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Use of Recombinant OspC from *Borrelia burgdorferi* for Serodiagnosis of Early Lyme Disease

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Infection with *Borrelia burgdorferi*, the etiologic agent of Lyme disease, is associated with an early and dominant humoral response to the spirochete's 23-kDa outer surface protein C (OspC). We have cloned and expressed OspC as a fusion protein in *Escherichia coli* and have shown that patient serum samples react with it in an enzyme-linked immunosorbent assay (ELISA) (S. J. Padula, A. Sampieri, F. Dias, A. Szczepanski, and R. W. Ryan, Infect. Immun. 61:5097–5105, 1993). Now we have compared the detection of *B. burgdorferi*-specific immunoglobulin M antibodies in 74 individuals with culture-positive erythema migrans by a whole-cell ELISA, immunoblot, and the recombinant OspC (rOspC) ELISA. Seventy-six negative controls were also studied. With all of the tests, there was a statistically significant association between the duration of disease and the frequency of a positive result. With the rOspC ELISA, the predictive value of a positive test was 100% and the predictive value of a negative test was 74%. Similar results were obtained with the whole-cell ELISA and with the immunoblot using as the source of test antigen a strain of *B. burgdorferi* which expresses abundant levels of OspC. We conclude that the use of rOspC in an ELISA is a convenient, readily automated, and easily standardized test for the serodiagnosis of early Lyme disease.

Lyme disease is a multisystemic illness caused by the tick-borne spirochete *Borrelia burgdorferi* (4, 7, 26). Early manifestations of the infection include an expanding, erythematous rash at the site of the tick bite, called erythema migrans (EM), and a nonspecific flu-like illness (25). Recognition of the distinctive EM rash is important, as prompt treatment with appropriate antibiotics usually prevents the late sequelae of neurologic, cardiac, or musculoskeletal organ involvement (27). EM, however, may not be recognized in some cases of Lyme disease (25). Failure by the patient to recognize the rash, an atypical appearance of the rash, and possibly the lack of development of a skin lesion may account for the variable recognition of EM in patients with Lyme disease.

In patients without recognized EM but suspected of having Lyme disease, establishment of the diagnosis is usually dependent on serologic confirmation of exposure to *B. burgdorferi*. Accurate serodiagnosis, however, has been complicated by delayed humoral response after infection and by antibodies to other bacteria which cross-react with proteins from *B. burgdorferi* (19, 24). Both the lack of standardization of antigen preparations used for testing and nonconformity in interpretation of results have also contributed to the confusion surrounding the serodiagnosis of this disease (15).

Using immunoblots of lysates from *B. burgdorferi* 2591, we found predominant immunoglobulin M (IgM) reactivity to a 23-kDa protein in serum samples from patients with early Lyme disease (10, 20). We cloned and sequenced the gene encoding this protein and found that it is OspC, an outer surface protein (20). We expressed recombinant OspC (rOspC) as a fusion protein in *Escherichia coli* and showed that serum samples from patients with Lyme disease react in an enzyme-linked immunosorbent assay (ELISA) with rOspC (20). In the present study, we compared the detection of *B. burgdorferi*-specific IgM antibodies in patients with culture-positive EM by a whole-cell (WC) ELISA, immunoblot, and the rOspC ELISA.

MATERIALS AND METHODS

Study groups. Group 1 consisted of serum samples from 74 individuals with EM. In all of these cases, *B. burgdorferi* was cultured from a biopsy specimen of the lesional skin. Serum samples were obtained from the Centers for Disease Control and Prevention, Fort Collins, Colo. The individuals had contracted the infection in the northeastern and midwestern United States. This group included group 1a, with serum samples from 20 patients at the time of EM. These individuals could not give a reliable estimate of the duration of the rash, but the rash was present when serum samples were obtained. Groups 1b, 1c, and 1d consisted of serum samples from 54 individuals who were able to accurately state the number of days between the onset of EM and the time sera were obtained: group 1b, 19 serum samples at 1 to 7 days; group 1c, 20 serum samples at 8 to 19 days; and group 1d, 15 serum samples at 20 to 90 days. All of the patients had received treatment with antibiotics upon presentation with EM. Group 2 consisted of controls as follows: group 2a, serum samples obtained from 20 healthy individuals with no prior history of Lyme disease; group 2b, serum samples obtained from 50 healthy volunteer donors living in areas where Lyme disease is not endemic; and group 2c, serum samples from 6 patients with severe periodontitis. Patients with periodontitis have chronic exposure to high levels of oral spirochetes and can develop antibodies which cross-react with proteins from *B. burgdorferi* (19). All serum samples were stored at −20°C prior to analysis.

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Specimens were tested without knowledge of the status of the donor.

Antigen preparation. *B. burgdorferi* 2591, originally isolated from a white-footed mouse caught in East Haddam, Conn., was obtained from the Connecticut Agricultural Experiment Station, New Haven, Conn. (2). The spirochetes were grown in BSK II medium in a closed flask at 33°C for 10 to 14 days as previously described (3). The gene encoding OspC was cloned from *B. burgdorferi* 2591 as previously described (20). rOspC was expressed as a fusion protein with glutathione *S*-transferase at the amino terminus to facilitate its purification from *E. coli* proteins as previously described (20). Glutathione *S*-transferase was also expressed and purified in exactly the same fashion as the fusion protein for use as a control in the ELISA.

WC ELISA. The ELISA for IgM and IgG anti-*B. burgdorferi* antibodies was performed as previously described with minor modifications (16, 18). Briefly, *B. burgdorferi* 2591 spirochetes were collected from growth medium by centrifugation at 35,000 × *g* for 35 min, washed three times, and resuspended in Dulbecco's phosphate-buffered saline (DPBS) (Sigma, St. Louis, Mo.) at a concentration of 10^6 spirochetes per ml. Fifty microliters of the spirochete solution was added to alternate wells of a 96-well, flat-bottom microdilution plate (Nunc-Immunoplate; Marsh Biomedical Products, Rochester, N.Y.). Fifty microliters of DPBS was added to the remaining wells as a control for nonspecific binding. The plates were dried overnight at 37°C before the assay was performed. Patient serum samples were tested at dilutions of 1:160 and 1:320 in DPBS–0.05% Tween 20 (DPBS-T). Positive and negative control serum samples were also included on each plate. Goat anti-human IgG or IgM conjugated to horseradish peroxidase (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) was used as secondary antibody. Plates were read when the net absorbance value (the difference in optical density between the antigen-coated well and the DPBS-containing well) of the 1:160 dilution of the positive control serum sample was 0.5 for IgM and 1.0 for IgG. A test serum dilution was considered positive if the net absorbance value was ≥3 standard deviations above the mean net absorbance of the negative serum controls. Serum samples containing positive dilutions of ≥1:160 for IgM or ≥1:320 for IgG were reported as positive. Positive IgG-containing serum samples were further titered to end point by serial twofold dilutions to 1:5,120. Further titration of positive IgM-containing serum samples was not performed.

rOspC ELISA. The ELISA to detect IgM antibodies to rOspC was performed essentially as described previously (20). Preliminary checkerboard titration studies with serum samples containing IgM antibodies to native OspC by immunoblot determined the optimal concentration of the rOspC fusion protein to be 300 ng per well. Sixty microliters of the rOspC fusion protein (5 μg/ml) in DPBS was added to alternate wells of the plate for 12 h at 4°C. An equimolar amount of glutathione *S*-transferase in DPBS was added as a control antigen to the remaining wells. Patient serum samples serially diluted twofold from 1:40 to 1:1,280 in DPBS-T were added. Plates were read by the microplate spectrophotometer when the net absorbance value (optical density of well with fusion protein minus that of well with glutathione S-transferase) of the 1:160 dilution of the positive serum control was 0.5. A serum dilution was considered positive if the net absorbance was ≥3 standard deviations above the mean net absorbance of the negative serum control wells. Most sera were tested twice in separate assays performed on different days to confirm reproducibility of the results.

Immunoblot assay. The IgM and IgG immunoblot assays using a sonicate of *B. burgdorferi* 2591 were performed as previously described with some modifications (20). Sixty micrograms of spirochetal proteins in loading buffer containing a final concentration of 0.5% sodium dodecyl sulfate and 1.25% 2-mercaptoethanol was heated at 95°C for 5 min and electrophoresed in a discontinuous 12% polyacrylamide-sodium dodecyl sulfate gel (1.5-mm-thick gel, plates (8 by 18 cm), and 130-mm preparative comb) using a vertical-slab electrophoresis unit (model SE 640; Hoefer Scientific Instruments, San Francisco, Calif.) at 22°C for 2.5 to 3 h at 120 V with buffers described by Laemmli (14). Proteins were transferred from the gel to a nitrocellulose sheet (0.2-μm pore size; Hoefer Scientific Instruments) with a Hoefer TE 62X Transphor II at a constant current of 500 mA at 22°C for 2 h. The nitrocellulose sheets were incubated overnight at 4°C in DPBS with 2% powdered nonfat milk and cut into 3-mm strips. Serum samples diluted 1:100 in DPBS-T with 2% powdered nonfat milk were mixed with the nitrocellulose strips at 22°C for 1 h with agitation and washed three times with DPBS-T. Goat anti-human IgG and IgM conjugated to horseradish peroxidase (Sigma) were added to the strips for 1 h at 22°C and washed three times with DPBS-T. 3,3′-Diaminobenzidine solution (50 mg/100 ml of PBS and 10 μl of 40% hydrogen peroxide) (Sigma) was added and incubated at 22°C for 10 min. The strips were allowed to dry prior to interpretation. The molecular masses of the reactive bands were determined by (i) comparing their mobility with transblotted prestained molecular mass markers (Bio-Rad Laboratories, Hercules, Calif.) and (ii) comparing their mobility with bands recognized on immunoblots by a serum with well-characterized reactivity. Three different criteria for positivity on the IgM immunoblot were used: the presence of (i) three or more bands; (ii) three or more bands, one of which had to be the 23-kDa protein; or (iii) the 23-kDa protein alone or in combination with any other bands. For the IgG immunoblot, four or more bands were considered positive.

Statistical analysis. The association between the length of duration after onset of disease and the rate of positive serologic result was evaluated by the chi-square test. The predictive value of a test was calculated as follows: positive predictive value, number of true-positive test results/number of true-positive and false-positive test results with a particular assay; and negative predictive value, number of true-negative test results/number of false-negative and true-negative test results with a particular assay.

RESULTS

IgM antibodies in early Lyme disease. Nine of twenty serum samples from group 1a patients were positive by IgM immunoblot when three or more bands were used as the positive criteria (Table 1). Eight of the nine serum samples with positive IgM immunoblots demonstrated reactivity with the 23-kDa protein. One serum sample which did not meet the criteria for positivity was reactive with a single band of 23 kDa (patient 16). Six of twenty group 1a serum samples were positive by the IgM ELISA with WCs of *B. burgdorferi* 2591, whereas eight of the twenty serum samples were found to be reactive with rOspC by ELISA. In eight of nine serum samples which demonstrated reactivity with the 23-kDa antigen on immunoblot, reactivity with rOspC by ELISA was also found.

For the group 1b serum samples, 11 of 19 were positive by IgM immunoblot (Table 2). All 11 positive samples showed reactivity to the 23-kDa protein. None of the eight negative samples demonstrated reactivity to the 23-kDa protein. Of the

TABLE 1. IgM reactivity of group 1a serum samples drawn during EM

Patient	IgM reaction								
	ELISA		Immunoblot						
	WC	rOspC	21 kDa	23 kDa	34 kDa	39 kDa	41 kDa	60 kDa	66 kDa
1	−	−					+	+	+
2	+	+		+			+	+	+
3	−	+		+			+		+
4	+	+	+	+	+	+	+	+	+
5	+	+		+			+	+	
6	−	−		+			+	+	
7	+	+		+			+	+	+
8	+	+		+				+	+
9	−	+		+			+		+
10	+	−					+		+
11	−	−							
12	−	−							
13	−	−							
14	−	−							
15	−	−					+		
16	−	+		+					
17	−	−					+		
18	−	−					+		
19	−	−					+		
20	−	−							

TABLE 3. IgM reactivity of group 1c serum samples drawn 8 to 19 days after onset of EM

Patient	IgM reaction								
	ELISA		Immunoblot						
	WC	rOspC	23 kDa	31 kDa	39 kDa	41 kDa	45 kDa	60 kDa	66 kDa	88 kDa
40	+	+	+			+		+	+	
41	+	+	+		+	+	+	+	+	
42	−	+	+			+		+		
43	+	+	+			+		+	+	
44	+	+	+			+		+	+	
45	+	+	+			+		+	+	
46	+	+	+			+			+	
47	+	+	+			+		+	+	
48	+	+	+			+		+	+	
49	+	+	+			+			+	
50	+	+	+			+		+	+	
51	+	+	+			+		+	+	
52	+	+	+		+	+			+	
53	+	+	+	+		+		+	+	
54	+	+	+		+	+		+	+	+
55	+	+	+			+		+	+	+
56	+	+	+		+	+		+	+	
57	−	−				+				
58	−	−								
59	−	−								

19 samples, 9 were positive by WC IgM ELISA compared with 12 positive samples by rOspC ELISA.

Seventeen of twenty group 1c serum samples were positive by immunoblot (Table 3). All 17 samples contained antibodies which bound both the 23- and 41-kDa proteins. Testing with the WC ELISA yielded positive results with 16 of 20 samples, whereas 17 of the 20 samples tested positive by the rOspC ELISA. There was a perfect correlation between reactivity with the rOspC ELISA and the 23-kDa protein on immunoblot within this group.

Thirteen of the fifteen group 1d serum samples were positive by IgM immunoblot (Table 4). Twelve of fifteen serum samples were positive by the IgM WC ELISA, and ten of fifteen were positive by the rOspC ELISA.

For all three methods of testing, there was a statistically significant association between longer disease duration and greater frequency of a positive test (Table 5). There was no relationship seen between the results of serologic testing and

TABLE 2. IgM reactivity of group 1b serum samples drawn 1 to 7 days after onset of EM

Patient	IgM reaction							
	ELISA		Immunoblot					
	WC	rOspC	23 kDa	39 kDa	41 kDa	45 kDa	60 kDa	66 kDa
21	−	+	+				+	+
22	−	+	+		+		+	+
23	+	+	+		+		+	+
24	+	+	+		+			+
25	+	+	+		+		+	+
26	+	+	+				+	+
27	+	+	+		+		+	+
28	+	+	+		+	+	+	+
29	+	+	+		+		+	+
30	+	+	+	+	+	+	+	+
31	+	−	+		+		+	+
32	−	−						
33	−	−			+			
34	−	−			+			
35	−	−						
36	−	−						
37	+	+			+			+
38	−	−						
39	−	−					+	+

TABLE 4. IgM reactivity of group 1d serum samples drawn 20 to 90 days after onset of EM

Patient	IgM reaction								
	ELISA		Immunoblot						
	WC	rOspC	23 kDa	39 kDa	41 kDa	45 kDa	60 kDa	66 kDa	88 kDa
60	+	+	+		+		+	+	
61	+	+	+		+		+	+	
62	+	+	+		+		+	+	
63	−	−	+				+	+	
64	+	−	+	+	+		+	+	
65	+	−	+		+	+	+		+
66	+	−			+		+	+	
67	+	+	+				+	+	
68	+	+	+				+	+	
69	+	+	+	+	+	+	+	+	
70	+	+	+		+		+	+	
71	+	+	+		+		+	+	
72	+	+	+		+		+	+	
73	−	−							
74	−	+			+				

TABLE 5. Relation of duration of EM to IgM serologic testing

Patient group	No. of patients	% Positive[a]		
		Immunoblotting[b]	ELISA	
			WC	rOspC
1a	20	45	30	40
1b	19	58	47	63
1c	20	85	80	85
1d	15	87	80	67
2 (a, b, and c)	76	3[c]	0	0

[a] The association between longer disease duration and greater frequency of a positive test was statistically significant as follows: for immunoblotting, χ^2 = 10.730, 3 degrees of freedom, with P = 0.013; for WC ELISA, χ^2 = 14.282, 3 degrees of freedom, with P = 0.003; for rOspC ELISA, χ^2 = 8.821, 3 degrees of freedom, with P = 0.032.

[b] Positive result determined by the criterion of binding three or more bands.

[c] One patient was from an area where Lyme disease is not endemic, and another had no history of Lyme disease.

TABLE 6. Comparison of IgM tests for Lyme disease

Test	Indicator			
	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Immunoblotting[a]				
≥3 bands	68	97	96	76
23-kDa band and ≥2 bands	65	99	98	74
23-kDa band	66	64	64	66
ELISA				
WC[a]	58	100	100	71
rOspC	64	100	100	74

[a] *B. burgdorferi* 2591 containing OspC (23 kDa) was used as the source of antigen.

the geographic region where the patient had contracted the infection (data not shown).

IgG antibodies in early Lyme disease. Conceivably, in some of the patients with Lyme disease and negative IgM reactivity, class switching of the anti-*B. burgdorferi* antibodies to IgG may have occurred. Therefore, we tested the serum samples by IgG immunoblots and WC IgG ELISAs. Within group 1a, only 1 of the 20 samples was positive by IgG immunoblot, and it was also positive by IgM immunoblot (patient 4). For 1 of these 20 samples, the IgG WC ELISA was positive and the IgM WC ELISA was negative (patient 3). In group 1b, one serum sample was positive by both IgM and IgG immunoblots (patient 31). One of the three group 1c serum samples which tested negative by IgM immunoblot was reactive with the 23- and 41-kDa proteins by IgG immunoblot (patient 58). The other two IgM immunoblot-negative samples showed reactivity to only the 41-kDa antigen by IgG immunoblot. Both of the group 1d serum samples which were negative by IgM immunoblot were also negative by IgG immunoblot.

Negative control sera. To examine the specificity of the serologic testing, we studied 76 control serum samples (group 2). Nineteen of the twenty group 2a samples were negative by IgM immunoblot with the positive criterion of three or more bands. One serum sample bound four bands, including the 41-kDa protein and the 23-kDa antigen. Three of the negative samples bound the 23-kDa protein. Twelve of the twenty serum samples contained reactivity with the 41-kDa protein. The WC and rOspC IgM ELISAs were negative for all 20 samples.

Although these serum samples were obtained from healthy donors without a history of Lyme disease, we were concerned about whether there were some individuals within this group who may have had an asymptomatic infection and thus developed antibodies to *B. burgdorferi*. Therefore, we studied an additional 50 samples obtained from healthy volunteers from Iceland, an area where Lyme disease is not endemic (group 2b). Only 1 of the 50 serum samples met the criterion of three or more bands for a positive IgM blot (41-, 60-, and 66-kDa bands). Twenty of the fifty samples demonstrated reactivity with the 23-kDa antigen, albeit faintly, by IgM immunoblot. None of the 50 samples were positive by the WC and rOspC IgM ELISAs.

As an additional control group, we also studied serum samples obtained from six patients with severe periodontitis (group 2c). None of these samples were positive by IgM immunoblot with the positive criterion of three or more bands. Five serum samples reacted with the 41-kDa flagellar protein, and three samples had reactivity with the 23-kDa antigen. All six serum samples were negative for IgM reactivity by the WC and rOspC ELISAs.

There were no group 2 serum samples which were positive by IgG immunoblot or IgG WC ELISA, including the two samples found to be positive by IgM immunoblots.

In Table 6, predictive values of the different serologic tests as determined by the results obtained with the 74 Lyme disease serum samples and the 76 negative serum controls are compared. The results of the immunoblot analysis using three different criteria for a positive test are presented. There were only two Lyme disease samples which did not bind the 23-kDa antigen, but they still fulfilled one of the criteria for a positive blot by binding three or more bands. There was only one example among the Lyme disease culture-positive serum samples of binding to the 23-kDa protein but not binding three or more bands. Because of these infrequent findings, there was no essential difference in the sensitivities of the IgM blots with three different criteria for a positive assay. There was, however, a significant drop-off in specificity when binding of the 23-kDa protein was used as the sole criterion for a positive immunoblot. This reflects the relatively frequent observation of binding to the 23-kDa antigen (usually faintly) among the negative serum controls. In contrast, there were no false-positive results with the WC and rOspC ELISAs. A positive result by either of these two methods, both of which contain OspC, was therefore superior to a positive result by immunoblot, using the positive criterion of binding to the 23-kDa protein, for accurately predicting the presence of disease. The predictive value of a negative result was essentially equivalent for all three methods.

DISCUSSION

Diagnosis of Lyme disease in patients with early disease who present with distinctive EM is usually not a problem. However, in a significant number of patients, EM may not be recognized and the only manifestation of infection may be nonspecific constitutional complaints. In these patients, serodiagnosis can play an important role in confirming the disease and leading to prompt and appropriate antibiotic treatment. Accurate serodiagnosis for early Lyme disease, however, has been complicated by delayed humoral response to the infection and by the fact that antibodies to other bacteria cross-react with proteins from *B. burgdorferi* (19, 24).

In hope of improving the level of detection of the antibody

response in early Lyme disease, studies have utilized enriched preparations and recombinant forms of the 41-kDa flagellar protein as the target antigen for serologic testing (5, 8, 13, 17, 21). This approach was prompted by initial immunoblot studies with *B. burgdorferi* lysates in which an early and predominant IgM antibody response to the flagellar protein was demonstrated (9). Use of flagellar protein-based serologic tests may be problematic, however, because of the relatively frequent finding of cross-reactive antibodies to conserved flagellar epitopes from commonly occurring commensal and pathogenic spirochetes, such as those found in the mouth (19, 22).

We and others have recently described another early and dominant antibody response in patients with Lyme disease which is directed to a protein of approximately 23 kDa (1, 11, 20). European investigators had previously described an immunodominant, early antigen of similar molecular mass called pC (12, 29, 30). These early targets of the immune response have now been determined to be OspC, a protein located in the outer membrane of the spirochete (12, 20, 28). Interestingly, stained gels of only a small percentage of lysates of strains of North American *B. burgdorferi* have revealed a major protein with the apparent molecular mass of OspC (29, 30). Included among the OspC-negative strains is B31, a widely distributed strain which is used as the source of test antigen in many clinical laboratories. Reasons for the variability in expression of OspC by these strains, which have been passaged for multiple generations in vitro, may be due to mutation, loss of the plasmid encoding the gene, or another as yet undefined mechanism(s) which is sensitive to varying growth conditions (20, 23, 28). The high prevalence of OspC-negative, in vitro-passaged strains probably accounts for the heretofore unappreciated reactivity of serum samples from patients with early Lyme disease with OspC. Nevertheless, frequent reactivity against OspC observed in serum samples from infected patients clearly argues that disease-causing spirochetes express OspC and that it is a dominant target of the early humoral immune response.

We have cloned and expressed OspC as a fusion protein in *E. coli* (20). In a previous study, we tested the feasibility of using rOspC in an ELISA for detection of Lyme disease (20). We showed that 15 of 15 serum samples from patients with clinically suspected Lyme disease had positive immunoblots and were also strongly reactive with rOspC. We also found that 5 serum samples from patients with syphilis, 10 containing rheumatoid factor with a high titer, 5 from patients with Epstein-Barr virus infection, and 10 from patients with systemic lupus erythematosus with antinuclear antibodies with high titers were all negative for anti-rOspC.

On the basis of those results, we have now carried out studies on a large panel of serum samples obtained from patients with culture-positive EM as well as a control group including patients from an area where Lyme disease is not endemic and patients with severe periodontitis.

As shown in Table 5, there was a positive correlation between duration after EM onset and frequency of positive results, regardless of the assay used. For the entire group of Lyme disease serum samples, the sensitivities of the immunoblot, regardless of the criterion used for a positive result, and the rOspC ELISA were equivalent (approximately 65%) and slightly better than that of the WC ELISA (58%) (Table 6). The specificities of all three tests, including the immunoblot with the criterion of three or more bands with or without the 23-kDa band, were 100% or nearly so (Table 6).

It is important to recognize that the source of antigen for the immunoblot and WC ELISA was strain 2591, a producer of abundant levels of OspC. Use of this strain may, therefore, have led to increased sensitivities with these assays compared with the use of a nonexpressing strain. We have observed poorer test performance when directly comparing B31 (OspC-negative)- and 2591-based assays (data not shown).

There were a number of serum samples in which there was a discrepancy between the observed reactivity to the 23-kDa protein by immunoblot and the rOspC ELISA. In most of these cases, including the negative controls from Iceland, there was faint reactivity found by immunoblot and negative reactivity by rOspC ELISA. This finding highlights the potential confusion introduced by subjective interpretation of intensity and apparent molecular size of a band on an immunoblot. Explanation of the less frequent situation of reactivity to rOspC and no band seen on the immunoblot (e.g., patient 6) is more difficult. Although strain 2591 served as the source of both the antigen used for the immunoblots and the gene used for expression of rOspC, there may be some differences in the expressed epitopes. For example, the rOspC is expressed as a fusion protein and coexpression of the glutathione *S*-transferase may influence the availability of immunoreactive sites. In addition, there is evidence that OspC is a lipoprotein in its native form, whereas rOspC likely lacks posttranslational modification (6, 12). Immunoblotting of serum samples with the rOspC preparation has not revealed reactivity with a contaminating protein derived from *E. coli* (data not shown).

Another consideration in this study was whether the rOspC from strain 2591, originally isolated in Connecticut, would be a useful target for testing serum samples from patients who are infected outside of that area. In this study, all of the Lyme disease samples tested were obtained from patients who contracted their infection outside of Connecticut. Despite this, the disease serum samples did demonstrate frequent and strong reactivity with the strain 2591 rOspC, suggesting there are common OspC antigenic epitopes shared by *B. burgdorferi* from different geographic sources. Also, we have recently found that antibodies in patients' sera are frequently directed against the carboxyl terminus of OspC, a conserved region of the protein among different strains of *B. burgdorferi* (data not shown).

The results of this study suggest that the use of rOspC in an ELISA is a convenient, readily automated, and easily standardized test for the serodiagnosis of early Lyme disease. The rOspC assay compared favorably in sensitivity and specificity to the much more difficult and labor-intensive immunoblot using a lysate of a strain which produces abundant levels of OspC.

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