Fatal Granulomatous Meningoencephalitis associated to Mycobacterium Mucogenicum-like Microorganism: a Case Report

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Mycobacterium mucogenicum is rarely associated to human infections. However, in the last year, a few reports of sepsis and fatal cases of central nervous systems have been documented. Here we report a fatal case of granulomatous meningoencephalitis of three weeks of evolution where DNA from a M. mucogenicum-like microorganism was identified post-mortem in samples of brain tissue.

Keywords: M. mucogenicum, CNS, immunocompetent, Meningoencephalitis

Since the early 1980's there has been an increase in diseases caused by nontuberculous mycobacteria (NTM); mycobacteria other than Mycobacterium tuberculosis and M. leprae. Mycobacterium mucogenicum is a common environmental mycobacterium found in water and soil with a worldwide distribution. Until its identification as a new taxon, it was known as Mycobacterium chelonae-like organisms (1). It was first identified as causing diseases in humans in 1982, during two outbreaks of peritonitis associated with peritoneal dialysis in the United States (2). Recent reports have identified this mycobacterium in immunocompetent patients. Multiple infections were described in a patient with the diagnosis of Münchausen syndrome most likely due to self-inoculations (3). The Central Nervous System is rarely involved in M. mucogenicum symptoms, however, two unrelated fatal cases, one of lymphocytic meningitis and other of a cerebral thrombophlebitis in immunocompetent patients have been described (4). Molecular techniques to identify NTM include nucleotide amplification followed by restriction fragment length polymorphism (RFLP) or phylogenetic reconstructions. The ß subunit of RNA polymerase (rpoB gene) (nucleotide sequence 2573 to 3337) showed the highest genetic heterogeneity within the clinical isolates of M. mucogenicum and M. abscessus (5-6).

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Case Report

Case of a 42 year-old male who presented himself at the emergency room on Day 0 with history of headache, fever, chills, dizziness, myalgias and prostration of three weeks duration. In the previous three weeks he sought ambulatory treatment and was treated with analgesics for the headaches. On the day of admission he complained of fever, headache, blurred vision and feeling ataxic. Physical examination was remarkable for neck tenderness. Babinski, Brudzinski and Kernig signs were negative. He also brought results of a head CT scan performed five days before which revealed changes suggestive of old lacunar infarcts and a hypodense cystic area on posterior aspect of right internal capsule. He was admitted with a diagnostic (vs. clinical) impression of meningoencephalitis. On day 1, neurologist noted mild sixth cranial nerve palsy. There was no photophobia. Spinal tap revealed clear yellow fluid with increased protein 457 (15-45 mg/dL), reduced glucose 27 (40-80 mg/dL), diminished chloride 109 (119-129 meq/l), increased RBC's 140.8 (0-2 cmm), and leukocytosis with monocytic predominance 84%. That same day a brain MRI showed tiny areas of hyperintensity in basal ganglia and right frontal location but no evidence of encephalitis. Patient was started on Acyclovir, Ampicillin and Azithromycin IV. On day 2, serology and microbiology results were reported as negative. On day 3 Herpes B serology (Cercopithecine herpesvirus 1) was ordered due to occupational exposure to non-human primates. That same day, spiking fevers were noted by attending physician. On day 4, the patient became disoriented with respect to time and place. On day 6, Babinski reflex was elicited. That same afternoon, patient's speech became unintelligible; he was unable to

be fully aroused by painful stimuli and sixth nerve palsy worsened as per physical examination. A head CT scan revealed sudden dilatation of the lateral and third ventricles and patient was transferred to the Intensive Care Unit due to increased aggressiveness, disorientation, and abrupt onset of diplopia, blurred vision and vertigo. Because of an anecdotic report of exposure to Mycobacterium tuberculosis more than 14 years ago, he was also started on Isoniazid, Rifampin, Ethambutol, and Pyrazinamide. On day 7, full neurological examination disclosed no spontaneous respiration, no response to painful stimuli, no decerebrate nor decorticate posturing; fixed, dilated pupils unresponsive to light, no response to ice water irrigation, no corneal or gag reflexes and no deep tendon reflexes. An electroencephalogram was subsequently performed yielding an isoelectric line, a finding compatible with brain death. Patient's condition continued to deteriorate and death ensued on the ninth day after his admission.

Results

Negative microbiology results included Herpes simplex PCR, Cryptococcus antigen, spinal fluid bacterial culture (Gram and Ziehl-Neelsen acid-fast staining), HIV 1 and 2 serology, blood culture, spinal fluid fungi culture, urine culture, anti-streptolysin O titer, VDRL-RPR and Cytomegalovirus IgG. Immunoglobulin quantification assays revealed low IgG and IgM values (595.00 mg/dL and 60.5 mg/dL, respectively). A leukocytosis with neutrophilic predominance of 80.3-88 % was present in all complete blood counts.

At the autopsy the most significant findings were located in the brain, heart, lungs and liver. Examination of the heart revealed cardiomegaly, left ventricular hypertrophy, subendocardial necrosis, early fibrosis and increased lipofuscin pigment , consistent with hypertensive vascular disease despite negative history. Lungs were heavy and edematous but there was no evidence of pulmonary tuberculosis or granulomatous disease. Liver showed mild macrovacuolar steatosis, cholestasis and scattered areas of hepatic necrosis. Examination of the brain demonstrated extensive, diffuse lymphoplasmatic leptomeningeal infiltrates accompanied by multinucleated giant cells, non caseating granulomas and necrosis (Figure 1). There were perivascular inflammatory infiltrates with occasional hemosiderin laden macrophages extending into the Virchow-Robin spaces. There were microglial nodules present on the sections of the hippocampus, pons and medulla. No viral cytopathic changes were seen. Autopsy results were confirmed by the Armed Forces Institute of Pathology staff.

In order to conduct molecular biology studies, several parts of the brain were processed for DNA extraction. Samples were obtained both from paraffin embedded and formalin fixed samples. DNA was subject to PCR amplification for herpes B virus, amoebas and mycobacteria. This patient worked with non-human primates during 15 years as veterinary supervisor technician. Because of that, special emphasis was placed in the diagnosis of herpes B virus. Both serological and PCR studies were negative when tested in our Virology Laboratory and at the National Reference B virus Laboratory, Atlanta, GA. PCR for Acanthamoebas, Balamuthia and Naegleria were negative when tested in our laboratory (7-9). Stained slides were examined at the Division of Parasitic Diseases, National Center for Infectious Diseases, CDC, Atlanta GA, for Acanthamoebas, Balamuthia, Naegleria and Sappinia and structures compatible with these organisms were not found. Immunofluorescence conducted on unstained slides using anti-amoebic sera (Acanthamoebas, Balamutia and Naegleria) was also negative. Patient serum was tested for antibodies to Acanthamoebas and Balamuthia and titers (1:32 and 1:16 respectively) were not indicative of infection with these amoebas.

After amplifying DNA extracted from 35 different parts of the encephalon, a robust PCR band at the expected size (360 bp) was obtained from two samples representing the temporal brain lobe. To characterize the mycobacterium a RFLP was done essentially according to Lee, et al (10). The restriction pattern produces five MspI bands (157, 95, 57, 45, 21 bp) and four Hae III bands (197, 107, 32, 63 bp) (results not shown). Because RFLP pattern from other M. mucogenicum strains were not available for comparison, nucleotide sequencing was performed to clarify the origin

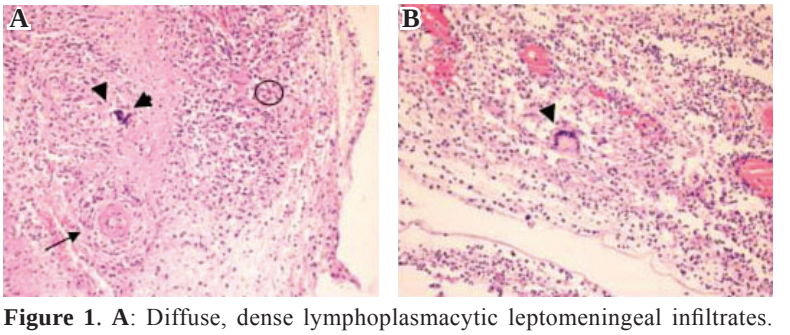

Figure 1. **A**: Diffuse, dense lymphoplasmacytic leptomeningeal infiltrates. Note meningeal congestion (right) and a non caseating granuloma (arrow) with associated multinucleated giant cell (upper left, arrow head). There are also occasional hemosiderin laden macrophages (inside circle) and perivascular inflammatory infiltrates, also seen in B. **B**: Multinucleated giant cell (arrow head) within a dense lymphoplasmacytic infiltrate (20 X).

of this PCR band. The amplified sequence showed the maximal homology of 86% percent with M. mucogenicum (data not shown).

Then we amplified a region of the rpoB gene that has been used for phylogenetic reconstruction and identification of interspecies strains. For the first and the nested PCR we used the pair of primers MycoF and MycoR and MycoseqF and MycoSeqR respectively (5). The PCR was sequenced using internal vector primers on an ABI Prism 3100 Genetic Analyzer (Perkin-Elmer Applied Biosystem). The resulting sequence was aligned with available sequences in the GeneBank using the program Clustal W (11). Phylogenetic reconstruction was performed using the PHYLIP (version 3.66) software package (12). The amplified sequence from this case clustered with M mucogenicum sequences as defined by Neighbor-joining (NJ) and Parsimony (PARS) methods with a 99.6% and a 73.7% of bootstrap values respectively (Figure 2).

Discussion and Conclusions

M. mucogenicum is considered a rapidly growing mycobacterium (RGM) and an environmental organism. However, like other species of this group it was initially isolated from a patient. In the present case, a potential contamination from tap water during the autopsy could be suggested as the original source of this agent. However, if that were the case, we could expect more than two positive tissue samples from those 35 tested. Additionally, the identification of this agent in tap or drinking water required a concentration process of 500 to 50 ml of water followed by subsequent bacterial cultures. (13-15). The count of nontuberculous mycobacteria in water samples have been determined to be 1 to 20 colonies in 500 ml (13). The running water from the autopsy room was only in contact with the encephalon surface while washing it. After that, water residues were displaced by the buffers used: embedding in paraffin or fixed by formalin. Since after this process, only few milligrams of formalin-fixed tissue or paraffin embedded tissues were used for the DNA extraction, it is unlikely we would get a positive PCR directly from the samples. In addition, the 1 ml water sample from the autopsy room used as control was negative.

The microorganism was not isolated because at the moment of the PCR identification, adequate samples for culture were not longer available. However, several facts support this microorganism as etiological agent. The clinical presentation of neurological symptoms without meningeal signs, as well as the fulminant evolution resemble the cases of the two previously reported patients (4). In addition, the cerebrospinal fluid profile of this patient is consistent with the presence of bacterial growth, while the negative standard bacterial culture supports the presence of an uncommon microorganism. The presence of a mycobacterium is reinforced by the exclusion of other potential agents like amoebas and the presence of a granulomatous reaction and a lymphoplasmacytic infiltrate in the brain sections.

There was no evidence of immunodeficiency in this patient. However, roommates reported daily alcohol intake for the last 14 years. Despite that, this person had no impaired social nor vocational functioning. It is known that the acute and chronic use of alcohol dampers the immune system. Impaired host defense after alcohol exposure appears to be linked to a combination of decreased inflammatory response, altered cytokine production, and abnormal reactive oxygen intermediate generation (16-17). The cellular immunity, which is essential for an effective immune response to intracellular pathogens like mycobacterium, is particularly affected (18).

The source of infection with this microorganism is quite difficult, if not impossible, to determine. Because of the generalized symptoms present and the febrile syndrome three weeks before the admission, it is unlikely that the infection was nosocomial.

In our opinion, after considering all circumstances including clinical presentation, the CSF profile of this patient, the anatomic-pathological findings and the phylogenetic reconstruction results, we conclude that a Mycobacterium mucogenicum-like organism is the most plausible causal agent involved in the clinical evolution (and subsequent demise) of this patient.

To our knowledge, this is the first report of a M. mucogenicum-related organism associated with a Granulomatous Meningoencephalitis.

Resumen

Desde los comienzos de los años 80 se ha reportado un incremento en las enfermedades causadas por micobacterias no-tuberculosas (MNT) y otras micobacterias distintas a M. leprae. Mycobacterium mucogenicum es una micobacteria ambiental que se encuentra fundamentalmente en los suelos y aguas alrededor del mundo. Hasta su identificación como un grupo taxonómico independiente, fue clasificado como microorganismo relacionado al Mycobacterium chelonae. Su primera asociación con una enfermedad en humanos fue en 1982, Estados Unidos, donde se identifico como agente causal en dos brotes de peritonitis durante diálisis peritoneal. Reportes mas reciente han encontrado este agente en pacientes inmunocompetentes. En un caso de infecciones múltiples en una paciente

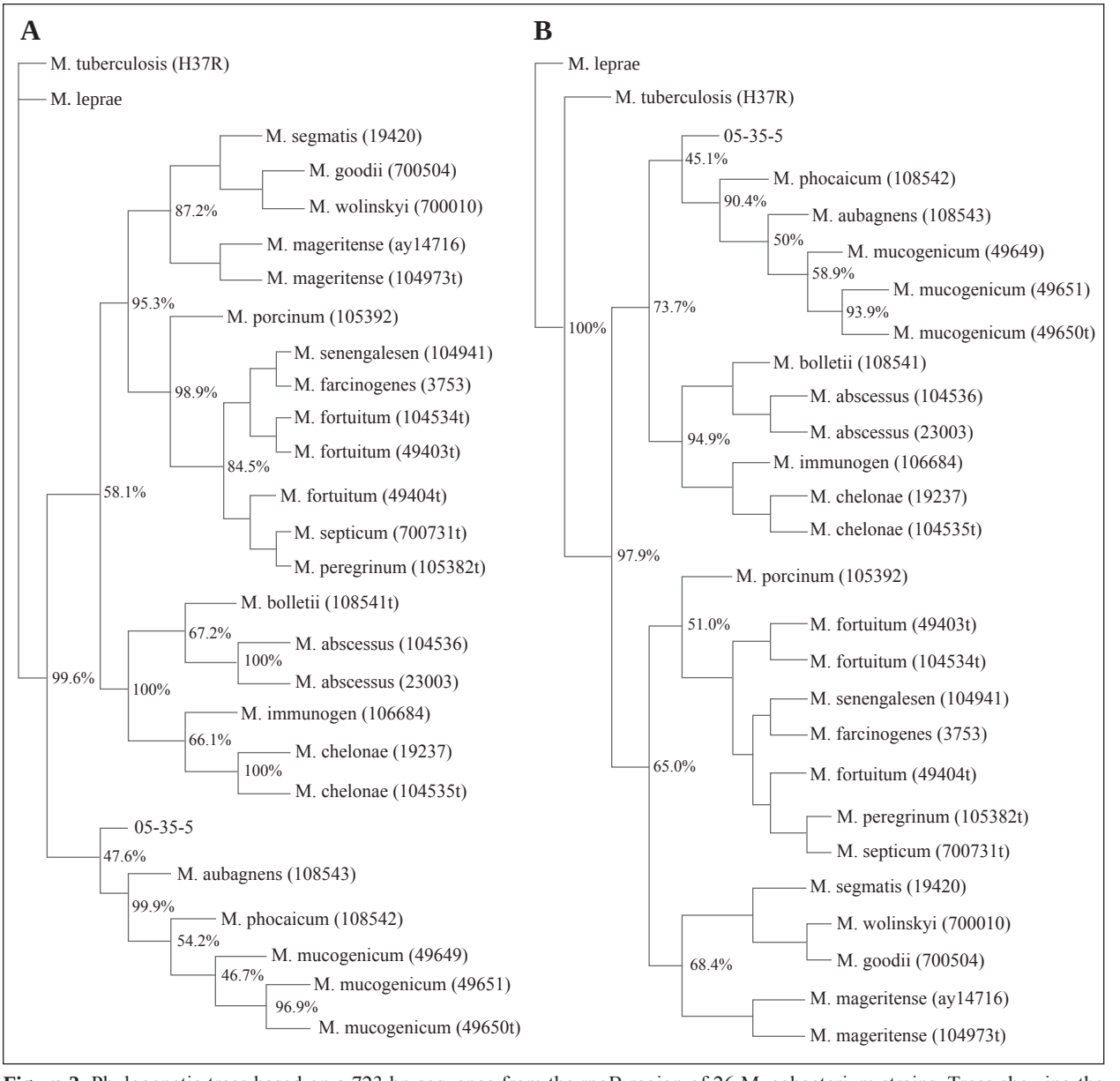

Figure 2. Phylogenetic trees based on a 723 bp sequence from the rpoB region of 26 Mycobacterium strains. Trees showing the segregation of the 05-35-5 sequence amplified in this work along other M mucogenicum sequences. Phylogeny was determined (A) by use of Kimura 2-parameters analysis for distance estimates or (B) by use of the DNAPARS program included in the PHYLIP software package. In A the tree was constructed by use of the Neighbor-joining method. A bootstrap analysis (1000 repeats) using M tuberculosis and M leprae as outgroups was performed to evaluate the topology of the phylogenetic trees. Bootstrap values (out of 1000) are given at each relevant node. Values above 70 % were considered significant. 05-35-5 is an arbitrary designation of the sequence amplified on this work. In parenthesis, EMBL's accession numbers of the other sequences used for this analysis are given. Gene Bank Accession number for MMQ-5 sequence: EU018141.

con síndrome de Münchausen, probablemente debido a lesiones auto inflingidas t aunque manifestaciones del sistema nerviosos central han sido asociada solo en raras ocasiones al Mycobacterium mucogenicum, al menos dos casos de fatales se han reportado también en pacientes inmunocompetentes. Uno de meningitis linfocítica y otro con una tromboflebitis cerebral. Entre las técnicas moleculares utilizadas para identificar a las MNT se encuentran la reacción en cadena de la polimerasa, el análisis de fragmentos de restricción y la reconstrucción

filogenética. El segmento geonómico mas utilizado, por presentar el mayor por ciento de heterogeneidad dentro de los aislados de M. mucogenicum y M. abscessus, es el correspondiente a la subunidad ß de la RNA polymerasa (gen rpo ß). En este trabajo, aplicando estas tecnicas, nosotros describimos un caso de meningoencefalitis granulomatosa con un desenlace fatal en tres semanas asociado a la detección de ADN de un microorganismo relacionado al M. mucogenicum en muestras de tejido cerebral tomadas post-mortem.

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