 70% of mice with severe combined immunodeficiency inoculated intranasally died of CNS infection and failed to develop an inflammatory response, whereas only 10% of similarly infected immunocompetent BALB/c mice died of CNS infection.

Thus, it appears that *B. mandrillaris* is an opportunistic organism that is capable of infecting the healthy host. Of the 13 adult patients now described in the literature, only one had AIDS, one had diabetes mellitus, and one had chronic renal failure and was undergoing hemodialysis [3]. Five of the 13 patients were noted to abuse alcohol, and two were intravenous drug users. None of the patients described in the pediatric literature were immunocompromised. *B. mandrillaris* also has been identified outside the CNS, including a culture specimen from a thigh lesion [22]. Sites of involvement outside the CNS have included the skin in two previously healthy patients [22, 31] and the kidneys and adrenal glands in a patient with AIDS [32]. Our patient had granulomatous inflammation of the lung, but there was no evidence of amebae; examination of an acid-fast-stained smear of the lung tissue disclosed an moderate number of mycobacteria.

The pathogenesis of infection with *Balamuthia* remains unclear. The route of infection is not known, but the organism was found in a submandibular lymph node of an infected sheep, thus suggesting the site of entry may have been the oral cavity, with subsequent spread to the brain [13]. In a patient with AIDS, multiple foci of infection suggested hematogenous dissemination [32].

Although successful treatment of naegleria encephalitis with intravenous and intrathecal amphotericin B has been reported [33], there is no known effective therapy for balamuthia infection. Treatment with miconazole and 5-fluorocytosine was administered to one patient with *B. mandrillaris* infection [12]; a repeated brain biopsy 9 days after the initiation of treatment demonstrated a diminished number of trophozoites, more cyst forms, and better development of granulomatous inflammation, thereby suggesting a possible therapeutic effect. In vitro testing of a number of antimicrobial agents suggested that pentamidine isethionate may be most effective against *B. mandrillaris* (90% inhibition after 6 days of exposure), but the drug was amebicidal in the axenic system at the highest concentration tested (10 μg/mL) [29]. Fluconazole and ketoconazole were much less effective inhibitors of amebic growth, requiring 10 μg/mL for a significant effect. Amphotericin B was marginal in its effects, and azithromycin, clarithromycin, and trimethoprim-sulfamethoxazole had no effect [29].

*B. mandrillaris* infection should be included in the differential diagnosis of intracerebral mass lesions in both the immunocompetent and the immunosuppressed host. Although *Balamuthia* has not yet been demonstrated in CSF, consideration of this entity should prompt examination of a wet-mount speci-
men and culture of this fastidious organism on appropriate axenic media and cell monolayers. Biopsy has been necessary in the past for diagnosis and should be pursued early. Immunohistological testing of tissue sections is invaluable in the definitive identification of this ameba. Much remains to be learned about the pathogenesis, epidemiology, virulence factors, and optimal treatment of this heretofore invariably fatal parasitic infection.

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References