**Borrelia spielmanii**

**Erythema Migrans, Hungary**

To the Editor: Lyme disease is the most frequent tickborne human infection in the northern hemisphere. At least 5 species of the Borrelia burgdorferi sensu lato complex, B. burgdorferi sensu stricto, B. afzelii, B. garinii, B. bissettii, and B. lusitaniae, have a pathogenic role in human Lyme disease in central Europe (1–3). A sixth pathogenic strain, A14S, has been isolated from 1 Dutch (4) and 2 German patients with erythema migrans (5). This strain was also detected in 4 questing Ixodes ricinus ticks in Germany (6,7) and 1 in the Czech Republic (8). A14S has recently been described as a new species, B. spielmanii (9); its main reservoir host is probably the garden dormouse (Eliomys quercinus), but B. spielmanii could not be detected in mice or voles. Richter et al. (9) could not find ticks harboring B. spielmanii in 3 of 5 examined areas in Germany. They were present almost exclusively in a single area where the prevalence of infection with this genotype was 15 (6%) of 251. We describe the isolation of this novel Lyme disease spirochete from a human patient with erythema migrans in Hungary.

Since 1999, we have regularly isolated Borrelia burgdorferi sensu lato from skin biopsy specimens of erythema migrans and acrodermatitis chronica atophicans taken from patients at the Center for Tick-borne Disease, Budapest, Hungary. To identify the Borrelia species occurring in Hungarian Lyme disease patients, we have started to molecularly analyze cultured isolates that originate from erythema migrans of different patients. DNA was isolated from 8 bacterial pellets by using QIAamp DNA mini kit (Qiagen, Hilden, Germany). Primers BSL-F and BSL-R were used; these amplify an 250-bp region of the outer surface protein (osp) A gene from all Lyme disease spirochetes (10). We added 2 µL extracted DNA to a 20-µL reaction mixture composed of 1.0 U HotStart-Taq DNA polymerase, 200 µmol/L of each dNTP, 25 pmol of each primer, and 1.5 mmol/L MgCl₂ (HotStartTaq Master Mix, Qiagen). An initial denaturation step at 94°C for 15 min was followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s. Final extension was done at 72°C for 5 min. Amplified DNA was subjected to electrophoresis in a 1.5% agarose gel that was prestained with ethidium bromide and viewed under UV light. After purification, the dyeoxy chain termination (Applied Biosystems Division, Foster City, CA, USA) was used for sequencing. Obtained sequences were checked with Chromas v.1.45 and compared to sequence data available from GenBank by using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). New sequences were submitted to GenBank.

Six sequences (DQ00729, DQ00-7299, DQ007300, DQ007301, DQ007302, DQ007303) showed 100% homology to B. afzelii Khab 625 strain (AY502599). One (DQ007297) of the remaining 2 samples showed 99.6% similarity with B.burgdorferi B31 (AE000790), and the other (AY995900) showed 99.21% similarity with B. spielmanii (AF102057).

The patient whose culture showed B. spielmanii was a 42-year-old woman with a homogenous erythema migrans, diagnosed on September 24, 1999. The erythema was 10 cm in diameter on the front surface of the knee at the first visit (see online Figure, available at http://www.cdc.gov/ncidod/EID/vol11no11/05-0542-G.htm). The immunoglobulin M (IgM) and IgG Borrelia immunoblot that applied B. afzelii (ACA1) antigen was negative in serum drawn on the seventh day after the appearance of erythema migrans. The patient did not remember a tick bite and had not traveled abroad during the previous 6 months. She complained of an “extremely unusual,” intense, serious nasal discharge that started 3 weeks before the appearance of erythema migrans and of a moderate headache; both disappeared spontaneously 2 weeks before treatment.

Our results show at least 3 distinct species of B. burgdorferi sensu lato in Hungary. In addition to B. burgdorferi sensu stricto and B. afzelii, known throughout Europe, we detected the recently described species B. spielmanii among randomly selected samples. Together with 2 previous publications (4,5), our observation also suggest that B. spielmanii has a pathogenic role in human Lyme disease. Although B. spielmanii is distributed more focally than other species of the B. burgdorferi sensu lato complex (9), it occurs from the Netherlands through Germany and Czech Republic to Hungary (4,5,7,8).

Acknowledgment

We acknowledge the advice and the positive control samples provided by Martin J. Kenny.

Gábor Földvári,* Róbert Farkas,* and András Lakos†

*St. István University Faculty of Veterinary Science, Budapest, Hungary; and †Center for Tick-borne Disease, Budapest, Hungary

References


Address for correspondence: András Lakos, Center for Tick-borne Diseases, Visegrádi u. 14. H-1132 Budapest, Hungary; fax: 36-1-329-3898; email: alakos@t-online.hu

Proiling Mycobacterium ulcerans with hsp65

To the Editor: Mycobacterium ulcerans is an emerging human pathogen responsible for Buruli ulcer, a necrotizing skin disease most commonly found in West Africa, but outbreaks have also been reported in the Americas, Australia, and Asia (1). Environmental sources of infection and mode of transmission are not completely known. M. ulcerans grows slowly at 32°C, requiring 6–8 weeks for colonies to be visible in primary culture. Differentiation from M. marinum, which also causes skin infections, is important, since M. marinum can usually be treated with antimicrobial agents, whereas M. ulcerans most often does not respond favorably to drug therapy, and treatment is usually by surgical excision (2). M. shinshuense, initially isolated from a child in Japan, is phenotypically and genetically related but biochemically distinct from M. ulcerans (3).

In the last decade, several DNA-based techniques for mycobacterial identification have been developed. Rapid molecular detection and differentiation of organisms that cause skin infections directly from tissue or exudates could be of great value for early treatment. Some techniques, especially those that include nucleic acid amplification, could be used directly on clinical samples. The accepted standard for molecular identification of mycobacteria is sequencing analysis of 2 hypervariable regions identified in 16S rRNA gene. M. marinum and M. ulcerans share identical 5′-16S rDNA and 16S-23S rRNA gene spacer sequences (4). Polymerase chain reaction (PCR)-dependent methods are based on the 16S rRNA gene (5), the hsp65 gene (6) or the insertion sequence IS2404 (7).

Recently, a novel category of variable number tandem repeats that could distinguish M. marinum and M. ulcerans genotypes has been described (8).

Polymorphisms in the 3′-16S rDNA region discriminate M. ulcerans from M. marinum and M. shinshuense (5). These polymorphisms also allow the separation of M. ulcerans into 3 subgroups according to geographic origin and variable phenotypic differences. IS2404 discriminates M. ulcerans from M. marinum (7). It has been used in restriction fragment length polymorphism analysis applied to a comparable number of M. ulcerans and M. marinum strains, confirming that this sequence is present in high copy numbers in M. ulcerans but absent in M. marinum. Nevertheless, an unusual mycobacterium was recently isolated that is closely related to M. marinum by phenotypic tests, lipid pattern, and partial 16S rDNA sequencing but presents low copy numbers of this element (9).

PCR-restriction enzyme analysis (PRA) of a 441-bp fragment of the hsp65 gene is a rapid, easy, and inexpensive method for identifying mycobacteria (10). Devallois et al. (6) described the PRA-hsp65 pattern of 1 M. ulcerans strain ATCC 33728 that originated in Japan. This isolate was considered a new species that resembled M. ulcerans and was named M. shinshuense (3).

We report here the usefulness of PRA-hsp65 to differentiate M. ulcerans strains from different geographic areas. Since Buruli ulcer cases have been reported on 5 continents, we studied 33 M. ulcerans strains that originated from Africa (Benin, Zaire, Ghana, Congo, Angola, Côte d’Ivoire, Togo), Asia (China, Malaysia), Australia (Papua New Guinea, Australia), the Caribbean (Mexico, Surinam, French Guiana), 1 M. shinshuense from Japan, 1 M. marinum isolate and 1 IS2404-positive M. marinum isolate from France (9). All strains were identified at the Institute.