Clinical Findings for Patients with Lyme Borreliosis Caused by *Borrelia burgdorferi* Sensu Lato with Genotypic and Phenotypic Similarities to Strain 25015

Franc Strle, Roger N. Picken, Yu Cheng, Joze Cimperman, Vera Maraspin, Stanka Lotric-Furlan, Eva Ruzic-Sabljic, and Maria M. Picken

In the course of performing culture isolation of *Borrelia burgdorferi* sensu lato for the diagnosis of Lyme borreliosis in Slovenia, we encountered nine patients who were infected with atypical strains. Molecular analyses of these strains suggested that they were more closely related to the North American tick isolate, strain 25015 (which belongs to the DN127 genomic group of *B. burgdorferi* sensu lato), than they were to the three species (*B. burgdorferi* sensu stricto, *Borrelia garinii*, and *Borrelia afzelii*) hitherto found to be associated with European Lyme borreliosis. Review of the case histories of these patients revealed some atypical clinical features and variability in clinical presentation. In this study, we present the clinical findings for these patients and discuss their significance for the diagnosis of Lyme borreliosis. The DN127 genomic group shares with *B. burgdorferi* sensu stricto the distinction of being present in both the Old and New Worlds.

Lyme borreliosis is a multisystem infection caused by spirochetes of the genus *Borrelia* and is transmitted by the bite of ixodid ticks [1]. Protean clinical manifestations are a noted feature of the disease. Although the most common early manifestations are skin lesions, the disease can disseminate to involve other organs including the nervous system, joints, heart, other muscles, and the eye [1–3]. A chronic skin manifestation (acrodermatitis chronica atrophicans), which is more prevalent in European patients, has also been described [2, 4].

Intensive molecular analysis of the etiologic agent over the last 5 years has demonstrated that Lyme borreliosis may be caused by several closely related genomic groups of organisms, collectively referred to as *Borrelia burgdorferi* sensu lato [5] (which includes three species pathogenic for humans: *B. burgdorferi* sensu stricto, *Borrelia garinii*, and *Borrelia afzelii* [6, 7]). Other related genomic groups of spirochetes have also been identified in investigations of ixodid ticks and reservoir animals, some of which have been accorded species status. Examples of the former include the DN127 and VS116 genomic groups of organisms [8], while *Borrelia andersonii* and *Borrelia japonica* exemplify the latter [9, 10]. The worldwide distribution of species is not homogeneous. Of the species pathogenic for humans, only *B. burgdorferi* sensu stricto has thus far been detected in the United States [8, 11], whereas *B. garinii* and *B. afzelii* are also present in Europe [6, 7].

Recent extensive characterization of nine isolates of *B. burgdorferi* sensu lato that were recovered from patients with Lyme borreliosis who resided in Slovenia revealed that they appear to be most closely related, in molecular terms, to a member of the DN127 genomic group of organisms, strain 25015 [12]. These findings represent the first indication that this genomic group, known hitherto to be isolated only from ticks and reservoir animals, is pathogenic for humans. We present the clinical findings for the nine patients from whom these isolates were recovered.

**Patients and Methods**

**Patients**

The nine patients included in the present study presented to the Department of Infectious Diseases, University Medical Centre, Ljubljana, Slovenia, between July 1992 and December 1993. The University Medical Center serves as a reference center for Lyme borreliosis, treating patients with early disease manifestations from the surrounding area and patients with suspected disseminated disease from Slovenia as a whole. Patient histories showed that all nine patients acquired their disease in Slovenia.

**Serological Testing**

Titers of IgM and IgG antibodies to *B. burgdorferi* sensu lato were determined by immunofluorescence assay (IFA) as
Table 1. *Borrelia* species and strains used in a study of Lyme borreliosis caused by *Borrelia burgdorferi* sensu lato with genotypic and phenotypic similarities to strain 25015.

<table>
<thead>
<tr>
<th>Strains, species</th>
<th>Strain designation</th>
<th>Biological origin</th>
<th>Geographic location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference strains</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Borrelia burgdorferi</em> sensu stricto</td>
<td>B31 (ATCC 35210)</td>
<td>Tick (<em>Ixodes scapularis</em>)</td>
<td>USA</td>
</tr>
<tr>
<td><em>Borrelia garinii</em></td>
<td>20047</td>
<td>Tick (<em>Ixodes ricinus</em>)</td>
<td>France</td>
</tr>
<tr>
<td><em>Borrelia afzelii</em></td>
<td>PGau</td>
<td>Skin (ACA)</td>
<td>Germany</td>
</tr>
<tr>
<td><em>B. burgdorferi</em> sensu lato</td>
<td>25015</td>
<td>Tick (<em>I. scapularis</em>)</td>
<td>USA</td>
</tr>
<tr>
<td><em>B. burgdorferi</em> sensu lato</td>
<td>CT39</td>
<td>Mouse (<em>Peromyscus leucopus</em>)</td>
<td>USA</td>
</tr>
<tr>
<td>Strains from patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. burgdorferi</em> sensu lato</td>
<td>SL-70</td>
<td>Skin (EM)</td>
<td>Slovenia</td>
</tr>
<tr>
<td><em>B. burgdorferi</em> sensu lato</td>
<td>SL-73</td>
<td>Skin (EM)</td>
<td>Slovenia</td>
</tr>
<tr>
<td><em>B. burgdorferi</em> sensu lato</td>
<td>SL-74</td>
<td>Skin (EM status post)</td>
<td>Slovenia</td>
</tr>
<tr>
<td><em>B. burgdorferi</em> sensu lato</td>
<td>SL-75</td>
<td>Skin (EM status post)</td>
<td>Slovenia</td>
</tr>
<tr>
<td><em>B. burgdorferi</em> sensu lato</td>
<td>SL-76</td>
<td>Skin (EM)</td>
<td>Slovenia</td>
</tr>
<tr>
<td><em>B. burgdorferi</em> sensu lato</td>
<td>SL-78</td>
<td>Skin (lymphocytoma)</td>
<td>Slovenia</td>
</tr>
<tr>
<td><em>B. burgdorferi</em> sensu lato</td>
<td>SL-79</td>
<td>CSF</td>
<td>Slovenia</td>
</tr>
<tr>
<td><em>B. burgdorferi</em> sensu lato</td>
<td>SL-80</td>
<td>CSF</td>
<td>Slovenia</td>
</tr>
<tr>
<td><em>B. burgdorferi</em> sensu lato</td>
<td>SL-90</td>
<td>CSF</td>
<td>Slovenia</td>
</tr>
</tbody>
</table>

NOTE. ACA = acrodermatitis chronica atrophicans; ATCC = American Type Culture Collection; EM = erythema migrans.

Described previously [13]; a local *B. afzelii* isolate was used as antigen. All patient serum samples were tested for the presence of antibodies at the time of skin biopsy; CSF samples from some patients were also tested at the same time for the presence of intrathecal antibodies by using the same IFA.

**Culture Isolation of Patient Strains**

Sites and processing of skin biopsy and CSF specimens as well as culture isolation of spirochetes have been described previously [14]. Three isolates were from extant erythema migrans (EM) lesions, two were from normal appearing skin at the site of resolved EM lesions, one was from a lymphocytoma, and three were from CSF. Patient isolates are listed in table 1.

**Characterization of Strains**

Isolates were characterized by the following procedures: species typing by 16S rRNA–specific PCR analysis with use of primers designed to differentiate *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii*; pulsed-field gel electrophoresis (PFGE) of undigested or *Mlu*I-digested total genomic DNA; and SDS-PAGE separation of total proteins. These procedures have been described previously [15]. Reference strains of *B. burgdorferi* sensu lato that were used for comparison are also listed in table 1. The source of reference strains has been described previously [16].

**Results**

**Characteristics of Strains**

By 16S rRNA–specific PCR analysis, all nine isolates typed as *B. burgdorferi* sensu stricto. Further analysis by PFGE and SDS-PAGE demonstrated that the isolates were similar in terms of their molecular characteristics to two North American isolates of *B. burgdorferi* sensu lato, strains 25015 and CT39; these two strains are derived from an *Ixodes scapularis* tick and a white-footed mouse, respectively. Although the latter isolates also type as *B. burgdorferi* sensu stricto by 16S rRNA–specific PCR analysis, they are known to belong to a distinct genomic group (the DN127 genomic group).

The genetic and phenotypic similarities of the nine patient isolates, both to each other and to strains 25015 and CT39 are shown in figure 1. All isolates except strain SL-80 shared the same *Mlu*I digestion pattern as strains 25015 and CT39 (figure 1A); strain SL-80 appears to possess a variant form of this pattern. In addition, all isolates except strain SL-79 possessed the same plasmid profile as strain 25015 (figure 1B); strain SL-79 lacks one plasmid. A distinctive feature of the nine patient isolates was their expression of prominent proteins with molecular masses of ~27 kD (figure 1C). Higher-molecular-mass proteins show differences among individual strains in their levels of expression but correspond in size to those seen in each of the nine patient isolates and strains 25015 and CT39. Further molecular analyses of the nine strains confirmed their identity as *B. burgdorferi* sensu lato and revealed other genotypic similarities to strains 25015 and CT39 [12].

**Clinical Findings for Patients**

Patient 1. A previously healthy 46-year-old woman presented to the medical center in August 1993. She first noticed an erythema of <1 cm in diameter on her left thigh 10 days before the visit; she also recalled several recent tick bites but none at the site of the lesion. She reported only mild local...
Figure 1.  

A. Results of pulsed-field gel electrophoresis (PFGE) of MluI digested genomic DNA from *Borrelia burgdorferi* strains 25015 and CT39 and the nine patient isolates of *B. burgdorferi* sensu lato with the MLx large restriction fragment pattern (LRFP) [12]. *Borrelia afzelii* strain PGau, which has the very similar MLa1 LRFP, was included for comparison. Strain designations are listed above their respective lanes. Lane M, DNA molecular size markers (λ DNA concatemers of 49–485 kilobases [kb]).

B. Results of PFGE of undigested genomic DNA from the *B. burgdorferi* sensu lato isolates shown in A. Strain designations are shown above their respective lanes. Lane M, DNA molecular size markers of 8.3, 8.6, 10.1, 12.2, 15.0, 17.1, 19.4, 22.6, 24.8, 29.9, 33.5, 38.4, and 48.5 kb (these markers are too closely spaced to be shown at the side of the figure); Chr>, the position of the 950-kb chromosome; D>, diffuse band of DNA representing randomly sheared, chromosomal breakdown products; LLP|, the position of the largest linear plasmids (49 and 56 kb).

C. Results of SDS-PAGE of total cellular proteins from *B. burgdorferi* sensu lato strains 25015 and CT39 and the nine patient isolates of *B. burgdorferi* sensu lato with the MLx LRFP. Strain designations are shown above their respective lanes. Lane M, prestained protein molecular mass markers of 21.9–142.9 kD.
itching at the site of the erythematous rash. Physical examination revealed a homogeneous, circular erythematous area on her leg that was ~5 cm in size. A biopsy specimen from the margin of the erythematous lesion was obtained; culture of this specimen yielded *B. burgdorferi* sensu lato. This isolate was subsequently designated SL-70 (table 1). Serological testing by IFA [13] on the day of the biopsy revealed a negative titer of IgM antibody to *B. burgdorferi* sensu lato and a titer of IgG antibody to *B. burgdorferi* sensu lato of 1:256.

The patient was treated with azithromycin (500 mg twice a day for the first day followed by 500 mg once a day for the next 4 days). The skin lesion disappeared in 6 days. A protocol rebiopsy [14] at the site of the original lesion was performed 8 weeks after the initial evaluation; culture of the biopsy sample from the lesion yielded *B. burgdorferi* sensu lato. This isolate was designated SL-74 (table 1). Serological tests for antibodies to *Borrelia* that were performed 1 year after the first appearance of EM were negative.

**Patient 2.** A 51-year-old woman with a history of hyperthyroidism and depression presented to the medical center in mid-August 1993. Three days before her visit, she had noticed a large (25 × 10 cm) area of redness behind her right knee that was slowly enlarging. Over the 3-day period, she had experienced itching and burning sensations at the site of the erythematous lesion as well as generalized malaise and fatigue. She had no recollection of a tick bite. Physical examination revealed a typical elliptic EM lesion behind the right knee that was ~25 × 15 cm in size. A skin biopsy specimen from the advancing margin of the lesion was obtained; culture of the specimen yielded *B. burgdorferi* sensu lato. This isolate was subsequently designated SL-73 (table 1). Serological tests for IgM and IgG antibodies to *B. burgdorferi* sensu lato by IFA were negative.

The patient was treated with azithromycin for 5 days (total dose, 3 g). The skin lesion disappeared on the fourth day of treatment. No additional symptoms or signs of Lyme borreliosis were reported or apparent during a 1-year follow-up. A protocol rebiopsy [14] at the site of the original lesion was performed in October 1993; culture of the specimen was negative for spirochetes.

**Patient 3.** A 50-year-old previously healthy man presented in September 1993 (this patient has been previously described [14]). He recalled a tick bite in the right axilla 2 months before evaluation. He subsequently developed a 10-cm erythematous rash at the site of the tick bite that persisted for 10 days before resolution. He was without further symptoms for ~6 weeks. Subsequently, he experienced a severe headache followed 1 day later by a lack of dorsiflexion of his left foot. This difficulty continued until the time of presentation 2 weeks later.

Physical examination at the time of presentation was unremarkable with the exception of the left footdrop. Cultures of CSF were negative for *B. burgdorferi* sensu lato. Routine CSF parameters were also unremarkable. Electromyograms revealed a mild peroneal nerve abnormality. However, culture of a skin biopsy specimen from the site of the previous EM rash yielded *B. burgdorferi* sensu lato. This isolate was eventually designated SL-74 (table 1). IFA revealed negative titers of antibody to *B. burgdorferi*. Western blotting of serum with use of strain SL-74 as antigen demonstrated responses to both IgM and IgG antibodies, with the IgM response predominating [14].

The patient received a 14-day course of ceftiraxone therapy; his clinical symptoms abated completely within 5 weeks of initiating treatment. The subsequent clinical course was uneventful. A protocol rebiopsy [14] at the site of the previous lesion was performed 8 weeks after the initial evaluation; culture of the specimen was negative for spirochetes.

**Patient 4.** A 61-year-old man with a history of hypertension presented to the medical center in June 1993. He reported that he had removed a tick from his leg in April 1993. Ten days later, he noticed a circular rash (~2 cm in diameter) at the site of the tick bite that slowly increased in size and itched occasionally. Physical examination revealed an annular erythematous lesion with a diameter of ~10 cm. Culture of a biopsy sample from the lesion yielded *B. burgdorferi* sensu lato. This isolate was later identified as *B. afzelii*. Serological testing by IFA at the time of biopsy revealed a titer of IgM antibody of 1:128 and a negative titer of IgG antibody.

The lesion disappeared 12 days after the start of treatment with azithromycin. However, when a protocol rebiopsy [14] of normal appearing skin at the site of the previous EM lesion was performed 70 days after the first visit, culture of the specimen yielded spirochetes. This isolate was subsequently designated SL-75 (table 1). In view of the positive culture results, the patient was treated with doxycycline (100 mg twice a day for 2 weeks) despite being asymptomatic. Culture of a third skin biopsy specimen obtained 4 months later was negative. The patient remained asymptomatic.

**Patient 5.** A 51-year-old woman with a 2-year history of arthralgias and myalgias presented to the medical center in late August 1993. The patient recalled a tick bite in July 1993. A few days later, she noticed a redness of the skin on her left leg that was ~25 × 10 cm. A skin biopsy specimen from the lesion was obtained; culture of this specimen yielded *B. burgdorferi* sensu lato. This isolate was eventually designated SL-76 (table 1). Serological testing by IFA did not detect IgM or IgG antibodies to *B. burgdorferi* sensu lato. Culture of a biopsy sample from the erythematous lesion yielded *B. burgdorferi* sensu lato that was subsequently designated SL-76 (table 1). The patient was treated with azithromycin (total dose, 3 g). The skin lesion disappeared within 5 days of the start of therapy, and
additional symptoms disappeared a few days later. A protocol skin rebiopsy [14] at a proximal site was performed 64 days after the first procedure; culture of the specimen was negative for *B. burgdorferi* sensu lato. During 1 year of subsequent follow-up, no signs or symptoms compatible with Lyme borreliosis were apparent.

**Patient 6.** A 72-year-old woman undergoing treatment for heart failure due to mitral valve involvement (probably consequent to childhood rheumatic fever) and hypertension was referred to the medical center in September 1993. She recalled a tick bite on the left side of her chest in mid-May 1993. In June 1993, her left breast became tender. At this time, she noticed redness and edema of the left nipple. She was referred to an oncologist who determined that the lesion was not clinically suspicious for malignancy. In August 1993, the patient noticed a ringlike redness of the skin around her left breast. During the 2 months before presentation, she had also experienced intermittent headaches as well as arthralgias in the knees, left hip, and elbows.

At the time of physical examination, an elliptic annular erythematous lesion that was 28 × 14 cm in size was apparent on the left hemithorax. The left nipple was moderately edematous and slightly painful. In the area of the areola mammae of the left breast, a 2 × 1.5-cm nodule was palpated. Serological testing by IFA revealed a titer of IgM antibody to *B. burgdorferi* sensu lato of 1:128 and a negative titer of IgG antibody. Histological examination of the nodule revealed a dense polymyalphocytic infiltrate without germinal centers. Culture of a biopsy sample from the nodule yielded *B. burgdorferi* sensu lato. This isolate was subsequently designated SL-78 (table 1).

The patient was treated with azithromycin (total dose, 3 g). The EM disappeared 2.5 weeks after the start of treatment, and the lymphocytoma resolved 2 weeks later. During 1 year of follow-up, the patient reported intermittent arthralgias in the knees and ankles. Subsequent serological tests for *B. burgdorferi* sensu lato were negative.

**Patient 7.** A 51-year-old previously healthy woman who recalled several tick bites but no associated skin changes compatible with EM presented to our medical center in August 1993. In March 1993, she experienced difficulties in concentration and failing memory that was followed by increasing tiredness, occasional arthralgias and myalgias, and frequent headaches. No diagnostic abnormalities were apparent during a routine physical and neurological examination or from laboratory tests. In August 1993, she suffered a severe headache, fever (temperature to 38.8°C), nausea, and vomiting. On the following day, a lumbar puncture was performed; analysis of CSF was unremarkable. Serological examination of serum and CSF by IFA failed to detect IgM or IgG antibodies to *B. burgdorferi* sensu lato; however, culture of CSF yielded spirochetes. The isolate was subsequently designated SL-79 (table 1). MRI demonstrated several lesions in the white matter of the brain with diameters of up to 1 cm (findings suggestive of demyelination plaques).

The patient was treated with ceftriaxone (2 g/d intravenously for 14 days). After the first administration of ceftriaxone, the patient experienced fever compatible with a Jarisch-Herxheimer reaction. In the following months, the patient’s condition improved substantially, and she was able to resume work. However, during 3 years of follow-up, fatigue, concentration disturbances, and emotional changes (mood changes and nervousness) persisted and became intermittently more pronounced. The patient refused additional examinations by MRI and remained seronegative for *B. burgdorferi* sensu lato.

**Patient 8.** A 20-year-old previously healthy woman was admitted to our medical center in September 1993. She had noted several tick bites during the spring and summer of 1993. No skin changes at the sites of the tick bites were recalled by the patient. In July 1993, she was treated by her local doctor with phenoxymethyl penicillin for apparent pharyngitis. Although there was immediate improvement in her condition, one transient episode of fever, headache, and vomiting occurred 5 days after the initiation of therapy. Subsequently, intermittent headaches and low-grade fever returned, leading to her admission because of suspected meningitis in September 1993.

At the time of admission, she had a temperature of 37.7°C and mild meningeal signs. CSF examination revealed mild pleocytosis with a predominance of lymphocytes (lymphocyte count, 64 × 10⁶/L; neutrophil count, 11 × 10⁶/L), a borderline protein concentration (0.47 g/L), and a glucose concentration of 3.3 mmol/L (serum concentration, 4.8 mmol/L). An increase in the serum C-reactive protein level (43 mg/L) was found; results of all other routine laboratory tests were within normal ranges. ELISA of serum did not detect IgM antibodies to tick-borne encephalitis virus but did detect IgG antibodies (which is indicative of past infection). IFA of serum and CSF samples were all negative for antibodies to *B. burgdorferi* sensu lato. However, in view of her history of tick bites, CSF was cultured in borrelia medium.

The patient was initially treated symptomatically for presumed aseptic meningitis, but 3 days after admission, she experienced another episode of pharyngitis. Streptococcal pharyngitis was diagnosed and she was treated with phenoxymethyl penicillin (1 million U three times a day for 10 days). Her condition improved rapidly. Subsequently, however, the CSF culture yielded *B. burgdorferi* sensu lato, and this isolate was later designated SL-80 (table 1). Therefore, ~6 weeks after initial hospitalization, the patient was reevaluated.

At the time of reevaluation, CSF examination revealed no abnormalities, and titers of antibodies to *B. burgdorferi* sensu lato in serum and CSF samples remained negative. A second CSF culture was negative for *Borrelia*. However, in view of a positive result of the first CSF culture, the patient was given 14 days of therapy with ceftriaxone (2 g/d intravenously). Another examination 2 months later revealed no abnormalities. Serological tests for antibodies to *B. burgdorferi* sensu lato were again negative. The patient remained asymptomatic for 3.5 months.

---

**Clinical Findings for DN127 Genomic Group Infection**

CID 1997;25 (August)
Thereafter, she developed a right lumboischialgia. Analgesic therapy was unsuccessful. CSF analysis showed an increased protein concentration (0.98 g/L), but again no antibodies to \textit{B. burgdorferi} sensu lato were detected in serum and CSF samples. However, spirochetes were once again cultured from the CSF. The patient was treated again with ceftriaxone and subsequently recovered. The patient was lost to follow-up. The second CSF isolate was characterized by an extremely slow growth rate and a low yield of spirochetes; therefore, it was not analyzed. Subsequently, the isolate could not be detected in subcultures by dark-field microscopy or recovered from earlier frozen stocks.

**Patient 9.** A 26-year-old man presented to the medical center at the beginning of July 1992 because of severe meningoencephalitis. The patient had felt ill since the end of June 1992. He recalled several tick bites 1 month before feeling ill but had no recollection of skin changes. CSF examination revealed a leukocyte count of \(35 \times 10^6/\text{L}\) (54% neutrophils). Protein and glucose concentrations were normal. Serological testing by ELISA for antibodies to tick-borne encephalitis virus revealed high levels of specific IgM antibodies and seroconversion to IgG antibodies. Low-level titers of IgM and IgG antibodies to \textit{B. burgdorferi} sensu lato were also detected in serum by IFA.

Since the patient’s condition showed little improvement, it was considered that concomitant \textit{B. burgdorferi} sensu lato infection could be responsible for some of the clinical manifestations of his prolonged meningoencephalitis. Therefore, in August 1992, the patient was treated with ceftriaxone (2 g/d intravenously for 14 days). An improvement was observed after 10 days. Two months later, periodic headaches, vertigo, and memory disturbances occurred. Six months later, myalgias, fatigue, and intense sweating appeared. Repeated serological testing by IFA for antibodies to \textit{B. burgdorferi} sensu lato was negative.

One year later, all of the symptoms increased. Although results of routine examinations of CSF were normal, borderline titers of IgM and IgG antibodies to \textit{B. burgdorferi} sensu lato were detected in serum. Culture of CSF yielded spirochetes. This isolate was subsequently designated SL-90 (table 1). No new tick bites or EM lesions were reported by the patient during this time. The patient was again treated with ceftriaxone (2 g/d intravenously for 14 days). The improvement was substantial, but complete recovery was not achieved. The patient was last seen in November 1996, at which time he reported continuing fatigue, occasional myalgias, arthralgias, and intermittent headaches.

**Discussion**

Strains 25015 and DN127 were both isolated from ixodid ticks collected in North America in the 1980s, and although these strains were considered to be isolates of \textit{B. burgdorferi}, their protein profiles determined by SDS-PAGE suggested that they were atypical members of the species [17, 18]. Strain CT39 was isolated from a white-footed mouse captured in North America in 1990 [15]. Detailed molecular analysis of this strain demonstrated similarities to strain 25015 and a borderline taxonomic relationship of both isolates to other members of the species \textit{B. burgdorferi} [19–21]. Subsequently, studies of DNA relatedness among many North American and European isolates defined three distinct species that were associated with human Lyme borreliosis (\textit{B. burgdorferi} sensu stricto, \textit{B. garinii}, and \textit{B. afzelii}) [6] as well as other genomic groups containing strains isolated from ticks [8]. Strain DN127, strain 25015, and four \textit{Ixodes neotomae} isolates comprised one such group (the DN127 genomic group) [8]. Although strain CT39 was not included in these DNA relatedness studies, other evidence suggests that it is also a member of the DN127 genomic group [12, 15, 19–21].

Strain 25015 was previously found to be infectious but non-pathogenic by using a mouse model of Lyme borreliosis [22]. This fact and the fact that members of the DN127 genomic group had been isolated only from ixodid ticks and small reservoir animals suggested that these organisms may not be pathogenic for humans either. However, a later study using a different murine system [23] showed that strain 25015 was mildly arthritogenic. Molecular analysis of isolates from the nine patients described here showed that they belonged to the general taxon \textit{B. burgdorferi} sensu lato and possessed many genotypic and phenotypic similarities to strains 25015 and CT39 [12]. Therefore, our findings enlarge the number of known isolates associated with the DN127 genomic group, expand their known geographic distribution to Europe as well as North America, and demonstrate an association of these strains with human disease.

The isolates were obtained from extant EM lesions, CSF, a lymphocytoma, and normal appearing skin at the site of resolved EM lesions. Isolates from extant EM lesions and CSF have been widely reported [24–33]. However, patient 7 (one of the three patients from whom a CSF isolate [SL-79] was recovered) was unusual in that she had no apparent CSF abnormalities. For this patient, the only indication of borrelial infection was culture isolation of the organism. A similar finding has been reported previously [34]. Moreover, for all three patients from whom CSF isolates were recovered, no intrathecal antibody production could be demonstrated. Our findings also add to the rare reports of isolation from a lymphocytoma [35] or resolved EM lesions [36].

It is of interest that the clinical presentations of the nine patients varied considerably, although in some cases the clinical features could have been influenced by possible coinfections (patients 8 and 9), a preexisting illness (patient 5), or delay in treatment (patients 3, 5, 6, 7, and 8). Four patients (patients 1, 2, 3, and 4) appeared to have a relatively benign course of illness. However, in some patients (nos. 7, 8, and 9), the illness was quite severe. In addition, some patients (nos. 1, 2, 5, 7, 8, and 9) had variable and unpredictable serological responses,
including an apparent lack of immunologic response despite disseminated disease (patients 7 and 8). In some cases, this could be related to the prompt initiation of antibiotic therapy. Alternatively, the negative serological findings might be related to the use of a local isolate of B. afzelii as antigen in IFAs; the antigenic profile of this isolate might be expected to differ markedly from that of strains in the DN127 genomic group.

Findings for patient 3 seem to support this hypothesis. IFA revealed that titers of IgM and IgG antibodies were both negative; however, western blotting with use of the patient’s autologous isolate as antigen (SL-74) demonstrated responses to both IgM and IgG antibodies [14]. Similar inconsistent serological findings have recently been reported by other investigators [37]. Thus, direct detection continues to prevail as the ultimate confirmatory method for the diagnosis of Lyme borreliosis.

These patient histories also indicate the persistence of DN127 genomic group isolates of B. burgdorferi sensu lato despite antibiotic treatment (patient 8 and possibly patients 4 and 9). Isolation of Borrelia from the CSF of a patient previously treated with phenoxymethyl penicillin (as in the case of patient 8) is not implausible since this antibiotic—which is available only for oral administration—does not achieve any substantial level in the CSF or CNS. However, Borrelia organisms were isolated from the CSF of two patients treated with ceftriaxone (2 g/d intravenously for 14 days) (patients 8 and 9). This antibiotic regimen is well established for the treatment of neuroborreliosis and is generally considered to be effective for eradication of the organism. In spite of this fact, similar failures of treatment with various antibiotics that are active against Borrelia species both in vitro and in vivo (including ceftriaxone and the other antibiotics used in this study) have previously been described by other investigators [24, 29, 31–33, 38, 39].

Reasons for the persistence of Borrelia have not been completely defined. For example, no species typing of such patient isolates has thus far been reported. However, Preac Mursic et al. [38] demonstrated differences between species in their in vitro susceptibility to most of the antibiotics commonly used in clinical practice and even found variations within one species. It may be conjectured that the same correlations pertain in vivo, but this supposition has yet to be confirmed by experimental observation.

In the case of patient 4, B. burgdorferi sensu lato was isolated both from the extant EM lesion and from normal appearing skin at a proximal biopsy site after resolution of the lesion. The first isolate was later shown by PFGE to possess an MluI digestion profile typical of B. afzelii, while the second isolate (SL-75) had the same PFGE profile as strains 25015 and CT39. Similar findings have recently been reported by Oksi et al. [37] who described a patient with bilateral facial palsy and meningitis in whom concomitant infection with B. garinii and B. afzelii was proven by culture. Demaerschalck et al. [40] used species-specific PCR analysis to demonstrate that the prevalence of infection with more than one species of B. burgdorferi sensu lato may be higher than expected; of 18 patients with neuroborreliosis who were examined in their study, eight were shown to have pluri-infections. Alternatively, the findings for patient 4 could represent reinfection. Slovenia is a region where Lyme borreliosis is endemic. In a recent analysis of clinical data for patients residing in this region (authors’ unpublished data), we found that of 2,273 patients presenting with typical EM, 91 (4%) had prior clinically documented infection with B. burgdorferi sensu lato and 163 (7.2%) claimed that they had previously had Lyme borreliosis.

We conclude that B. burgdorferi sensu lato of the DN127 genomic group are present on the European continent and can be responsible for causing Lyme borreliosis in humans. The isolation of organisms from this group in Europe is of interest in view of recent speculation concerning the global distribution of Lyme borreliosis [41]. Hitherto, B. burgdorferi sensu stricto has been considered the only Borrelia species present in both the Old and New Worlds. Thus, the DN127 genomic group organisms represent the second Borrelia species to be found on both continents. However, although considered a separate species, these organisms have been shown to be taxonomically closely related to B. burgdorferi sensu stricto. Therefore, these findings may be considered consistent with results of recent phylogenic analyses of North American and European isolates [42] that suggest that B. burgdorferi sensu stricto (and presumably its close relatives) constitutes the ancestral stock and that the exclusively European species evolved later. It remains to be seen whether North American DN127 genomic group organisms will eventually be found to be associated with human disease.

References
of rRNA genes and their intergenic spacers in *Borrelia japonica* sp. nov. and genomic group 21038 (*Borrelia andersonii* sp. nov.) isolates. J Clin Microbiol 1995;33:2427–34.


