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Inflammatory brain changes in Lyme borreliosis

A report on three patients and review of literature

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Summary

Despite a rapid increase in the number of patients with Lyme neuroborreliosis (LNB), its neuropathological aspects are poorly understood. The objective of this study was evaluation of neuropathological, microbiological, and magnetic resonance imaging (MRI) findings in three patients with the Borrelia burgdorferi infection and neurological disease from whom brain tissue specimens were available. Perivascular or vasculitic lymphocytic inflammation was detected in all specimens. Large areas of demyelination in periventricular white matter were detected histologically and by MRI in one patient. The disease had a fatal outcome in this patient. Brain MRI suggested malignancies in two patients before histopathological studies were carried out. One of these two patients was a child with sudden hemiparesis. Another was a 40-year-old man presenting with epileptic seizures and MRI-detected multifocal lesions, which disappeared after repeated courses of antibiotics. We conclude that cerebral lymphocytic vasculitis and multifocal encephalitis may be associated with B. burgdorferi *infection. The presence of B. burgdorferi DNA in tissue samples from areas with inflammatory changes indicates that direct invasion of B. burgdorferi may be the pathogenetic mechanism for focal encephalitis in LNB.*

Keywords: *Borrelia burgdorferi*; Lyme disease; neuroborreliosis; neuropathology; vasculitis

Abbreviations: BBB = blood–brain barrier; ESR = erythrocyte sedimentation rate; H and E = haematoxylin and eosin; HSV = herpes simplex virus; LNB = Lyme neuroborreliosis; PCR = polymerase chain reaction

Introduction

The meninges, spinal nerve roots and cranial nerves are most commonly involved in LNB. Chronic CNS involvement of LNB may mimic diseases such as neurosyphilis, meningoencephalitis of viral, fungal or mycobacterial origin, multiple sclerosis, brain tumor, autoimmune disease, stroke or Alzheimer's disease.

Despite a rapid increase in the number of patients with LNB its neuropathological aspects are poorly understood (Garcia-Monco and Benach, 1995). In principle, the brain lesions can be caused either directly by the spirochaete or indirectly via biologically active substances secreted by host cells on stimulation with the spirochaete. *Borrelia burgdorferi* is able to adhere to and penetrate through the endothelium of CNS blood vessels and then adhere to the glial cells (Garcia-Monco *et al.*, 1989; Comstock and Thomas, 1989; Szczepanski *et al.*, 1990). This invasion can already occur at early stages of the infection, even without signs of inflammation in the CSF (Garcia-Monco *et al.*, 1990; Luft *et al.*, 1992). After invasion of the CNS, the spirochaete may cause direct damage to oligodendroglial cells, which may lead to demyelination (Baig *et al.*, 1991). However, there is increasing evidence that inflammatory changes in CNS blood vessels, such as diffuse or focal vasculitis or cerebrovascular injury, may be an important factor in the development of the CNS lesions and dysfunction in LNB (Midgard and Hofstad, 1987; Uldry *et al.*, 1987; Weder *et al.*, 1987; Wokke *et al.*, 1987). It is conceivable that Lyme borreliosis may also cause vascular damage in the CNS, because it is known to cause vasculitis and perivascular inflammation in several other organs outside the CNS (Johnston *et al.*, 1985; Camponovo

and Meier, 1986; Duray, 1989*a, b*; Meurers *et al.*, 1990; Moody *et al.*, 1990*a*; Olson and Esterly, 1990; Smith *et al.*, 1991; Karma *et al.*, 1995). The factors involved in the development of the vascular changes in LNB are not known. It is evident that the spirochaete occurs in very low numbers especially in the CNS, since its presence in brain tissue has been a rare finding (MacDonald and Miranda, 1987; Weber *et al.*, 1988; Pachner *et al.*, 1989; Kuntzer *et al.*, 1991; Millner *et al.*, 1991; Miklossy *et al.*, 1994), suggesting that CNS lesions are also caused by inflammatory mediators and not only by the direct action of the spirochaete itself. One recent study showed that *B. burgdorferi* is capable of direct activation of vascular endothelium promoting recruitment of leucocytes to perivascular tissues (Sellati *et al.*, 1995).

Modern imaging techniques, such as CT and MRI, are used successfully for the detection of vascular lesions in the CNS of LNB patients (Uldry *et al.*, 1987; Kohler *et al.*, 1988; Belman *et al.*, 1992), and together with sensitive gene amplification methods, such as polymerase chain reaction (PCR), they have improved the possibilities of studying the role of *B. burgdorferi* in the development of vasculitic lesions.

We describe three patients with CNS lesions shown by MRI and neurological symptoms. In two of the patients, *B. burgdorferi* DNA was detected by PCR in specimens from inflammatory CNS lesions; it was cultivated from the CSF of one of these two. In the child with hemiparesis and vasculitis in the brain, borrelia PCR yielded positive results with CSF.

Patients and methods

Patients

Two patients living in an area where Lyme borreliosis is endemic were examined at the Turku University Central Hospital. One patient, who had briefly visited an endemic area, was examined at the Oulu University Central Hospital.

Magnetic resonance imaging

All patients were examined using a high-field magnet (1.5 Magnetom, Siemens) with T_2 and T_1 sequences (TR 2500, TE 90 and TR 600, TE 15). Gadolinium enhancement was also used. Axial, coronal, and sagittal planes were imaged.

Assessment of borrelia antibodies

The IgM and IgG antibodies against sonicated *B. burgdorferi* were measured by an in-house ELISA using *B. burgdorferi* sensu stricto (B31, ATCC 35210) as the antigen (Viljanen and Punnonen, 1989). All steps of the ELISA were carried out automatically with an Auto-EIA II instrument (Labsystems, Helsinki, Finland). Serum samples were tested at a dilution of 1:100. The results were expressed as relative ELISA units. Seropositivity was determined by comparing antibody results from test serum samples with those of 110 healthy controls. The cut-off value for weakly positive results was the mean+(2×SD) of the controls. The IgM and IgG antibodies against flagellin were measured using a commercial ELISA test (Lyme Borreliosis ELISA Kit, 2nd generation; DAKO A/S, Glostrup, Denmark). The CSF samples were tested at a dilution of 1:10. The CSF IgM and IgG antibodies against sonicated *B. burgdorferi* were measured using the in-house ELISA (EIU units >10 were considered positive) and against flagellin by a commercial ELISA (DAKO).

Culture of B. burgdorferi

The specimens (biopsy, CSF or blood) were inoculated into tubes containing BSK-II medium and incubated at 30°C. The tubes were examined macroscopically twice weekly and passaged once weekly for at least two months. Dark-field microscopy was carried out if the colour of the culture medium indicated growth. The final identification of cultured spirochaetes was based on PCR.

Extraction of DNA for the PCR

A 1 ml sample (i.e. minced biopsy specimen, plasma, serum or CSF) was centrifuged (Eppendorf Microfuge, 13000 r.p.m., 10 min), 800 µl of the supernatant was removed, and the remaining 200 µl was mixed with 300 µl of sodium dodecyl sulphate (SDS) solution (0.1 M NaOH, 2 M NaCl and 0.5%). After incubation at 80°C for 15 min, 200 µl of 0.1 M Tris–HCl (pH 8) was added. After the SDS treatments, DNA was extracted with phenol–chloroform, precipitated with ethanol and finally dissolved in Tris–EDTA buffer (Hance *et al.*, 1989).

Polymerase chain reaction

A 5 µl volume of extracted DNA was added into the reaction tube. The specific targets chosen for the PCRs were DNA fragments from the flagellin gene sequence of *B. burgdorferi*. The PCR from all specimens obtained from Patient 1, and from the brain tissue specimens of Patient 2, was run in two steps, first with external primers prB31/41–4 and prB31/41–5, resulting in a 730-bp PCR product (Wallich *et al.*, 1990) and then with nested primers WK1 and WK2, resulting in a 290-bp fragment (Krüger and Pulz, 1991). Finally, the PCR from specimens obtained from Patient 3, and the plasma of Patient 2 was run as described earlier with primers WK1 and FL7, resulting in a 497-bp PCR product (Krüger and Pulz, 1991; Picken, 1992; He *et al.*, 1994). Each PCR run included a positive control containing DNA extracted from a reference strain (B31) of *B. burgdorferi* sensu stricto (ATCC 35210). Control brain tissue samples were included in the PCR runs in a blinded manner and with negative results. Every sixth tube of each run was used as a negative control subjected to all above sample treatments. The negative controls remained negative in each run. One hundred blood donors living in the Turku area provided control samples for the PCR assay with primers WK1 and

Table 1 *Laboratory results of borrelia tests, treatments for LNB, and outcome of three patients with Lyme borreliosis associated with CNS inflammatory changes*

	Patient number		
	1	2	3
Serum antibodies	−	+	+
Lymphocyte proliferation	−	+	ND
CSF antibodies	−	−	ND
CSF culture	+	−	ND
CSF PCR	+	−	+
Blood culture	−	−	ND
Plasma PCR	+	+	ND
Bone marrow PCR	+	−	ND
Brain tissue PCR	+	+	−
Number of treatments for LNB	2	2	1
Interval between onset of symptoms and first treatment for LNB (months)	>39	1	3
Persistence of PCR positivity after onset of antibiotic treatment (months)	8	16	0
Outcome	D	A	B

+ = positive; − = negative; ND = not done; A = asymptomatic; B = significantly better than before treatment; D = dead.

FL7: one of their samples was positive. Similar studies on blood donors was not done for the other above mentioned PCR assays with different primer pairs. The sensitivity of the PCR with WK1 and FL7 primers (He *et al.*, 1994) was found to between 10 and 100 *B. burgdorferi* cells per reaction. The sensitivities of the other PCR modifications were at the same level as that with WK1 and FL7 primers. All PCRs used were found highly specific for *B. burgdorferi* s.l. Other *Borrelia* species (*B. hermsii*, *B. parkeri* and *B. turicatae*) and treponemes (*Treponema denticola*, *T. pectinovorum*, *T. socranskii* and *T. vincentii*) gave negative results.

Neuropathology

Surgical samples were fixed in 4% phosphate buffered formaldehyde and routinely processed into paraffin sections. Brains from autopsies were also fixed in the same fixative. During cutting of the brains, samples from areas judged as abnormal on brain imaging and from areas with macroscopic pathological features were collected and processed into paraffin sections. The specimens were stained using haematoxylin and eosin (H and E), and Luxol Fast Blue–Cresyl Violet stains. Inflammatory cells were demonstrated immunohistochemically using mouse monoclonal antibody to CD 45 and leucocyte common antigen (Dakopatts A/S, Glostrup, Denmark). Bound primary antibodies were detected applying an appropriate Vectastain (Vector Laboratories, Burlingame, Calif., USA) avidin–biotin–peroxidase detection kit with diaminobenzidine as chromogen.

Results

In two cases (Patients 1 and 2), the brain tissue specimen from areas with vasculitic lesions contained DNA of *B. burgdorferi* as evidenced by PCR amplification (Table 1). In one of them, culture as well as PCR of the CSF showed *B. burgdorferi* (Patient 1). In addition, the plasma of both of the patients (Patients 1 and 2) contained DNA of *B. burgdorferi*. In the remaining one patient, DNA of *B. burgdorferi* was amplified in the CSF (Patient 3). In all patients, a positive PCR result was obtained from more than one specimen taken and analysed at different times (Table 1). Two of the patients had antibodies against *B. burgdorferi* in the serum but none of the patients had them in the CSF. Circulating immune complexes were found in both two patients studied (Table 1).

In one patient (Patient 1), MRI of the brain showed large periventricular lesions in white matter and the disease had a fatal outcome. MRI findings in two patients (Patients 2 and 3) suggested malignancy before the histopathological studies were carried out. One of them was a child with sudden hemiparesis. Another was a 40-year-old man who presented with epileptic seizures; his MRI showed multifocal lesions, which disappeared after repeated antibiotic therapy.

Perivascular or vasculitic lymphocytic inflammation in the clinical or autopsy brain biopsy specimens was detected in all three patients. Large areas of demyelination in periventricular white matter were detected in one patient (Patient 1). Detailed neuropathological descriptions are included in the following case reports.

Patient 1

The patient was a 51-year-old woman with a history of progressive lymphedema of the left lower limb since 1954. She had suffered from erysipelas in the left lower leg and erythema nodosum in both legs, and had also had recurrent fever episodes several times a year. Lung fibrosis, heart insufficiency and chest pain atypical of coronary heart disease developed at the age of 30–35 years. She had received long-term corticosteroids and several courses of antimicrobial drugs.

In 1985, the patient had a 3 week period of fever and facial redness suggestive of lupoid erythema. Despite corticosteroid treatment, a spiking fever persisted. At hospital, no infection focus was found. Antimalarial drugs combined with methylprednisolone were given for two years, but episodes of mild fever reappeared. Antinuclear antibodies and antibodies against extractable nuclear antigens were repeatedly negative. Anti-DNA-antibodies were found slightly positive.

After September 1988, she was hospitalized several times for prolonged vomiting, fatigue, fever, dizziness and progressive walking difficulties with ataxia and short gait. In addition, impairment of memory, taste, and hearing occurred. In February 1989, the erythrocyte sedimentation rate (ESR) was 125 mm h^{-1}, serum C-reactive protein 83 mg l^{-1} (normal <10 mg l^{-1}), and leucocytes 9.2×10^9 l^{-1} with 96% granulocytes. Sinus X-ray showed sinusitis, and the brain CT showed an empty sella. Despite treatment with methylprednisolone and i.v. erythromycin, the ESR and C-reactive protein remained elevated. At CSF examinations (February 1989 and January 1991), leucocyte counts and protein concentrations were normal, as was the IgG/albumin ratio, but one or two subfractions were observed with protein electrophoresis. In January 1991, MRI of the brain showed enlarged ventricles, cortical atrophy, and marked degenerative changes in the periventricular areas (Fig. 1A). Total serum immunoglobulins were normal, but immune electrophoresis showed an M-component (IgG lambda). Circulating immune complexes also occurred. Rheumatoid factor, antinuclear antibodies, anticardiolipin, TPHA (*Treponema pallidum* haemagglutination test), anti-phospholipid antibodies, and antibodies against *B. burgdorferi* were negative in the serum. Leucocytes were 3.5×10^9 l^{-1} with an excess of band forms.

In August 1991, CSF examination showed no inflammatory cells, a slightly elevated protein concentration of 762 mg l^{-1}, and no antibodies against *B. burgdorferi*. Culture of CSF in BSK-II medium showed very slow growth of spirochaetes during 3 months. Using monoclonal antibodies, immunofluorescence and PCR, the spirochaete was identified as *B. burgdorferi* s.l.

In December 1991, antimicrobial treatment with ceftriaxone (2 g i.v. daily) was instituted. The patient improved slightly, and therapy was continued after 3 weeks with oral amoxicillin (500 mg every 8 h) and oral probenecid (500 mg every 8 h). After 1 week on amoxicillin, the patient developed urticaria. Oral doxycycline (100 mg every 12 h) was substituted and continued until July 1992. During this treatment, the walking difficulties and fever episodes recurred. All cultures for fungi and common bacteria were negative. In January 1992, brain MRI showed slight progression of the periventricular lesions from the image obtained 1 year earlier. In March and July 1992, subdural haemorrhages of unknown origin were evacuated.

On August 7, 1992, plasma and bone marrow specimens were positive for *B. burgdorferi* PCR. Treatment with ceftriaxone (2 g i.v. daily) was reinstituted, the patient

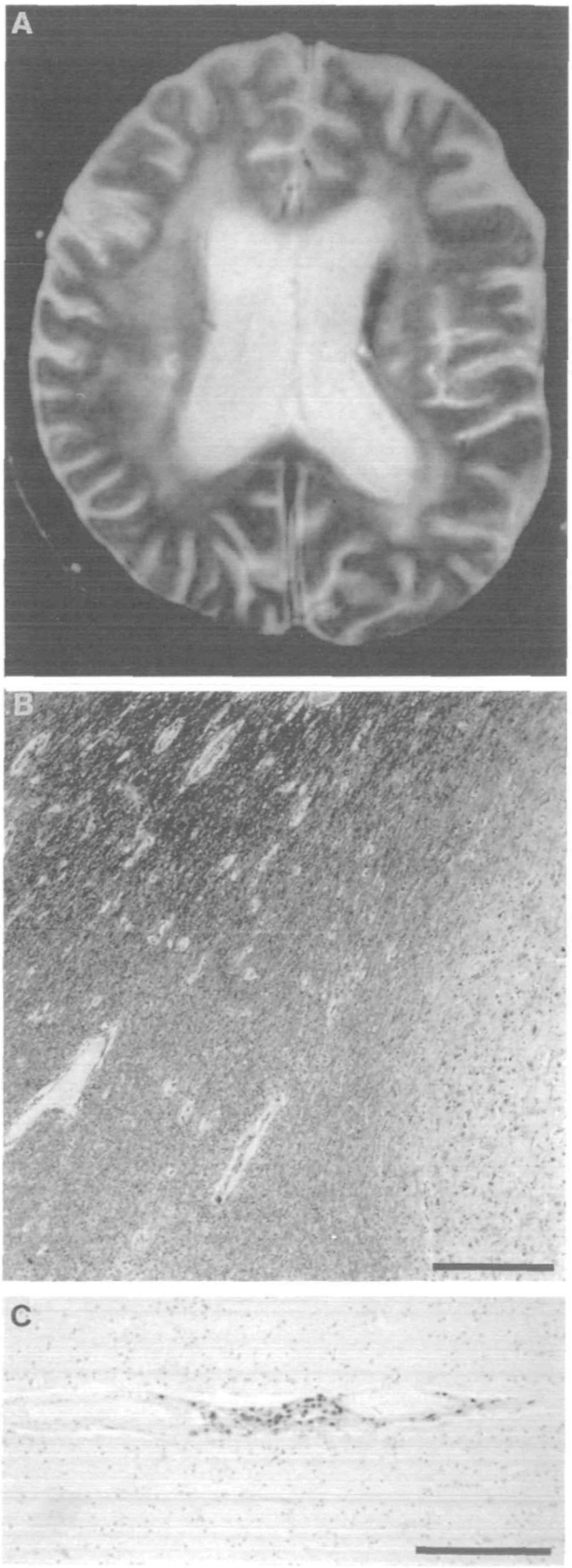

Fig. 1 (Patient 1). Enlarged ventricles, cortical atrophy and marked degenerative changes are seen in periventricular areas on T_2-weighted MRI of the brain after the first treatment (**A**). Within an area of diffuse demyelination in subcortical white matter (**B**; Luxol Fast Blue–Cresyl Violet stain; bar = 400 μm) there is mild perivascular inflammation (**C**; anti-CD45 immunostain + H and E

reacting with high fever. Empirical antifungal therapy with amphotericin B was also started. These treatments were continued until the patient died on September 12, 1992.

At autopsy, the pathological changes were slighter than expected. The spleen was slightly enlarged. Chronic liver stasis and mild pulmonary oedema were detected. No signs of fungal infection were seen. Neuropathological examination showed a chronic left-sided subdural haematoma. Its structure was compatible with the haemorrhages occurring 6 and 2 months before death. An increased number of plasma cells were present within the organizing connective tissue of the haematoma. In subcortical and periventricular white matter, diffuse demyelination with mild perivascular inflammation was seen (Fig. 1B and C). In one of the six analysed brain tissue specimens, *B. burgdorferi* DNA was detected by the PCR.

Patient 2

This 40-year-old man had previously been healthy, apart from reactivation of a genital herpes infection some weeks before. He recalled no tick bites or erythema migrans. On December 26, 1992, he had a generalized seizure and was admitted to the hospital. Another seizure occurred on the day of admission. Brain CT was normal. On admission, CSF examination showed an unremarkable increase of protein level (688 mg l^{-1}) with no inflammatory cells. The PCR assays for herpes simplex virus (HSV) and antibodies against viruses were negative in the CSF. Serum IgM antibodies against *B. burgdorferi* were found at a low level and IgG antibodies against *Chlamydia pneumoniae* were moderately elevated. Serum C-reactive protein was 50 mg l^{-1}, lactic dehydrogenase 927 U l^{-1} (normal value < 440 U l^{-1}) and bilirubin 36 μmol l^{-1} (normal value <20 μmol l^{-1}l), but the changes were transient. Other laboratory tests were normal including serum hepatitis B surface antigen, antibodies against HIV and herpes viruses. The EEG showed an irritative focus in the left hemisphere.

On December 30, MRI of the brain showed three small frontal lesions at the bottom of the left frontal lobe near the meninges. The imaging of these lesions was enhanced using contrast medium (Fig. 3A). In the right pleural cavity, a chest X-ray examination showed fluid, which disappeared in 2 weeks. The CT showed a central cystic lesion in the left kidney, but no abnormal findings were obtained in the mediastinum, lungs, or pleural cavities. On December 31, a CSF examination showed 4×10^6 l^{-1} lymphocytes, but protein (373 mg l^{-1}), and angiotensin convertase enzyme and lysozyme concentrations were normal. The CSF antibodies against herpesviruses, *B. burgdorferi*, and *Treponema pallidum* were negative as was antigen detection for HSV and PCR for *B. burgdorferi*. The PCR for HSV was positive with this specimen. Culture for viruses and mycobacteria remained negative.

On January 8, 1993, a frontally located brain lesion was resected for suspected malignancy. Histopathological studies

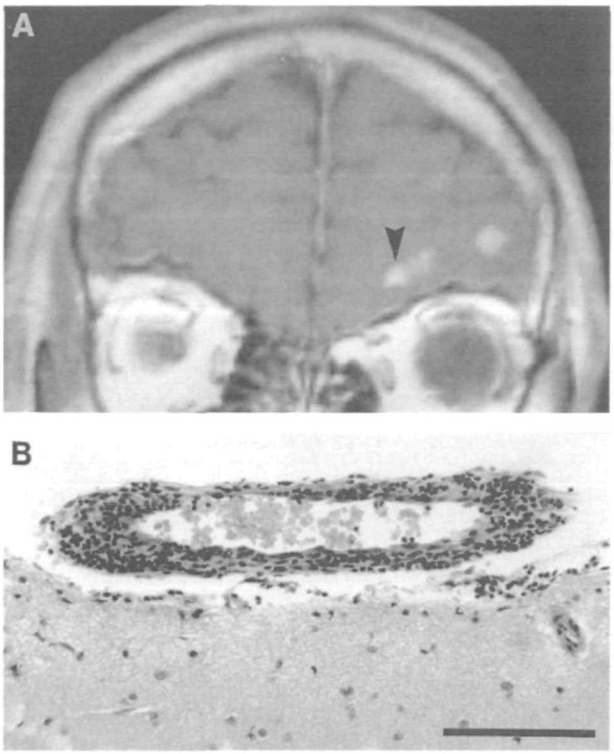

Fig. 2 (Patient 2). Gadolinium enhanced T_1-weighted MRI of the brain (December 1992) shows three small lesions at the bottom of the left frontal lobe (**A**). In the surgical sample of the lesion indicated with an arrowhead in **A** the wall of a leptomeningeal vessel is infiltrated by abundant lymphocytes (**B**; H and E; bar = 200 μm).

showed lymphocytes in the walls of leptomeningeal and small penetrating arteries as well as in the perivascular space of the latter (Fig. 2B). The adjacent cortex was slightly oedematous with very mild astrocytic gliosis. A PCR analysis of three separate brain specimens detected DNA of *B. burgdorferi*. The IgM (but not IgG) antibodies against *B. burgdorferi* were positive only in the first pretreatment serum sample but negative thereafter. The circulating immune complexes and complement activation products were positive. The IgG antibodies against *C. pneumoniae* were elevated at a constant level, but IgM antibodies remained negative, indicating that the IgG antibodies were of earlier origin. A neuropsychological investigation showed memory impairment affecting verbal function and slightly impaired fluency of verbal expression. Anticonvulsive therapy with carpamazepine was started.

Table 2 shows changes in the antimicrobial treatment schedule and the development of the brain lesions appearing on MRI. During the antibiotic treatment, MRI of the brain showed new lesions: one enhancing lesion (2 cm in diameter), suggestive of focal vasculitis, located medially from the postoperative area, and later, enhancing lesions at the bottom of the right frontal lobe and a frontal lobe sulcus (Fig. 3A and B). However, the initial lesion at the bottom of the left frontal lobe behind the orbita was now markedly smaller than at previous examinations. Later images showed that the

Table 2 *Development of brain lesions, PCR results for* B. burgdorferi, *and antimicrobial therapy given to Patient 2*

	MRI findings	PCR for *B. burgdorferi*	Therapy (duration)
Dec. 1992	A close group of three frontal lesions (Fig. 2).	Negative in serum and CSF.	Acyclovir (10 days)
Jan. 1993	Operative resection.	Positive in three brain tissue specimens.	Ceftriaxone 2 g daily i.v. (21 days) then amoxicillin 500 mg t.i.d. + probenecid 500 mg t.i.d. p.o. (20 days).
Feb. 1993	A new enhancing lesion medially from the postoperative area.	Negative in CSF.	Ceftriaxone 2 g daily i.v. (28 days) +azithromycin 250 mg daily p.o (21 days) +rifampin 600 mg daily p.o (21 days).
March 1993	Three new lesions distant from the primary lesions (Fig. 3).		Cefixime 200 mg t.i.d. p.o. + probenecid 500 mg t.i.d. p.o. (100 days).
April 1993	No new lesions, the first foci constantly reducing in size.		
July 1993	Only postoperative changes.		End of antibiotic therapy.
Dec. 1993	A new focus adjacent to the third ventricle (Fig. 3)		Doxycycline 150 mg t.i.d. p.o. (120 days).
Jan. 1994		Negative in CSF.	
March 1994			End of antibiotic therapy.
April 1994	The focus seen on previous MRI disappeared. A new focus in a frontal sulcus and a large peri-ventricular lesion (Fig. 3).		
May 1994		Positive in plasma.	
June 1994		Negative in plasma and bone marrow	Ceftriaxone 2 g daily i.v. (100 days).
Sept. 1994		Negative in plasma.	End of antibiotic therapy.
Oct. 1994	All lesions (including the periventricular ones) had disappeared.		
May 1995	No new lesions.	Negative in plasma.	

first lesion was constantly reducing in size, and five months after onset of antibiotic therapy all the new foci of the putative vasculitic process had also disappeared. The antibiotic therapy was discontinued on July 5, 1993.

The patient was asymptomatic at the end of therapy. Whole body bone scanning was carried out in June 1993 because of a history of pain in the thoracic spine some months earlier. Slightly increased uptake of isotope in the thoracic spine was seen, but the finding was considered unspecific. The EEG after sleep deprivation was normal in July 1993.

Five months after the end of antibiotic therapy, brain MRI showed a new focus located adjacent to the third ventricle (Fig. 3A and B). Oral antibiotic treatment was started (the patient was asymptomatic; *see*Table 2). The next MRI showed that the treatment had probably had a beneficial effect on the former lesions, but again, a new focus in a frontal sulcus and a relatively large pathological area in periventricular white matter were detected (Fig. 3B). On May 17, 1994, DNA of *B. burgdorferi* was detected by PCR in the patient's plasma specimen (Table 2). Intravenous antibiotic therapy was reinstituted and continued for 100 days. Thereafter, on MRI studies of the brain, all lesions and periventricular enhancement have disappeared, and no new lesions have developed to date (Table 2). The antiepileptic therapy has been discontinued, and no new seizures have occurred.

Patient 3

In the summer of 1993, this previously healthy 11-year-old girl had visited an area in Southern Finland where Lyme borreliosis is endemic. In September 1993, occasional episodes of hyperactivity followed by headache were observed by her family. On October 1, she developed paresis of the right lower limb. On October 7, she was admitted to a local hospital and 1 week later to the Oulu University Central Hospital. Standing on the right leg alone was difficult, and walking was slightly impaired.

On October 13, CT of the brain showed a periventricular low density enhancing lesion, 10×6 mm^2 in diameter, and located in left parietal lobe white matter. The lesion was suggestive of a neoplasm. On the next day, using MRI, the dimensions of the enhancing lesion were found to be

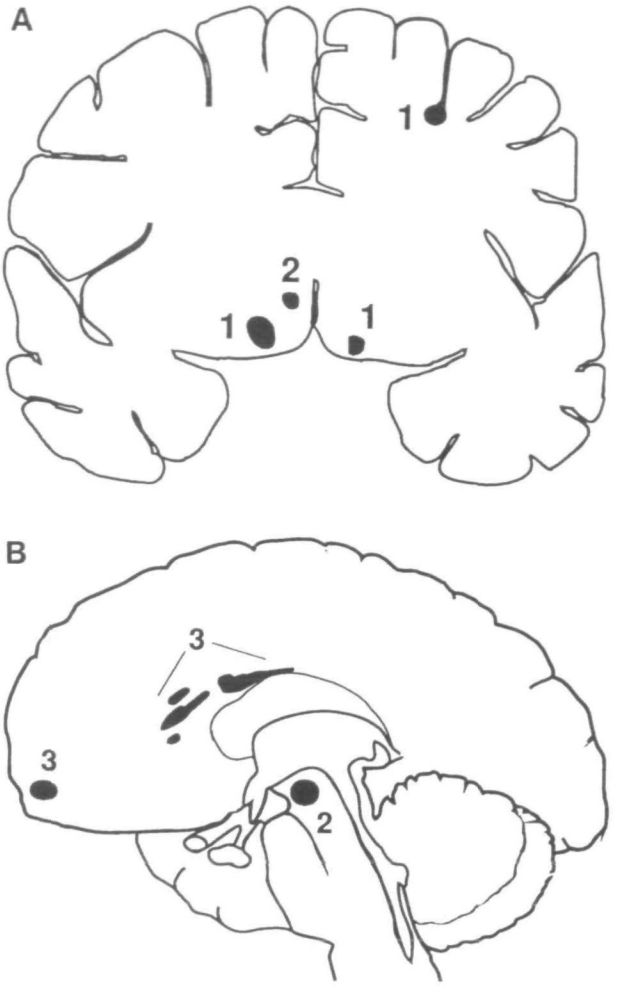

Fig. 3 (Patient 2). New enhancing lesions during antibiotic therapy (March 1993, *see* Table 3.), marked with '1' in frontal schematic view summarized from three close MR images (**A**). The initial lesions (not shown) were now markedly smaller than in previous images. In July 1993, at the end of antibiotic treatment, the MRI showed only postoperative changes in the left frontal lobe while all other lesions were no longer detectable. In December 1993 the MRI showed a new focus (marked with '2') adjacent to the third ventricle (**A** and **B**). Again, the next MRI in April 1994 showed a new focus (marked with '3') in the frontal sulcus and a large lesion (marked with '3') in periventricular white matter (**B**). In October 1994 the MRI demonstrated the disappearance of all lesions after vigorous antibiotic treatment (*see* Table 2).

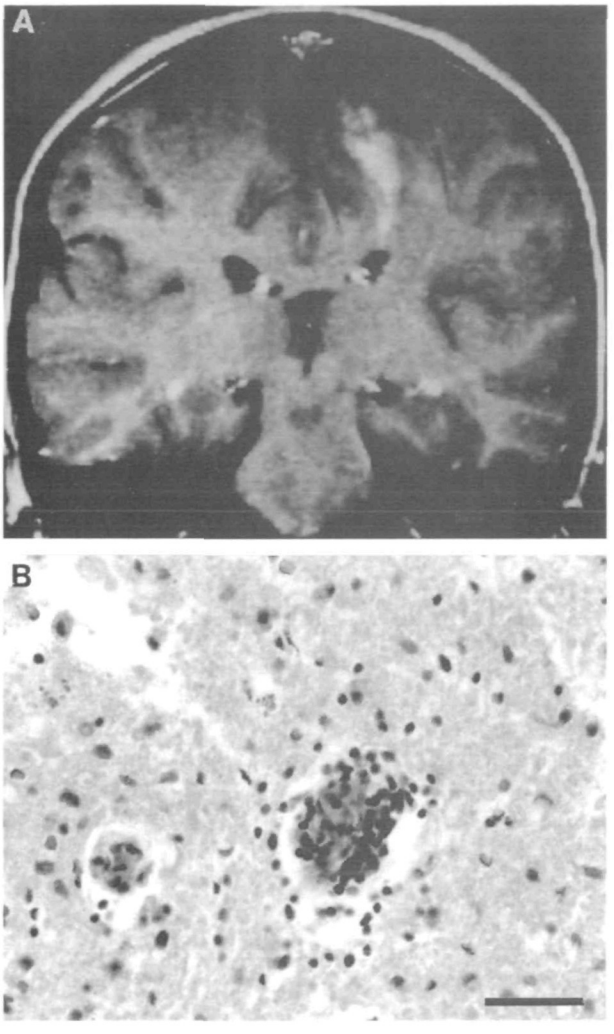

Fig. 4 (Patient 3). A periventricular, strongly enhancing lesion with surrounding oedema suggestive of a neoplasm and located in left parietal lobe white matter on T_1-weighted MRI (**A**). A blood vessel and its perivascular space within oedematous necrotized brain parenchyme is infiltrated by lymphocytes (**B**; H and E, bar = 50 μm).

$40\times20\times8$ mm^3 and the surrounding oedematous area was 20–30 mm thick (Fig. 4A). The EMG was normal. Abdominal ultrasonography showed mild splenomegaly.

On October 22, a craniotomy was carried out. In the area of the enhancing lesion, shown by MRI, elastic and stretchy tissue with abnormal white colour was detected. On histological examination, focal necrotic areas were found, surrounded by foamy macrophages, reactive astrocytes and oedema. (Fig. 4B). An increased number of small vessels with thickened walls and prominent endothelial cells were also seen. Lymphocytes occurred in the walls of some vessels.

Haemoglobin, ESR, and serum C-reactive protein values were normal. Serum total immunoglobulins were normal, except for a slightly increased value of IgM at 1.94 g l^{-1} (normal value 0.35–1.63 g l^{-1}). Serum rheumatoid factor, antinuclear antibodies, extractable nuclear antigens, anti-DNA- and anti-phospholipid antibodies were negative, and so were antibodies against several viruses. On November 4, lumbar puncture was carried out. The CSF specimen gave a negative virus culture and HSV PCR but *B. burgdorferi* PCR was positive with two separate CSF specimens (detected at two separate runs).

December 21, 1993, antibiotic therapy with ceftriaxone (2 g i.v. daily) was started for 4 weeks followed by therapy with oral amoxicillin (500 mg every 8 h) combined with oral probenecid (500 mg every 8 h). On February 1, the antibiotic therapy was stopped because of bloody diarrhoea. Culture and toxin detection for *Clostridium difficile* were negative. The diarrhoea was cured with oral metronidazole.

On February 1, 1994, MRI of the brain showed reduction of abnormal tissue around the operative area. At this time, no enhancement was seen in the walls of the cavity. At a follow-up of 1 year, recovery was observed with only a slight abnormality in walking. No new symptoms had developed.

Discussion

In this study, the evidence for the presence of *B. burgdorferi* was mainly based on positive PCR results. However, in one patient the spirochaete was also cultured from the CSF. The PCR detected DNA of *B. burgdorferi* directly in the brain lesions of two patients, and in the CSF, plasma or bone marrow of all three patients. In two patients, a positive PCR result was obtained from more than one type of specimen. Based on the results of control specimens and on the rigorous contamination control we consider our PCR results reliable. Since two of the patients had no previous antibiotic treatment before the first PCR-positive samples were obtained, it is highly probable that the PCR results indicate an ongoing infection. The CSF sample obtained from the third patient with a history of several previous antibiotic courses for other reasons was also culture-positive. The causative role of borrelial infection was supported by constriction or disappearance of the lesions in two patients after antimicrobial therapy directed against *B. burgdorferi*.

Both clinical and experimental studies have shown that *B. burgdorferi* can rapidly and effectively penetrate the CNS (Garcia-Monco *et al.*, 1990; Luft *et al.*, 1992). Interaction with the intraluminal endothelial surface by the spirochaete, directly or via systemic cytokines, was necessary for the increase of the permeability of the blood–brain barrier (BBB). The dissemination of *B. burgdorferi* to various organs depends on its ability to adhere to and penetrate the endothelium, and the BBB as well (Garcia-Monco *et al.*, 1990). In an *in vitro* model, *B. burgdorferi* was shown to adhere to the endothelial surface or to an exposed subendothelial basement membrane and migrate between endothelial cells, either at their junctions or in an area with endothelial damage (Comstock and Thomas, 1989; Szczepanski *et al.*, 1990). Recent findings indicate that *B. burgdorferi* can acquire proteolytically active host components. This mechanism could facilitate the dissemination and localization of spirochaetes to sites of vascular injury (Klempner *et al.*, 1995). The affinity of the organism for astrocytes, the nearest neighbours of brain capillaries, could facilitate its access to the nervous system (Benach and Garcia-Monco, 1992). The breakdown of the BBB noted after i.v. inoculation of *B. burgdorferi* (Garcia-Monco *et al.*, 1990) may be due to either generalized or focal production of inflammation mediators after adhering of spirochaetes to the endothelium. Focal vasculitis may then be developed by activation of endothelial cells and further release of inflammation mediators. Involvement of the CNS is associated with scattered perivascular mononuclear cell infiltrates in the cerebral cortex, mainly consisting of T-helper cells (Meurers *et al.*, 1990). The infiltrates are sometimes accompanied by mild, spongiform changes, a focal increase in microglial cells, and a modest infiltration of lymphocytes and plasma cells in the leptomeninges (Duray, 1989*a*).

Our observations support results from both experimental and clinical investigations suggesting that the number of *B. burgdorferi* spirochaetes in the CNS is very low. The spirochaete could be cultivated from the CSF of only one of our patients. To date, almost all attempts to isolate *B. burgdorferi* from human brain tissue have failed. Culture of *B. burgdorferi*-like organisms from brain tissue affected by Alzheimer's disease has been reported twice (MacDonald and Miranda, 1987; Miklossy *et al.*, 1994). A few other reports have been published showing the agent in biopsy or autopsy specimens. In one patient with subacute encephalitis, a brain biopsy specimen showed spirochaetes morphologically compatible with *B. burgdorferi* and microgliosis without inflammatory infiltrate (Pachner *et al.*, 1989). In a newborn whose mother had suffered from Lyme borreliosis in early pregnancy and who died during the first day after birth, *B. burgdorferi* could be demonstrated by staining and immunocytochemistry in the brain and liver (Weber *et al.*, 1988). One child with a history of severe arthritis for several months died during a protracted seizure which was her first neurological manifestation of the disease; histological studies of brain tissue showed general vasculitis, and *B. burgdorferi* was demonstrated by silver staining (Millner *et al.*, 1991). Brain involvement during experimental infection with *B. burgdorferi* has been reported in several animal models (Johnson *et al.*, 1984; Burgdorfer and Gage, 1987; Barthold *et al.*, 1988; Garcia-Monco *et al.*, 1990; Moody *et al.*, 1990*b*; Pachner and Itano, 1990; Barthold *et al.*, 1992), but even in these experiments the presence of borreliae in the brain or CSF has often been short-lived.

The pathogenesis of CNS manifestations in Lyme borreliosis is inadequately known. Our observations support earlier reports suggesting that vasculitis may be one of the primary pathophysiological mechanisms in neuroborreliosis (Camponovo and Meier, 1986; Midgard and Hofstad, 1987; Kohler *et al.*, 1988; Meier and Grehl, 1988; Mokry *et al.*, 1990; Meurers *et al.*, 1990). This is only logical, because *B. burgdorferi* infection frequently causes perivascular inflammation or vasculitis (e.g. retinal vasculitis) in affected organs other than the CNS (Duray, 1989*a*, *b*; Olson and Esterly, 1990; Smith *et al.*, 1991; Karma *et al.*, 1995). In general, micro-organisms causing retinal vasculitis have been found to be much the same as those causing cerebral vasculitides (Somer and Finegold, 1995). In the peripheral nervous system, inflammatory vascular changes or vasculitis seem to cause axonal degeneration in Lyme borreliosis (Camponovo and Meier, 1986; Meurers *et al.*, 1990). Studies on experimental borrelia infections have also shown prominent lymphoplasmacellular infiltration in the microvasculature, endarteritis obliterans, and spirochaetes in and around blood vessels of synovial and myocardial tissues (Johnston *et al.*, 1985; Moody *et al.*, 1990*a*). Vasculitis also is a predominant finding in *T. pallidum* infection, another

spirochetal disease with CNS invasion (Kohler *et al.*, 1988; Coyle and Dattwyler, 1990). Syphilitic endarteritis may cause multiple small infarctions in the CNS, or involve the vasa vasorum of large or medium-sized vessels, and lead to aneurysms or ischaemic infarction months or years after onset of infection.

Our study supports a sporadic causative role for *B. burgdorferi* infection in stroke-like diseases or vasculitis in agreement with previous studies (Midgard and Hofstad, 1987; Uldry *et al.*, 1987; Veenendaal-Hillbers *et al.*, 1988; May and Jabbari, 1990; Millner *et al.*, 1991; Hammers-Berggren *et al.*, 1993). This is also in agreement with the recent finding that patients with chronic Lyme disease encephalopathy have multifocally reduced blood perfusion to the cerebral hemispheres, particularly in white matter, and that these patients show objective improvement in brain perfusion after antibiotic treatment (Steere *et al.*, 1994).

The brain autopsy specimens of Patient 1 showed extensive demyelination in periventricular areas, and *B. burgdorferi* was cultivated from the CSF. This finding, combined with the long history of her disease (decades), indicates that *B. burgdorferi* may occasionally cause pronounced demyelination, possibly via damage to microvasculature, similar to that seen in neurosyphilis. Several studies have found an association between demyelinating disease and *B. burgdorferi* infection (Reik *et al.*, 1985; Kohler *et al.*, 1988; Pachner *et al.*, 1989; Clavelou *et al.*, 1993), although in multiple sclerosis *B. burgdorferi* infection rarely seems to be the trigger (Coyle, 1989; Baig *et al.*, 1991; Coyle *et al.* 1993). Direct damage to oligodendroglial cells may cause demyelination because *B. burgdorferi* very actively binds to them (Baig *et al.*, 1991). This binding may partly explain the long-term persistence of *B. burgdorferi* in the CNS and explain why the spirochaete is seldom isolated from the CSF (Garcia-Monco *et al.*, 1989; Pachner and Delaney, 1993). Galactocerebroside, a component of myelin, and other glycosphingolipids are possible binding structures for *B. burgdorferi* and other related spirochaetes causing disease in the central and peripheral nervous systems (Garcia-Monco *et al.*, 1992; Backenson *et al.*, 1995). Autoimmune reactions, triggered by *B. burgdorferi* infection, may also cause demyelination. B-cells capable of responding to myelin basic protein have been a common finding in the CSF of patients with LNB (Baig *et al.*, 1991). Because nervous tissue is considered an immunologically privileged site, autoreactive B-cells, and autoantibodies as their products, may arise as a result of host response to neuronal antigens exposed by tissue damage. Another explanation to autoreactivity could be molecular mimicry and cross-reactions between neuronal autoantigens and antigens of *B. burgdorferi* (Sigal and Tatum, 1988; Sigal, 1993). Experimental studies on SCID (severe combined immuno-deficient) mice, however, show that at least arthritogenesis is not necessarily dependent on an intact immune system, since chronic arthritis is inducible in these mice (Barthold *et al.*, 1992).

Brain lesions in Patient 2 developed in previously intact areas during or after treatment, the last one of them 16 months after onset of prolonged antibiotic therapy. Several reports have been published on the occurrence of new foci and paradoxical enlargement of CNS lesions during the treatment of mycobacterial CNS infections (Afghani and Lieberman, 1994). The direct effects of mycobacterial products or the host's immunological reactions elicited by microbial components have been offered as the most likely explanation for the appearance of new foci in mycobacterial infections (Afghani and Lieberman, 1994). Similar mechanisms could explain the development of new lesions during therapy in Patient 2. However, DNA of *B. burgdorferi* in the plasma of the patient over 16 months after the onset of first antibiotic treatment suggests the presence of ongoing infection. The route of entry to the new sites could have been the vascular wall after occurrence of subclinical spirochaetemia. Another explanation for the onset of new lesions is that the spirochaetes were already at the site of lesions before the antibiotic treatment. Because of metabolic inactivity, they were not affected by the antibiotics. After a latent period, the uneradicated spirochaetes could have activated and caused changes visible on brain MRI. The disappearence of all lesions after repeated therapy further supports the theory that the lesions were directly related to *B. burgdorferi* infection. Our experience with this patient suggests that, in rare cases, extended or repeated antibiotic treatments may be necessary to eradicate the spirochaete from sites where it has aquired a latent state.

The diagnosis of LNB has usually been based on nonspecific findings, serological testing and other indirect methods. Stereotactic biopsy or a surgical operation are necessary for direct demonstration of an etiological agent in vasculitis or brain lesions. However, evidence for the presence of *B. burgdorferi* could be obtained by culture or PCR of the CSF or plasma specimens in all three of our patients. This is consistent with the earlier finding that small numbers of spirochaetes or their structures occasionally circulate in the blood not only in early infection but also during the later stages of Lyme borreliosis (Viljanen *et al.*, 1992; Nadelman *et al.*, 1994; Oksi *et al.*, 1994 and 1995*a, b*; Oksi, 1995). Thus, MRI findings compatible with vasculitis associated with a positive PCR result from the CSF or plasma, even without direct demonstration of the spirochaete in the brain lesions, might be an indication for antimicrobial treatment directed against *B. burgdorferi*.

Our patients had no borrelial antibodies in their CSF. This result is in contrast with most published studies on European neuroborreliosis patients, in whom intrathecal antibody production has usually been detected. Indeed, intrathecal antibody production has been one of the criteria for the diagnosis of neuroborreliosis (Steere *et al.* 1990; Baig *et al.*, 1991; Hansen and Lebech, 1991, 1992). By contrast, American patients with LNB often show no intrathecally produced antibody (Steere *et al.*, 1990; Kuntzer *et al.*, 1991; Luft *et al.*, 1992). It is not known whether the differences reported between American and European LNB patients are

due to patient selection or some other factor (Steere *et al.*, 1990). However, our results indicate that the differences between European LNB and its North-American counterpart may not be as great as has been suggested. Three plausible explanations for the lack of intrathecal antibody production in LNB may be suggested. First, the CNS can be considered an immunoprivileged site where the spirochaete can lie latent out of reach of the host immune system (Guy and Turner, 1989; Pfister *et al.*, 1989; Halperin *et al.*, 1991). Second, studies with T cell clones suggest that *B. burgdorferi* may shift the immune response of the host towards cell-mediated immunity at the expense of antibody production (Yssel *et al.*, 1991). This is advantageous from the spirochaete's point of view, because antibodies are obviously the major factor in the host defense against *B. burgdorferi* infection (Fikrig *et al.*, 1992). We have also obtained evidence that *B. burgdorferi* infection can cause suppression of Th2 cells and activation of Th1 cells in patients with late Lyme borreliosis (Oksi *et al.*, 1996). Third, immune complex formation and binding of antibodies to complexes may be a mechanism underlying the negative results of routine antibody assays (Schutzer *et al.*, 1990).

Hemiparesis (Uldry *et al.*, 1987; Veenendaal-Hilbers *et al.*, 1988; May and Jabbari, 1990), which was the main manifestation in one of our patients, and epilepsy (Millner *et al.*, 1991; Mourin *et al.*, 1993), the dominating symptom of another patient, have also been reported to be associated with LNB. The almost complete recovery of our patient from the hemiparesis, and the complete disappearence of brain foci and recovery from the epilepsy in another patient after antimicrobial treatment may suggest infectious etiology. We recommend exclusion of Lyme borreliosis as a trigger of disease in selected patients with demyelinating disease, hemiparesis, or epilepsy.

We conclude that cerebral lymphocytic vasculitis and multifocal encephalitis may be associated with *B. burgdorferi* infection. The presence of *B. burgdorferi* DNA in tissue samples from areas with inflammatory changes indicates that direct invasion of *B. burgdorferi* may be the pathogenetic mechanism for focal encephalitis in Lyme neuroborreliosis.

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