March 2000 (Volume 41, Number 1)

Detection and Genotyping of Borrelia burgdorferi sensu lato by Polymerase Chain Reaction

Mirna Šitum, Blaženka Grahovac[1], Stribor Markoviæ[1], Jasna Lipozenèiæ, Gorazd Poje[2], Ivan Dobriæ[3], Branka Marinoviæ, Smiljana Bolanèa-Bumber, Ljiljana Mišiæ-Majerus4
Department of Dermatovenerology, Zagreb University Hospital Center, Zagreb; [1]Croatian Institute of Transfusion Medicine, Zagreb; [2]Department of Otorhinolaryngology, Zagreb University Hospital Center, Zagreb; [3]Division of Dermatovenerology, Dubrava University Hospital, Zagreb; and [4]Koprivnica General Hospital, Koprivnica, Croatia

Aim. To isolate and genotype Borrelia burgdorferi genospecies in serum samples of Croatian patients with erythema migrans.
Methods. DNA isolates from sera of patients with erythema migrans were analyzed by nested polymerase chain reaction (PCR), amplifying a segment of flagellin gene with primers encompassing the conserved region of the gene. To screen PCR products for heterogeneity, we performed single-stranded conformation polymorphism (SSCP) analysis. The samples showing differences in SSCP patterns were sequenced, and the sequence compared in the GeneBank for sequence homology with known Borrelia burgdorferi genospecies. We also constructed phylogenetic tree of all known borrelial sequences.
Results. The nested PCR method using specially designed flagellin gene primers, achieved the sensitivity of 10 genome copies (0.01 pg of purified Borrelia burgdorferi DNA from culture) by dilution analysis. The assay specificity was confirmed by amplification of a part of the flagellin gene from different bacterial species. The primer pairs successfully amplified only Borrelia burgdorferi flagellin gene. The genome of Borrelia burgdorferi sensu lato was detected in the sera of all 10 tested patients with erythema migrans. Sequence data and phylogenetic analysis confirmed that all amplified samples belonged to Borrelia afzelii genospecies.
Conclusion. Phylogenetic tree analysis placed the borrelial isolates together with Borrelia afzelii sequences into a single group. This finding was additionally supported by sequence homology analysis, which produced a homology score of 99%. In patients with erythema migrans who come from the northwest Croatia, an endemic area for Lyme borreliosis, Borrelia afzelii was the cause of skin manifestations of Lyme borreliosis.

Key words: bacterial gene proteins; bacterial typing techniques; biotyping, bacterial; Borrelia burgdorferi; Croatia; erythema chronicum migrans; flagellin; gene products, bacterial; polymerase chain reaction; nested PCR
