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Case Report

Recurrent transverse myelitis following neurobrucellosis: Immunologic features and beneficial response to immunosuppression

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The authors report the clinical course and immune system response of a patient with disease-associated recurrent transverse myelitis (TM) following cerebral infection with *Brucellosis melitensis*. The patient suffered four recurrences of his TM (each at a distinct spinal cord level) over the course of 2 years following his initial presentation, which ultimately progressed to quadriplegia. He had progressively declining cerebrospinal fluid (CSF) brucella antibody titers, suggesting a postinfectious, rather than an infectious, etiology. The authors simultaneously examined the expression of multiple cytokines in the CSF of this patient using cytokine antibody arrays and found a marked elevation of interleukin (IL)-6, IL-8, and macrophage chemoattractant protein (MCP)-1 levels relative to controls. Quantitative enzyme-linked immunosorbent assay (ELISA) analysis of the CSF confirmed a 1700-fold elevation of IL-6 and more modest elevations of IL-8 and MCP-1. IL-6 levels returned to baseline following treatment of the patient with intravenous cyclophosphamide and plasma exchange and the patient experienced a significant and sustained recovery of function. *Journal of NeuroVirology* (2005) **11,** 225–231.

Keywords: cytokine; IL-6; immunosuppression; neurobrucella; plasma exchange; recurrent; transverse myelitis

Introduction

Transverse myelitis (TM) is characterized by focal inflammation within the spinal cord and clinical manifestations are due to impaired neurotransmission of neurons and axons within the inflamed area (Krishnan *et al*, 2004). TM may exist as part of a multisystem or multifocal CNS disease, or as an isolated disease-associated or idiopathic entity. Some cases of TM are due to direct infection of the spinal cord with agents such as measles, rubella, mycoplasma, or a herpes virus, and are classified as infectious TM (Kerr and Ayetey, 2002). Postinfectious TM follows a systemic infection and likely occurs as a consequence of an aberrant immune response triggered by the infection (Kerr and Ayetey, 2002). Eighty percent to 85% of TM patients have a single inflammatory episode whereas the rest may have recurrent TM (Kim, 2003; Pandit and Rao, 1996).

Brucellosis is a disease transmitted from animals, such as camels, sheep, and goats, to humans who are the secondary hosts. The disease is endemic in Saudi Arabia and neighboring countries where the gram-negative organisms are transmitted to humans through the consumption of uncooked meat or unpasteurized dairy products. Nervous system manifestation of human brucellosis occurs in up to 13% of patients and may cause meningitis, meningoencephalitis, optic neuritis, radiculitis, or leukoencephalopathy (Kochar *et al*, 2000; Seidel *et al*, 2003).

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Here we describe the clinical course of a case of recurrent, disease-associated TM following intracranial *Brucella melitensis* infection, and characterize markers of immune response of this patient during treatment.

Case report and results

As reported previously (Seidel *et al*, 2003), a 65-year-old male Iranian immigrant living in the United States with no prior history of significant medical or neurologic disease was in his usual state of good health until a return visit to Iran 15 months prior to his diagnosis. Two months into his visit to Iran he developed bitemporal headaches and hearing loss. The patient was diagnosed with an ear infection and treated with oral antibiotics without effect. In the ensuing months the patient continued to have headaches and hearing loss and developed anorexia with weight loss and double vision. A magnetic resonance imaging (MRI) with gadolinium revealed diffuse, confluent T2-hyperintensities within the subcortical and periventricular white matter bilaterally with leptomeningeal enhancement. Cerebrospinal fluid (CSF) analysis revealed white blood cell (WBC) (100% mononuclear) at 13/mm^2 with red blood cell (RBC) at 2/mm^2, normal protein and glucose, and two oligoclonal bands. Because of the magnitude of white matter lesion burden the patient underwent a brain biopsy. Pathologic analysis revealed a florid reactive astrogliosis and marked activation of microglia in association with an extensive infiltration of cytotoxic T lymphocytes, although no organisms were detected. The patient had a postoperative cerebral abscess in the region of the previous biopsy, which when drained grew *Brucella melitensis*, thereby confirming the diagnosis.

The patient underwent 6 months of antibiotic therapy (Table 1), which resulted in marked improvement in his encephalopathy. Six months after the brain biopsy the patient presented to the hospital with fever, back pain, and rapidly progressive paraplegia over 1 to 2 days. The patient suffered four recurrences of his TM (each at a distinct spinal cord level) over the course of the subsequent 2 years. His

Table 1 Paraclinical findings

Time (months)	*Clinical sensory level*	*Motor function*	*CSF WBC, protein*	Brucella *titer*	*Spinal MRI*	*Brain MRI*	*Treatment*
0	None	Normal	13, 35	—	—	Diffuse leukoencephalopathy Gad: none	Doxycycline, rifampicin, gentamicin
4	None	Normal	—	1:64	—	Brain abscess with enhancement; multifocal leukoencephalopathy	Sulfa, gentamicin, minocycline
10 (TM no. 1)	T3	Paraplegia, resolved to independent ambulation	—	1:32	T2: T3–T5 Gad: T3–T5	Stable T2 signal abnormality in white matter Gad: None	Piperacillin and tazobactam; IV MP
16 (TM no. 2)	T5	Paraparesis, resolved to assisted ambulation	4, 79	1:2	T2: T1–T9 Gad: T3–T6	—	IV MP
19	T5	—	—	—	T2: T5–T9 Gad: none	Resolving T2 signal abnormality Gad: none	—
21 (TM no. 3)	T1	Quadriparesis, some hand weakness	1, 31	1:2	T2: C6–C7 Gad: C6–C7	—	IV MP
23 (TM no. 4)	C5	Quadriplegia	—	1:4	T2: C1–T9 Gad: C2–T4	—	IV MP
24.5	C5	Quadriplegia	5**, 59	1:2	T2: C1–T9 Gad: C2–T4	—	Cyclophosphamide, PLEX
25	T3	Improved quadriparesis	8***, 59	—	—	—	
25.5	T3	Improved quadriparesis	4****, 25	1:1	T2: C2–T6 Gad: none	Further resolution of T2 signal abnormality Gad: none	None
33	T3	Standing with assistance	—	1:1	T2: C3–C5 Gad: none	—	none

PLEX = plasma exchange; IV MP = intravenous methylprednisolone; Gad = gadolinium.
**CSF sample taken pre-cyclophosphamide/PLEX (Figure 2B).
***CSF sample taken 2 weeks post-cyclophosphamide/PLEX (Figure 2C).
****CSF sample taken 4 weeks post-cyclophosphamide (Figure 2D).

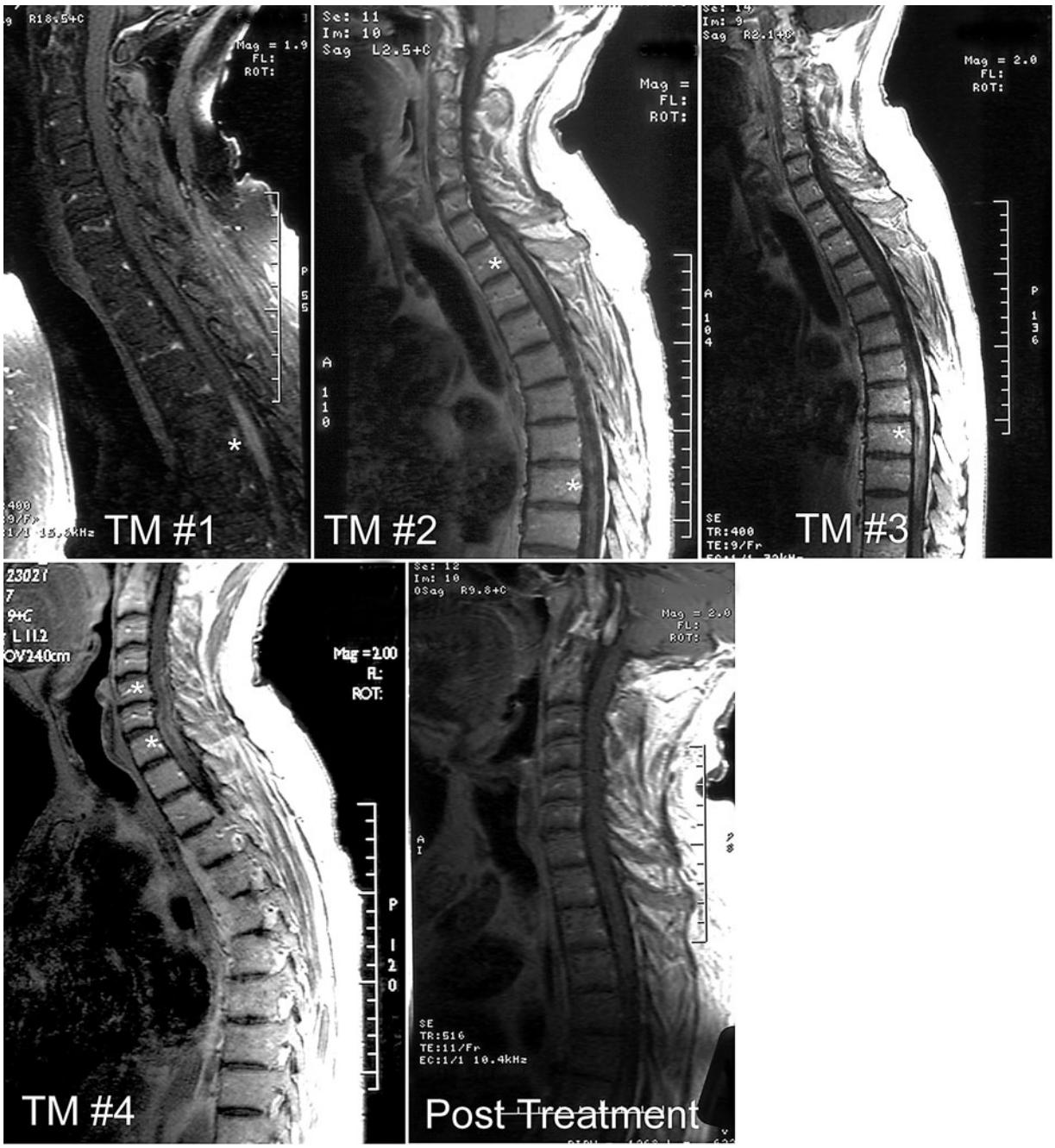

Figure 1 T1 Sagittal MRIs of the spinal cord after gadolinium contrast administration. Representative images of each of the patient's four episodes of TM are shown. Asterisks denote regions of abnormal gadolinium enhancement. The post-treatment sagittal MRI showed no abnormal gadolinium enhancement.

course of recurrent myelitis is detailed in Table 1. With each recurrent episode, the patient experienced new clinical deficits associated with new gadolinium enhancing lesions within the spinal cord (Table 1; Figure 1). All viral, bacterial, and fungal cultures of the CSF were negative. Viral polymerase chain reaction (PCR) analysis of the CSF was also negative. *Brucella* antibody titers were obtained from the patient's CSF collected at various points during his clinical course, and continued to decline over time from 1:64 to 1:1 at the last analysis. With his initial TM exacerbation the patient had a good recovery following intravenous (IV) methylprednisolone treatment. Subsequent TM recurrences, however, were progressively more refractory to intravenous steroid treatment, and the patient continued to accrue disability with each attack.

The protein expression profile of multiple cytokines from the spinal fluid obtained from the patient during his fourth recurrence of TM was

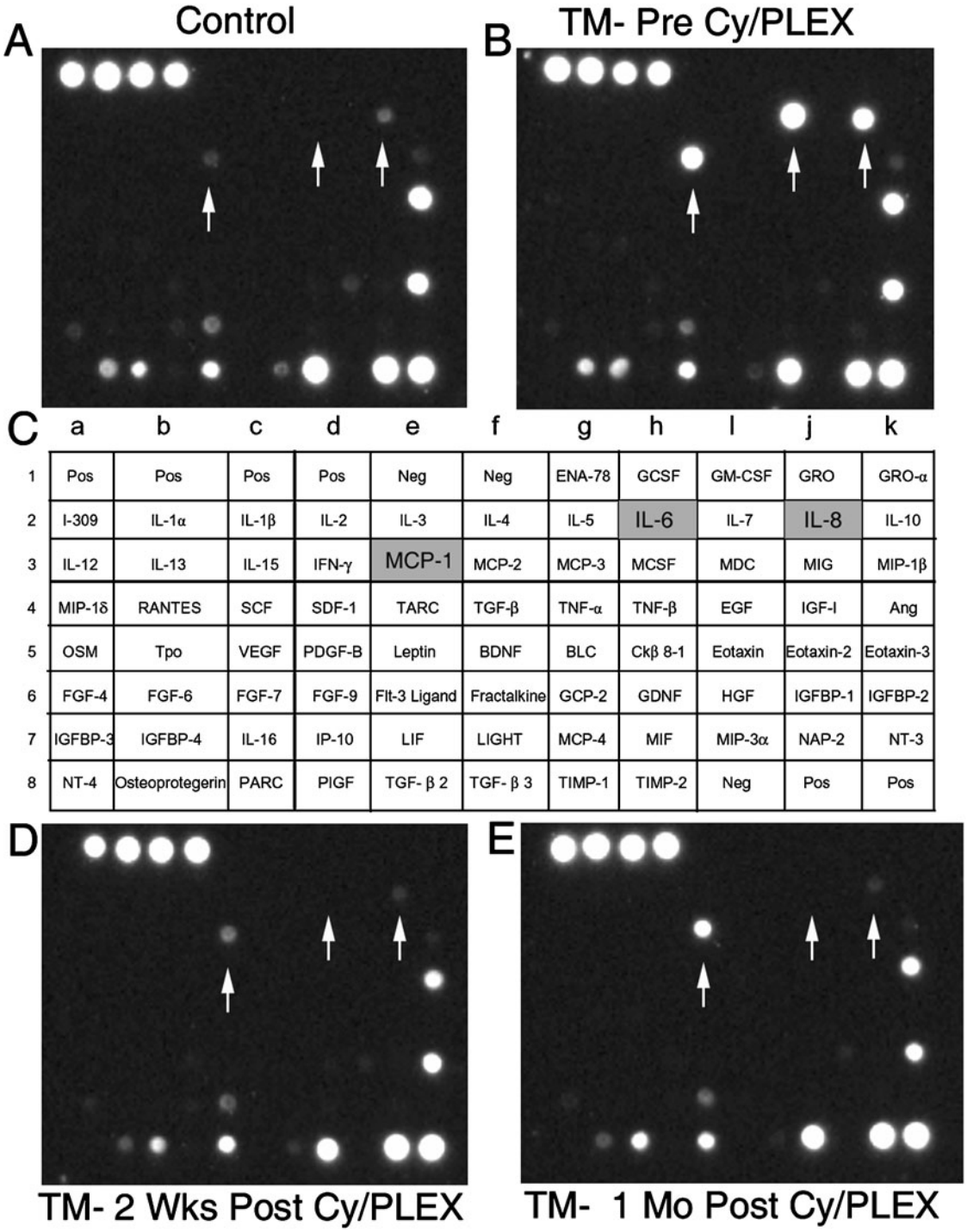

Figure 2 Analysis of the CSF humoral immune profile of patient with recurrent TM. (**A**) Sample cytokine antibody array on CSF from a control patient. (**B**) Cytokine array of described patient prior to treatment with cyclophosphamide and plasma exchange (PLEX). Cytokines that are differentially represented between the two are indicated with arrows. (**C**) Schematic illustration of the multiple cytokine array used. Shaded boxes show the locations of IL-6, IL-8, and MCP-1. (**D** and **E**) Cytokine array analysis of CSF 2 weeks (**D**) and 4 weeks after treatment (**E**).

examined using a cytokine antibody array (Figure 2). The relative expression of cytokines were determined by comparing signal intensities from the cytokine antibody array profile of CSF from the TM patient with the profile of CSF from a control patient with a noninflammatory, vascular myelopathy. Of the 79 inflammatory proteins compared, only 3 were elevated greater than 2.5-fold in the CSF sample from the TM patient: interleukin (IL)-6 (1517-fold), IL-8 (11-fold), and macrophage chemoattractant protein (MCP)-1

(24-fold). Elevated CSF IL-6 levels were confirmed and quantitated using enzyme-linked immunosorbent assay (ELISA) analysis that demonstrated a 1700-fold increase in CSF IL-6 in the TM patient relative to the control (3675 ± 121 pg/ml versus 2.16 ± 0.82 pg/ml, respectively). Data represent the average of three measurements for each sample. This experiment was repeated twice with the same results.

Despite declining CSF *Brucella* antibody titers, the patient had a worsening course of inflammatory myelitis, and we pursued a more aggressive course of immunomodulatory treatment following his fourth TM exacerbation (Table 1). Within 4 days of initiating treatment with IV cyclophosphamide (1000 mg/m^2) and plasma exchange (PLEX; 1.1 plasma volumes every other day for five exchanges), the patient began to move his fingers and could adduct both legs slightly. Fourteen days after the initiation of treatment he began to move his shoulders. Twenty-eight days after the initiation of treatment he had 4/5 strength in his arms and hands, and 2/5 strength in his legs. Fourteen and 28 days following treatment we repeated the analysis of the patient's CSF by employing a cytokine antibody array analysis. The analysis of the CSF revealed that IL-6 and IL-8 levels were at or below baseline levels. MCP-1 levels declined to a 2.4-fold elevation at 14 days and then a 9.6-fold elevation at 28 days relative to control CSF levels (Figure 2D, E). Quantitation of CSF IL-6 protein levels measured by ELISA confirmed levels of 2.36 ± 1.38 pg/ml and 1.98 ± 0.25 pg/ml at 14 and 28 days post treatment, respectively. In the first month following treatment with cyclophosphamide/PLEX, the patient recovered the ability to sit unassisted and to transfer with minimum assistance. The patient received four more courses of IV cyclophosphamide over the next 9 months. By 18 months after initiating treatment, the patient could stand with assistance and could transfer independently. He had no further clinical exacerbations and had no recurrent gadolinium-enhancing lesions on either of two follow-up MRIs (12 and 18 months after cyclophosphamide/PLEX).

Discussion

The diagnosis of neurobrucellosis in this case has extensive support based on the epidemiological, clinical, and radiological findings. Brucellosis is endemic in Saudi Arabia and neighboring Middle Eastern countries, and this patient contracted his disease during an extended visit to Iran. Early in his course the patient experienced a progressive bilateral sensorineuronal hearing loss, which had a prevalence of 90% in a recent case series of neurobrucellosis (Al Sous *et al*, 2004). In this same case series, 30% of patients were found to have white matter changes manifested as hyperintense lesions on T2-weighted images, which were consistent with the findings in the patient reported here. A recent example of neurobrucellosis that presented with progressive hearing loss and a chronic course similar to the case described here was also noted to involve diffuse, confluent white matter lesions on T2-weighted and FLAIR MRI (Koussa and Chemaly, 2003). In these cases of neurobrucellosis previously reported, as well as in the case presented here, there were no intraparenchymal enhancing lesions as is often seen in multiple sclerosis (MS), acute disseminated encephalomyelitis (ADEM), and Lyme disease. Moreover, in these reported cases of neurobrucellosis, the corpus callosum that commonly undergoes white matter changes in MS is spared. The nature and cause of these white matter changes are not known, but it has recently been speculated that they are due to an autoimmune reaction to CNS *Brucella* infection (Al Sous *et al*, 2004). Further support for an autoimmune mechanism in this patient's neurologic disease comes from the finding of oligoclonal bands in his CSF. Though common in patients with MS, oligoclonal bands were recently found to be present in all six reported patients with neurobrucellosis (Pascual *et al*, 1988), suggesting an expansion and activation of B-lymphocyte clones within the CNS. Finally, the finding of *Brucellosis melitensis* cultured from our patients CNS abscess leaves little doubt about the diagnosis in this case.

The evidence in this case supports a postinfectious etiology of the patient's recurrent TM. Despite improving significantly while on a prolonged course of IV and oral antibiotics to treat his neurobrucellosis, the patient presented 6 months later with an acute case of TM while still being treated with antibiotics. *Brucella* antibody titers from the patient's CSF continued to decline over time from 1:64 to 1:1 at the last analysis, despite his worsening course of recurrent TM and the cessation of his antibiotic treatment. The abundance of immune cells and inflammation seen on brain biopsy despite the absence of visible organisms argues for an immunopathogenic mechanism of CNS injury. Finally, and perhaps most compellingly, the patient's deteriorating course was dramatically improved after initiating cyclophosphamide and PLEX treatment, both of which would not be expected to assist a case of infectious TM. We suspect, therefore, that similar immunopathogenic events may underlie other forms of TM, many of which are para-infectious or are idiopathic (de Seze *et al*, 2001).

*Brucella*e are facultative, nonmotile, intracellular, gram-negative coccobacilli organisms that evade immune detection and replicate within cells of the monocyte lineage. Within macrophages, *Brucella* may impair cellular apoptosis in order to perpetuate a "Trojan Horse" state. However, *Brucella*-infected macrophages have been shown to secrete cytokines and chemokines, including IL-6 and IL-8, perhaps to facilitate auto-activation and activation of T-helper cells or $\gamma\delta$ T lymphocytes (Dornand *et al*, 2002).

In the case presented here, the most dramatic biological correlate of patient's worsening clinical course was the relatively selective and dramatic increase in the level of CSF IL-6 in conjunction with more modest elevations of CSF IL-8 and MCP-1. IL-8 and MCP-1 are chemokines that play a role in the initiation and propagation of an immune response within the CNS (Ransohoff *et al*, 2002). IL-8 is a proinflammatory chemokine that plays a pivotal role in acute inflammation by recruiting and activating neutrophils. MCP-1 stimulates IL-6 expression in monocytes. IL-8 and MCP-1 function by establishing a chemical gradient that recruits cells expressing the complementary receptors to the origin of chemokine expression. They alter the expression of adhesion molecules at the blood-brain barrier such that inflammatory cells can gain entry to the CNS more easily (Olson and Ley, 2002). It is plausible that IL-8 and MCP-1 production within the spinal cord was triggered by infected macrophages or microglial cells during active CNS infection with *Brucella*, but that sustained expression of these proinflammatory molecules following treatment with antibiotics led to recurrent immune cell recruitment into the spinal cord. This recruitment could have resulted in the ensuing recurrent TM.

IL-6 is a glycoprotein cytokine that mediates signal transduction between immune cells, induces acute-phase protein synthesis, and controls growth and differentiation of cells of the immune and hematopoietic systems (Gruol and Nelson, 1997). IL-6 produces its effects on CNS cells by binding to IL-6 receptors, which in turn form complexes with transmembrane proteins called gp130. Recent evidence suggests that IL-6 is an important regulator of cellular functions in the CNS (Gadient and Otten, 1997; Gruol and Nelson, 1997), but many important actions of IL-6 in the CNS remain to be elucidated. IL-6 levels in the adult CNS are usually low or undetectable under baseline conditions, but increase dramatically in response to injury or inflammation. Elevated IL-6 within the nervous system stimulates production of glucocorticoids, generates fever, modulates pain response, and has trophic effects on neural cell survival. There has been a growing appreciation of the destructive potential of elevated levels of IL-6 in the CNS in various disease states, such as Alzheimer's disease, Parkinson's disease, MS, major depression, traumatic brain injury, and human immunodeficiency virus (HIV) dementia. IL-6 may induce injury within the spinal cord through induction of inducible nitric oxide synthase (iNOS) and the subsequent generation of nitric oxide (NO) (Yu *et al*, 2003). We hypothesize that CNS IL-6 production was stimulated initially by the *Brucella* infection and that the markedly elevated IL-6 levels induced injury to the spinal cord, leading in turn to further immune cell recruitment and spinal cord inflammation. Future work will examine whether a cascade of inflammation involving cytokine production, subsequent tissue injury, and further immune-system activation plays a role in the genesis of idiopathic TM.

Materials and methods

Multiple cytokine antibody arrays were purchased (RayBio Human Cytokine Antibody Array V—H0108005, 79 cytokines detected; http://www.raybiotech.com/map/human_V_map.pdf, RayBiotech, Inc., Norcross, GA) and carried out according to the manufacturer's recommendations. A total of 1000 μl of CSF was utilized per Panomics blot. Control CSF samples in the Panomics assay were from patients with normal pressure hydrocephalus. Signal was analyzed and quantitated by using a Fuji chemiluminescent detection system.

Quantitative IL-6 ELISA assay kits and total NO assay kits were purchased from R&D Systems and analyses were carried out according to the manufacturer's instructions. All samples were measured in triplicate and average values were determined.

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