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Recombinant Low-Molecular-Mass Proteins pG and LA7 from *Borrelia burgdorferi* Reveal Low Diagnostic Sensitivity in an Enzyme-Linked Immunosorbent Assay

Serological testing is the most common way of confirming a clinical diagnosis of Lyme disease. There have been numerous efforts to improve the sensitivity and specificity of serological tests by employing purified, recombinant antigens which are immunodominant and specific for *Borrelia burgdorferi* (2, 3). The aim of this study was to determine the prevalence of antibodies with specificity for the in vivo-expressed 22-kDa pG protein (4) as well as the 21.8-kDa lipoprotein LA7 (5) (both from the European *B. burgdorfei* sensu stricto strain ZS7) in patients with various stages of Lyme disease. Both antigens comigrate in the low-molecular-mass region of sodium dodecyl sulfate-gel electrophoresis; when immunoblot analyses are performed, these antigens may be confused with each other as well as with the immunodominant outer surface protein C (OspC; 18 to 25 kDa). Identification of LA7 and OspC may be impossible in immunoblots employing whole-cell extract of bacteria as the antigen. Since pG is not expressed by in vitro-propagated spirochetes, antibody to this antigen will be overlooked in routine serology. To avoid these problems, purified, recombinant antigens were used in this study.

Both antigens were expressed as glutathione *S*-transferase fusion proteins and purified by affinity chromatography (4). Microtiter plates were coated with 0.5 μg of each antigen per ml. The cutoff for optical density (OD) readings at 492 nm was set at two standard deviations above the mean ODs of 135 control serum samples. The control serum samples were obtained from students living in a region where borreliosis was endemic. They were selected on the basis of no current clinical symptoms and of no prior history of Lyme disease. We studied 42 sera from patients with clinically diagnosed erythema migrans (EM), including 24 patients with a disease duration of longer than 5 weeks. EM patients were diagnosed on the basis of a characteristic skin rash. Sixteen of 42 (38%) EM patients revealed a positive borrelia-specific immunoglobulin G (IgG) titer in conventional serology. Most (11 of 16) of the IgG-positive EM patients had a disease duration of longer than 5 weeks. In addition, 36 sera from patients with acrodermatitis chronica atrophicans (ACA) and 60 patients with neuroborreliosis (NB) were tested. ACA and NB patients were diagnosed by typical clinical symptoms and a clear positive borrelia-specific IgG antibody titer in a commercially available borrelia-specific IgG enzyme-linked immunosorbent assay (ELISA) with a whole-cell extract as the antigen. All patients with NB revealed borrelia-specific intrathecal antibody synthesis. Results are given in Table 1 as the percentage of sera with a positive reaction in the appropriate ELISA.

TABLE 1. Percentage of positive ELISA results with pG and LA7 antigens in sera from three groups of patients with Lyme disease

Patient group (no. of sera)	% Positive ELISA results			
	pG		LA7	
	IgM	IgG	IgM	IgG
EM (42)	2	10	7	19
NB (60)	0	7	0	8
ACA (36)	3	15	0	33

IgM and IgG antibodies with specificity for the pG and LA7 antigens could be detected in only a few sera from patients with various manifestations of Lyme disease. Most prevalent were IgG antibodies reactive with LA7 in patients with dermatological manifestations (patients with ACA, 33%; patients with EM, 19%) of Lyme disease. A reactivity rate of 33% of sera from patients with late-stage Lyme disease (ACA) with the LA7 antigen is in agreement with the results of an earlier study (1) which reveals 35% positive results in sera from patients with Lyme arthritis. The surprising high percentages of IgG antibodies against LA7 (19%) and pG (10%) in the EM group can be explained by the high percentage of EM patients with a disease duration of longer than 5 weeks. All sera from EM patients which showed IgG antibodies against LA7 or pG were also positive by standard serology. Sera from five ACA and two EM patients revealed high concentrations of anti-LA7 IgG antibodies, whereas none of the sera from NB patients showed strong reactions. So far we have no explanation for the discrepant reactivities to LA7 in the study groups. However, the analysis of different spirochetal isolates on the basis of restriction fragment length polymorphism revealed considerable genotypic heterogeneity of the two antigens (4, 5). Since we tested recombinant pG and LA7 antigens derived from the sequence of only one borrelial strain, the discrepancies that were revealed may be explained by the sequence variability of the different genomospecies of *B. burgdorferi* in Europe. In addition, we cannot determine any correlation between high concentrations of anti-LA7 antibodies and the clinical course because the study was performed retrospectively. In conclusion, considering the low sensitivity of the pG antigen, it does not appear to be useful to include recombinant pG protein as a target antigen in serological tests for routine diagnostic application; with a sensitivity of about 33%, with regards to IgG antibodies in ACA patient sera, recombinant LA7 may be of limited use for diagnostic purposes in late-stage Lyme disease.

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Sebastian Rauer*
Department of Neurology
Albert Ludwig University
79106 Freiburg, Germany

Reinhard Wallich
Department of Immunology
Ruprecht-Karls-University
69120 Heidelberg, Germany

Uwe Neubert
Department of Dermatology
Ludwig Maximilian University
80337 Munich, Germany

*Phone: 49-761-270-5001
Fax: 49-761-270-5245
E-mail: rauer@nz11.ukl.uni-freiburg.de