# Mycoplasmal cerebral vasculopathy in a lymphoma patient: presumptive evidence of Mycoplasma pneumoniae microvascular endothelial cell invasion in a brain biopsy

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Mycoplasmal cerebral vasculopathy in a lymphoma patient:  presumptive evidence of *Mycoplasma pneumoniae* microvascular endothelial cell invasion in a brain biopsy

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Abstract

A 73-year-old man had episodic encephalopathy, ataxia and neuropathy. Symptoms largely resolved but adenopathy later lead to the diagnosis of a low-grade follicular lymphoma. The neurological symptoms soon recurred with new pontine calcifications identified by computed tomography. Brain biopsy revealed microvascular endothelial cell nuclear changes. Electron microscopy identified small polymorphic bacteria without a cell wall and with terminal and attachment organelles within endothelial cells and clustered in some microvascular lumina. Immunostaining was positive for *Mycoplasma pneumoniae* and convalescent serum enzyme immunoassay was positive for *M. pneumoniae* IgG. The patient again recovered and he was neurologically stable 33 months after the initial episode. The ultrastructural findings of the bacterial cells are distinctive of some mycoplasmal species when compared to other small bacteria. *Mycoplasma*-like organisms are reported in four autopsied patients who had chronic encephalopathy, movement disorders, and some of the same light- and electron-microscopic findings in the brain as our patient. Direct neuroinvasion by *Mycoplasma* species has been suggested, while anatomic observations in our patient and in the four autopsy cases show microvascular invasion but not parenchymal invasion. Most mycoplasmal encephalitis may be immune-mediated. The frequency of neurovascular invasion is not known. It may be rare and it may persist.

# 1. Introduction

Neurological symptoms due to an infection by a mycoplasmal species are documented in the first report of primary atypical pneumonia before the nature of the transmissible agent was appreciated [1]. Neurological findings include encephalopathy, psychosis, choreoathetosis, ataxia, brainstem encephalitis, optic neuritis, diplopia, Bell's palsy and other cranial nerve palsies, aseptic meningitis, meningoencephalitis, acute disseminated encephalomyelitis (ADEM), acute hemorrhagic leukoencephalitis (AHLE), transverse myelitis, polyradiculitis, brachial plexus neuropathy, motor neuropathy, and Guillain-Barré syndrome [2-11]. Neurological symptoms typically arise 1 to 2 weeks after a respiratory infection and they may be treated empirically with macrolide antibiotics, even though most macrolides penetrate the blood-brain barrier poorly, as well as with tetracycline and quinolones. There is some controversy concerning the need for antibiotic therapy since many mycoplasmal infections resolve spontaneously [8,12-15]. *Mycoplasma pneumoniae* is the best-studied and perhaps the most frequent mycoplasma in neurological disease [8,11]. Infections with this organism can persist for months and antibiotic treatment may not shorten the period of persistence [15]. Neurological as well as other sequelae can be debilitating or fatal and treatment has been recommended when there is laboratory evidence of a mycoplasmal infection [3,8,10].

The nervous system is a major extrapulmonary target of *M. pneumoniae* and nervous system involvement may occur without a preceding respiratory infection. This provides a diagnostic dilemma when no other family or community infection has been recognized and when the entire disease course is extrapulmonary. Extrapulmonary invasion by *Mycoplasma* species has an unknown frequency since it has been so seldom identified by laboratory testing until recently, particularly in adults [8,11].

Mycoplasmal encephalitis has long been considered to be immune-mediated, perhaps through molecular mimicry involving the plasma membrane and lipoglycan capsule, while a hypercoagulable state may lead to stroke [10-13]. However, direct neuroinvasion has been increasingly suggested from the results of immunoglobulin G (IgG), RNA hybridization, and polymerase chain reaction (PCR) testing of cerebrospinal fluid (CSF) and brain tissue [10,16-18]. These sensitive and specific tests provide indirect evidence that has not adequately addressed tissue or cellular localization.

Zu-Rhein et al. [19] report *Mycoplasma*-like organisms (MLO) in endothelial cell cytoplasm and in the lumen of central nervous system (CNS) microvessels in three patients who died after 2 to 11 years of a chronic, progressive motor and cognitive disorder. Ferreira [20] describes an additional case that fits into the same clinical and pathological parameters as those described by Zu-Rhein et al. [19]. We found ultrastructural features similar to

those first related by Zu-Rhein et al. [19] in a frontal lobe biopsy taken during an episode of encephalopathy. The findings in our patient are compared with the autopsy cases and with the ultrastructure of small bacteria, most without a cell wall.

## 2. Case presentation

The patient was a 73-year-old man hospitalized with new onset encephalopathy and ataxia. Two months prior to admission headaches began and increased in frequency and severity, sometimes accompanied by a visual aura. Tremors were reported for which primidone was given and this was later changed to propranolol. There was episodic confusion with word finding difficulty. Outpatient laboratory studies revealed a white blood cell (WBC) count of 4.8 x $10^9$/L (reference range: 3.4-11.2 x $10^9$/L), an absolute lymphocyte count of 1.3 x $10^9$/L (1.4-2.9 x $10^9$/L), monocytes 11.7% (2.0-11.0%), and platelets 101 x $10^9$/L (150-450 x $10^9$/L). Headaches ceased and then recurred. The patient was admitted after he awoke with more severe confusion and some balance difficulty. On examination he was afebrile and he had mild distress, garbled speech and episodic aphasia and cognitive changes. By the time of a formal neurological examination he had relatively fluent speech with difficulty in naming and comprehension. He had a normal cranial nerve examination, no focal weakness and a mildly unsteady gait. Admission laboratory studies showed WBC 4.1 x $10^9$/L (4.8-10.8 x $10^9$/L), absolute lymphocyte count 1.1 x $10^9$/L (1.5-3.5 x $10^9$/L), monocytes 13% (4-8%), and platelets 79 x $10^9$/L (130-400 x $10^9$/L). No platelet antibodies were found. Flow cytometry of blood revealed a mild decrease of the maturing myeloid population with absent CD64 and mild monocytosis with no aberrant immunophenotype. Blasts were present in the peripheral blood. These findings were interpreted as nonspecific. The serum anti-nuclear antibody level (1:80 titer) was borderline positive with a nucleolar staining pattern. Serum $B_{12}$ and folic acid levels, C-reactive protein, erythrocyte sedimentation rate, thyroid stimulating hormone level, free $T_4$, and serum ammonia were within references ranges. Negative serological studies included an anti-nuclear cytoplasmic antibody screen, rheumatoid factor, cryoglobulins and rapid plasma reagin as well as anti-cardiolipin, anti-SS-A/Ro, anti-SS-B/La, HIV and Lyme disease antibodies. Lumbar puncture revealed < 1 WBC x $10^6$/L (< 5 x $10^6$/L) without atypia, glucose 3.6 mmol/L (2.8-4.4 mmol/L), protein 400 mg/L (120-600 mg/L), no oligoclonal bands, and a normal IgG index and synthesis rate. Examination of CSF for varicella zoster virus (VZV), herpes simplex virus (HSV) and protein 14-3-3 was negative. Chest x-ray did not show any active disease and no respiratory symptoms were documented. CT and MRI showed a small remote left cerebellar infarct. Cerebral angiography did not show any evidence of vasculitis, but the left vertebral artery was occluded at

its origin.  Single-photon emission computerized tomography showed multifocal diminished uptake in the left

frontal, temporal and parietal lobes.  Electroencephalography revealed no focal abnormalities.  He developed

increasing confusion and ataxia during the hospitalization.

Vasculitis and infection were considered initially, but neither seemed to be confirmed.  His symptoms

improved significantly after 2 months without specific intervention and galantamine was started.  Positron-emission

tomography showed some diminished uptake in the frontal lobes and even less uptake in the temporal and parietal

lobes.  Eleven months after the headaches began he developed numbness over the dorsum of his feet and tightness of

the soles.  Electromyography and nerve conduction studies revealed an axonal motor-sensory neuropathy without

evidence of radiculopathy.  The patient continued to show cognitive improvement and he resumed driving and work-

related responsibilities.  Neuropsychological testing revealed variable impairment of executive function, visual and

verbal memory, and motor planning and programming.  A grade 1 follicular lymphoma was discovered in a bone

marrow biopsy 13 months after the initial headaches following investigation of extensive lymphadenopathy.  The

patient responded well to rituximab and high-dose steroids with no significant complications.  His neurological

status remained stable with sporadic mild headaches and no visual auras.  Sixteen months following the initial

headaches, and 2 months after lymphoma therapy was begun – and 4 days following an unremarkable follow-up

examination – he was admitted with increasing fatigue, visual changes, ataxia and confusion that resembled his

initial symptoms.  MRI did not reveal new findings, but CT showed new pontine calcifications (Fig. 1).  Testing for

serum autoantibodies to dsDNA, Scl-70, tissue transglutaminase, and voltage-gated potassium channels was

negative.  Lumbar puncture values remained normal.  CSF studies for VZV, HSV, JC virus, cytomegalovirus,

Epstein-Barr virus, enterovirus and cryptococcus were negative.  The patient improved once again without specific

intervention and he was discharged.  He was readmitted 2 days later with renewed aphasia, cognitive changes and

gait difficulty.  A brain biopsy was performed to investigate the possibility of an inflammatory disorder including

vasculitis, non-vasculitic autoimmune inflammatory meningoencephalitis, or changes related to lymphoma therapy.

The biopsy of right frontal neocortex revealed many scattered capillaries and small venules with enlarged

endothelial cell nuclei including some vessels with nuclear clusters.  Many of the enlarged nuclei had mildly

marginated chromatin or general pallor and a few were hyperchromatic.  The enlarged endothelial cells extended

into the vascular lumina (Fig. 2).  No fibrin strands were found.  With CD34 immunostaining the enlarged

microvascular nuclei were surrounded by stain and they were therefore in swollen endothelial cells (Fig. 3A).  A few

neurons were atrophic, hypoxic changes were minimal and there was mild gliosis.  There was no evidence of

lymphoma and both in-situ hybridization and immunostaining studies were negative for B cells. There were numerous CD3$^+$ T lymphocytes within many of the microvessels and a few appeared to be in narrow perivascular spaces but not demonstrably within the parenchyma. CD68 revealed a few intraluminal WBCs. CD68 also stained some scattered resident microglia and many perivascular microglial cells. All the microglia were relatively small with thin branching processes. There were no intranuclear or cytoplasmic inclusions. No white matter was present. A small region of leptomeninges present contained many scattered CD3$^+$ T cells. Dura was normal.

A presumptive diagnosis of mycoplasmal encephalitis was made, with electron microscopy and immunostaining for micro-organisms not yet undertaken. Low-toxicity treatment with azithromycin was initiated, although some improvement had already begun. Routine examinations performed in the patient's rehabilitation facility showed no evidence of pulmonary disease. Enzyme immunoassay for serum anti-*M. pneumoniae* IgG was positive (2.97 OD ratio; reference laboratory positive > 1.10 OD ratio) and serum PCR was negative for the organism. There was significant recovery of cognition and gait over the course of 8 weeks. The patient underwent further lymphoma therapy and he had no neurological deficits 33 months after the initial headaches.

Immunostaining was performed on the cerebral biopsy using anti-*M. pneumoniae* antibody (1:20, 1:50, 1:100, 1:200; rabbit polyclonal, cat. no. M9750-27A, U.S. Biological, Swampscott, MA, USA) with citrate-based antigen retrieval. Cytoplasm around atypical endothelial cell nuclei in a few microvessels and a few microvascular lumina were heavily stained (Fig. 3B). The intensity of the reaction product diminished slightly as dilution increased. The majority of cortical vessels were negative even when enlarged or clustered endothelial cell nuclei were present. Rare arachnoidal microvascular lumina were positive. Rabbit anti-calretinin control antibody gave light background staining in some vascular lumina, much below the heavy mycoplasmal staining, and a few vascular wall cellular nuclei were positive but not any cytoplasm. Rabbit anti-prostate specific antigen antibody staining only resulted in light luminal background staining in a few blood vessels. Immunostains for HSV, human herpesvirus type 8, cytomegalovirus (CMV) and adenovirus were negative. PCR of formalin-fixed, paraffin-embedded biopsy tissue performed at a reference laboratory was noninformative for *M. pneumoniae*.

Neocortical biopsy tissue was reprocessed for electron microscopy from the paraffin block. It revealed that most microvascular endothelial cell nuclei were enlarged and they often protruded along with swollen, lucent cytoplasm to compromise the lumen. Basement membrane duplication was present in most microvessels (Fig. 4A). No basement membrane densities of immune-complex type were found. A few of the enlarged nuclei were atypical with complex distortions of their outline due to deep invaginations or lobulation (Fig. 4B), but no multinucleated

endothelial cells were found.  Both the enlarged and the atypical nuclei had little remaining fine chromatin.  Many

capillaries contained red blood cells and a few microvessels contained mononuclear cells (Fig. 4C).  Some

microvascular lumina were partially empty (Fig. 4A), while many of the lumina were thin slits filled with fine,

dense, granular material (Figs. 4B and 5A).  No platelets or fibrin strands were present in any of the blood vessels.

The swollen, lucent endothelial cells had a paucity of organelles.  A few capillaries with thin, dense endothelial cell

cytoplasm were collapsed.  Rare hyperplastic microvascular complexes containing several lumina had nuclear

enlargement with loss of fine chromatin in endothelial cells, pericytes and smooth muscle cells, and there was no

adventitial collagen present.  There were a few neurons with apoptotic-appearing nuclei and a few reactive

astrocytes were seen.  A few small phagocytic cells with dense bodies were scattered in the parenchyma.

Small bacteria were in swollen endothelial cell cytoplasm of scattered capillaries and larger microvessels, and

some of the bacterial cells were clustered in the lumen.  Most bacteria were spherical, dense and in the 100–600 nm

size range.  Their plasma membrane was trilaminar and no cell wall material was identified.  One cell pair had the

appearance of undergoing binary fission.  Occasional dense attachment organelles were present.  Many

mitochondria in capillaries and in neural cells were mildly hyperplastic with a few parallel, flattened, lamellar

cristae.  Mitochondrial matrix degeneration was not apparent (Fig. 5).  A few bacterial cells were relatively lucent

but not entirely empty.  The smallest and some of the intermediate-size bacterial cells were filled with many dense

15-nm granules, most likely ribosomes, and with round structures 20 nm in diameter with walls of dense globular

subunits and a lucent 10-nm core.  These round structures tended to congregate just under the plasma membrane but

many cells had them more widely distributed.  A few bacterial cells up to 1 μm in length were curved or dumbbell-

shaped, including a few with nucleoids (Fig. 6A).  Several spherical to oval and flask-shaped bacterial cells had

long, thin, lucent or dense terminal organelles (Fig. 6B), one of which was attached to the endothelial cell basement

membrane by a dense attachment organelle (Fig. 6C).  Terminal organelles connected a few spherical bacterial cells

(Fig. 6D).  A large bacterial cell had regions of low and high density set off by a slight constriction (Fig. 6E).  It was

in a large perivascular cell together with curved and dumbbell-shaped cells.  An intraluminal macrophage contained

putative bacterial cells in phagosomes (Fig. 6F).  No viral particles or intranuclear inclusions were found.


## 3. Discussion

Mycoplasmas are small bacteria bounded by a plasma membrane and devoid of a cell wall [8].  *Mycoplasma*

species are referred to as MLO when serologic or genetic identification is lacking [19,21], but the ultrastructural

features of *Mycoplasma* species are unique and distinctive.  Most cells are in the 400 nm to 2 μm size range and they have variable granular and filamentous densities, 15-nm ribosomes and 20-nm circular arrays of globular densities with a lucent 10-nm core.  Large cells can be spherical, oval, elongated, dumbbell-shaped or flask-shaped.  Nucleoids are an inconstant feature [8,22-24].  Oval and elongated cells can have constrictions separating regions with antigenic and electron-density differences [22,25].  Long, thin terminal organelles may have an electron-dense core, which is characteristic of several species including *M. pneumoniae*.  Dense attachment organelles are at the leading end of mobile cells, including terminal organelles, and they attach to other *Mycoplasma* cells, host cells, and basement membranes.  Attachment organelles on cells without terminal organelles are focal membrane densities [8,23,25-29].

In our case, bacterial cells have no cell wall and they are spherical, oval, slightly constricted, elongated, dumbbell-shaped or flask-shaped.  They have the densities (cytoplasmic and nucleoid material), terminal organelles, attachment organelles, and size range distinctive of mycoplasmal cells.  Terminal organelles appear to connect some bacterial cells together and one terminal organelle attaches to a basement membrane by the attachment organelle.

*Ureaplasma urealyticum*, very closely related to the *M. pneumoniae* group [8], is uniformly oval, elongated or dumbbell-shaped in culture and it has no cell wall, but it does not have the polymorphic shapes or size range of *Mycoplasma* [30,31].  The order *Rickettsiales* contains small bacteria that can infect vascular endothelial cells [32], including *Ehrlichia* with a cell wall [33], spherical *Anaplasma* with no polymorphism [30], and *Rickettsia* species with a distinctive, spike-like outer microcapsular layer [34].  *R. sennetsu* has a very thin microcapsular layer that may be difficult to detect, but it lacks the polymorphism of mycoplasmal cells [31].  The only small bacteria without a cell wall that have a large size range, polymorphic and distinctive shapes, and terminal and attachment organelles are *Mycoplasma* species.

*M. pneumoniae*-specific IgG detected by enzyme immunoassay in our patient's convalescent serum and positive immunostaining for *M. pneumoniae* in cerebral microvessels suggest the identity of the organism found by electron microscopy.  However, serum IgG can be positive in an adult with a remote infection [8,11].  The antibody used for immunostaining is not reactive with human serum according to the manufacturer's specificity statement, and light background with control stains suggests that serum proteins are not labelled adventitiously, but the antibody to *M. pneumoniae* is not absorbed and it may react with related organisms.  While PCR in our patient is noninformative, a negative PCR result for *M. pneumoniae* is common in convalescent serum [8].  Negative PCR results are also common in formalin-fixed, paraffin-embedded tissue even with newer methods because of molecular

cross-linking by formalin, limited amounts of intact DNA in tissue sections, and the presence of PCR inhibitors [35]. With these results, we report the identification of *Mycoplasma* species by electron microscopy with presumptive species identification by serum IgG and immunostaining positive for *M. pneumoniae*.

The pathogenesis of mycoplasmal encephalitis is thought to include direct brain invasion, vascular injury, immune-mediated injury, a hypercoagulable state, and toxicity [10,11,13,36]. Direct *M. pneumoniae* parenchymal or vascular infection has not been sufficiently documented with IgG and PCR testing of CSF or with PCR of brain tissue [16-18,37]. Tsiodras et al. [10] suggest that transfer of antigen or DNA by antigen-presenting cells could result in false tissue localization. Mycoplasmal cells, antigen or DNA within WBCs responding to ADEM, AHLE, or less destructive immune-associated lesions in patients with a mycoplasmal infection are possible sources of microbial detection in brain tissue [4,6,17].

The clinical course in our patient and the pathological finding of neurovascular invasion resemble key findings in the four autopsy cases of MLO [19,20]. In our patient most bacterial cells are within endothelial cells, a few are clustered in capillary lumina, and a few intraluminal and perivascular phagocytes contain putative *M. pneumoniae*. No bacterial or inflammatory cells are identified outside the perivascular spaces in the neocortical biopsy. Zu-Rhein et al. [19, and personal communication] also demonstrate CNS microvascular endothelial cell invasion and intraluminal bacterial cells without finding parenchymal invasion. Zu-Rhein and colleagues do not identify inflammation in routine stains, which is also our finding. Immunostained T cells in our case are sparse in perivascular spaces and no vascular wall infiltration is detected so that vasculitis is not present. The leptomeninges contain enough T cells identified by immunostaining to reflect a mild chronic leptomeningitis associated with mycoplasmal vasculopathy. This might correlate with meningeal signs associated with some mycoplasmal infections [10].

Our remarks are focused on *M. pneumoniae* since it is the most likely species to cause these infections [8,11]. The rate of mycoplasmal encephalitis complicating *M. pneumoniae* infection is very low, but *M. pneumoniae* may be the cause of up to 10% of childhood encephalitis cases. Evidence of pediatric *M. pneumoniae* encephalitis is obtained from CSF, blood or tracheobronchial secretions [10,38]. Direct neuroinvasion in pediatric patients has been questioned since the other postulated mechanisms of nervous system damage have more support [39].

Our patient did not have respiratory symptoms during his disease course and he was recovering prior to antibiotic therapy so no laboratory testing of CSF or tracheobronchial secretions was done at his rehabilitation facility. Respiratory symptoms and laboratory evidence of respiratory tract *M. pneumoniae* can be absent in patients

with *M. pneumoniae*-related encephalitis, particularly cases with evidence of *M. pneumoniae* in the CSF [38]. It is of note that lymphocytic responsiveness increases in vitro to *M. pneumoniae* antigen in patients with *M. pneumoniae* pneumonia while lymphocytes do not respond above the control level in patients with acute *M. pneumoniae* encephalitis [40]. Most cases of mycoplasmal encephalitis are thought to be immune-mediated in association with a respiratory infection even though some patients have only extrapulmonary findings [8,10,36].

Perivascular lymphocytic infiltration is reported in anatomic studies of mycoplasmal encephalitis when there is no evidence of bacterial invasion and immune-mediated vascular injury is assumed [4,6,17]. The basis for a poor lymphocytic response in the rare cases of neurovascular invasion is not known. In our patient, effects of lymphoma, immunosuppressive therapy and CD64 deficiency could alter the cellular immune response. CD64 is a key receptor for antigen presentation [41] and for inflammatory cell attachment to endothelium [42]. The autopsy cases, on the other hand, have no known immunosuppression [19,20], and episodic mycoplasmal encephalitis is well known to occur in nonimmunosuppressed individuals [36]. The cases with neurovascular invasion are not observed at an early clinical stage and significant cellular inflammation may have been missed.

Endothelial infection by bacteria causes cytoplasmic downregulation with muted cytokine production and thrombogenic signalling [32,43]. *Mycoplasma* species secrete enzymes that cause oxidative and DNA damage to contribute to cellular dysfunction [26] and some *Mycoplasma* species produce toxins [10]. *M. pneumoniae* produces a cytotoxin in vitro [44], but there is no clinical evidence of a neurotoxin produced by *M. pneumoniae* [45]. Endothelial cell nuclear changes in mycoplasmal infection that resemble nuclear enlargement and clusters in some viral infections could be due to damage from mycoplasmal enzymes and possibly from a cytotoxin [19,46-48].

Tissue damage in our case is essentially limited to endothelial cell swelling and nuclear changes that narrow or close the lumen of most microvessels in the biopsy, even though mycoplasmal cells are found only in scattered vessels. The hyperplastic mitochondria are the type found in hypoxia which results from this type of injury [49,50]. The cytoplasmic swelling, like the nuclear injury, may have resulted from soluble factors as well as from direct infection. The only evidence of parenchymal injury is apoptosis in a few neurons and the presence of hyperplastic mitochondria in some cells. Resident microglial cells in the parenchyma have alterations no different than those found in the neocortex of normal elderly individuals [51] and astrocytosis is also no more than expected for age. Our patient's recovery without significant inflammation or ultrastructural evidence of fibrin or platelet thrombus formation is consistent with temporary vaso-occlusion, which has been suggested as a cause of resolving

mycoplasmal encephalitis even when direct brain invasion may not occur [12]. However, thrombi could be missed in our case from sampling error.

The initial course of the autopsy cases is similar to that of our patient, and a mycoplasmal persistence in microvessels may be shared by our case and the autopsy cases. Our patient's findings resemble animal models of acute hypoxia [52] while vascular remodelling with thrombosis and fibrosis in the autopsy cases is similar to models of chronic hypoxia [52,53]. Focal vascular hyperplasia without perivascular fibrosis in our case could have developed during the infection, but since this fits the description of glomerular loops or wickerworks in brains of elderly patients it may be a preexisting condition [54]. The pontine calcifications that developed in our patient are evidence of chronic damage where the earliest lesions are thought to occur in the autopsy cases [19]. Garbled speech and ataxia in our patient represent the most likely brainstem symptoms, but since encephalopathy and the specialized tomography studies demonstrate widespread brain changes, these symptoms do not necessarily reflect brainstem damage.

Circulating immune complexes in patients with both *M. pneumoniae* pneumonia and neurological symptoms are an indication of immune-mediated damage that may arise from macrophage infiltration and cytokine production [5,10,11]. We have no cytokine measurements in our patient and we find no immune-complex deposits in our patient's biopsy, but endothelial cell invasion may not have the same immune-system involvement that is found in patients with concomitant pneumonia, as suggested by lymphocyte immunostaining and in vitro responses and by immunomodulatory effects of neuroinvasive bacteria. In our patient, there remains the possibility of lesion extension, recurrence or late immune-mediated damage such as demyelination.

Intracellular *M. pneumoniae* has been studied in tissue culture [27] but it has not been demonstrated in a clinical infection [11]. Our case suggests that *M. pneumoniae* may be an intracellular human parasitic bacterium. *Mycoplasm*a species with the ability to invade human epithelial cells are in a protective niche [29], and perhaps invasion of brain endothelial cells also provides a survival niche.

The rare identification of mycoplasmal neurovascular invasion does not necessarily differentiate these five cases from all other cases of mycoplasmal encephalitis. There is limited anatomic evidence of mycoplasmal brain involvement, and neurovascular invasion, which will probably remain rarely demonstrated, should at least be a consideration in patients with episodic encephalopathy and movement disorders.

**Acknowledgements**

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Figure Legends

**Fig. 1.** Computed tomography without contrast performed just prior to the frontal biopsy shows focal pontine calcifications that were not present the previous year.

**Fig. 2.** Frontal lobe cortical biopsy.  A. Vascular endothelial cell nuclear enlargement is present in capillaries, H&E staining x 400.  B. A dilated blood vessel has enlarged, clustered endothelial cell nuclei, H&E staining x 600.

**Fig. 3.**  Frontal lobe cortical biopsy immunostaining.  A. The upper capillary has immunostained CD34 in swollen cytoplasm that, together with the nucleus, almost occludes the lumen.  In the lower vessel, CD34 is stained along endothelial cell membranes and in the cytoplasm around enlarged, mildly atypical nuclei.  The endothelial cells are crowded into the lumen, original magnification x 600.  B. Staining with anti-*M. pneumoniae* antibody (1:200 dilution) shows strong cytoplasmic reactivity of enlarged endothelial cells and possibly in the lumen of a small cortical blood vessel, original magnification x 400.

**Fig. 4.** Electron micrographs of frontal cortex blood vessels.  A. Capillary endothelial cells have enlarged nuclei (N) and swollen cytoplasm.  The capillary basement membrane is reduplicated and the lumen (L) is small and almost empty.  B. An atypical endothelial cell nucleus has deep invaginations or complex lobulation of its nuclear envelope. The lumen (L) is a thin slit filled with fine, electron-dense granular material and it is closed by the large nucleus and swollen endothelial cell cytoplasm.  C. A capillary lumen is filled by a red blood cell (*) and a mononuclear cell. Scale bars:  2 μm.

**Fig. 5.**  Electron micrographs of frontal cortex blood vessels.  A. A capillary has an enlarged, mildly atypical endothelial cell nucleus and swollen cytoplasm that closes the lumen (L).  Bacterial cells (arrows) are in the cytoplasm.  Perivascular glial cell mitochondria have mild hyperplastic changes (arrowheads).  B. The spherical bacterial cells vary in density.  They are in endothelial cell cytoplasm and clustered in the vascular lumen (L).  One has an attachment organelle (arrow).  C. Two bacterial cells in endothelial cell cytoplasm have trilaminar membranes and the appearance of having undergone binary fission.   Scale bars:  A, 2 μm; B, 500 nm; C, 100 nm.

**Fig. 6.** Electron micrographs of frontal cortex blood vessels. A. Bacterial cells within a large mononuclear cell in a perivascular space are fusiform and dumbbell shaped. Three have nucleoids (arrowheads). B. The smallest of the dense, spherical bacterial cells, about 100 nm in diameter, contrast with the large, flask-shaped cell with a terminal organelle and attachment organelle, emphasizing the size range and polymorphism of these bacterial cells. The densities in the small cells are of ribosomal size. C. Round (arrows) and oblong bacterial cells (arrowhead) are in an endothelial cell. An oblong bacterial cell (*) is attached to the basement membrane (bm) by its dense attachment organelle at the end of a long terminal organelle. The upper oblong cell may have a similar attachment without a terminal organelle. D. The lower bacterial cells are attached to one another through terminal organelles in this capillary endothelial cell. The capillary lumen contains a red blood cell (RBC). E. This large bacterial cell, in the same perivascular cell as those seen in 6A, has a prominent constriction that separates regions of different densities. F. A macrophage filling the lumen of a capillary contains several bacterial cells. Inset, bacterial cells in phagosomes. Scale bars: A, 100 nm; B–D, 500 nm; E, 100 nm; F, 2 µm; F inset, 100 nm.