New Recombinant and Conventional Antigens In the Laboratory Diagnosis of Lyme Borreliosis

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Jaana Panelius defended her thesis on December 20, 2002, at the Haartman Institute in Helsinki. Faculty Opponent was Professor Matti Viljanen (*left*), Department of Microbiology, University of Turku, Finland, and chairman was Associate Professor Risto Renkonen (*right*), Department of Bacteriology and Immunology, University of Helsinki, Finland.

To improve the serology of Lyme borreliosis (LB), genes for the borrelial proteins flagellin A (FlaA) and outer surface protein C (OspC) from three domestic borrelial species of *Borrelia burgdorferi* sensu lato were cloned and sequenced, and the respective recombinant antigens were evaluated in the serology of LB. Furthermore, in order to follow the effectiveness of the treatment of LB and to find a serological marker of disease activity, the half-lives of flagella antibodies were evaluated during a follow-up of patients with late LB.

The amino acid sequences of FlaA were highly homologous, having identity of 95% within the three domestic species of *B. burgdorferi* sensu lato. OspC was more heterogeneous, with protein sequence identities varying between 57 and 73% among the borrelial species studied. Both for FlaA and OspC, polyhistidine-tagged recombinant proteins were produced in *Escherichia coli*. Three variants of FlaA were applied in Western blot (WB) and enzyme-linked immunosorbent assay (ELISA). For IgG serodiagnosis of neuroborreliosis (NB) and Lyme arthritis (LA), FlaA appeared to be a sensitive antigen. Seventy-one or 74 percent of patients with NB were positive either with WB or with ELISA, respectively. The corresponding positivities for samples from patients with LA were 86 and 79%. Although the sequences of the three FlaA proteins were highly homologous, the immunoreactivity in Finnish patient sera was stronger against recombinant FlaA (rFlaA) from *B. garinii* and *B. afzelii* than against rFlaA from *B. burgdorferi* sensu stricto. In IgM serology, low sensitivity in early LB and poor specificity may constrain the use of FlaA to routine serodiagnosis.

For OspC, a biotinylated antigen on streptavidin-coated plates in ELISA was used. In IgM ELISA, 30 and 35% of patient samples of erythema migrans reacted with one to three variants of the recombinant OspC (rOspC) in the acute or convalescent phase, respectively. Of the patients, 53% with NB and 53% with LA had IgM antibodies to rOspC, whereas 33% with NB and 60% with LA had IgG antibodies to rOspC. Cross-reactivity existed in Epstein-Barr virus infections in IgM ELISA, but this could be reduced by using thiocyanate in serum dilution buffer. In IgM serology the immunoreactivity was stronger against rOspC from *B. afzelii* and *B. garinii* than against rOspC from *B. burgdorferi* sensu stricto. Because of the heterogeneity of OspC, a polyvalent antigen with

several OspC variants from at least *B. afzelii* and *B. garinii* is needed to improve the sensitivity of OspC ELISA in the serodiagnosis of LB in Europe.

Three variant recombinant antigens of decorin-binding protein A (DbpA), BBK32, and OspC, and *B. garinii* type IR_6 peptide were applied in the diagnosis of NB, using cerebrospinal fluid (CSF) and serum samples of 89 patients. Their performances in IgG ELISA were compared with that of a commercial flagella antigen. IgM ELISA was performed with rOspC and flagella. The new antigens confirmed the positive NB diagnoses aided by anti-flagella antibodies. More importantly, of those NB patients negative for anti-flagella antibodies in the CSF, 51% were positive for at least 3 new antigens and 68% for at least two new antigens (mainly DbpA and IR_6) in the CSF. Antibodies to rDbpA and IR_6 were observed in the CSF in early and late NB, while antibodies to rBBK32 were detected mainly early in the disease. New antigens performed well also in the serum analyses but the discriminatory power between patient and control samples was better in the CSF than in serum. Thus, the use of the new antigens at presentation of the disease seems to improve the accuracy of laboratory diagnosis of NB. In IgG ELISAs, the diagnostic sensitivity of assays with the new antigens was between 75 and 88%, but with the flagella antigen was only 52%. The specificity of the test varied between 92 and 98%. The results suggest that antibody positivity in the CSF to at least two new antigens could be used as a confirmatory laboratory marker for NB. Furthermore, antibodies to BBK32 might be suggested as a marker of early LB.

To find a serological marker of successful treatment of LB, consecutive serum samples from 68 treated patients with late LB were analysed during a 1 to 2 year follow-up period posttreatment. End-point ELISA titres of IgG_1, IgG_2, IgG_3, and combined IgG_{1+3} subclasses against a sonicate antigen of *B. burgdorferi* were determined and compared to the IgG antibody response against *B. burgdorferi* flagella. Individual half-lives of the antibody levels were calculated for each patient, and compared with the patients' clinical outcome. The half-life of flagella antibodies was found to be significantly shorter than that of whole cell lysate antigen after successful treatment of late LB. The decrease was observed mainly in the IgG_1 and IgG_4 responses to flagella. The results suggest that a rapid decrease in flagella antibodies may be used as a marker of a successful treatment of LB. A decrease in titre to half that of the pre-treatment level within 6 months suggests eradication of the disease.

In conclusion, assessment of antibodies to the new recombinant and peptide antigens alone or in parallel seems to increase the accuracy of laboratory diagnosis of LB. Still, the sensitivity and specificity, especially in IgM serology with single recombinant antigens, remained suboptimal. In European conditions, if immunogenic borrelial proteins are used as antigens in the serology of LB, sequence heterogeneity between species implies that variant proteins are needed to cover all the relevant borrelial species. Further studies with new antigens are needed to find an antibody test that would differentiate between active and past infection early during the follow-up of patients treated for LB.