HOST RESPONSE TO INFECTION

Comparative reactivity of human sera to recombinant VlsE and other *Borrelia burgdorferi* antigens in class-specific enzyme-linked immunosorbent assays for Lyme borreliosis

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A comparative study of human sera was conducted to determine which purified preparations of 11 recombinant antigens of *Borrelia burgdorferi sensu stricto* were diagnostically most important in enzyme-linked immunosorbent assays (ELISAs). To assess sensitivity, 20 serum samples obtained 1–6 weeks after onset of illness from 20 persons who had physician-diagnosed erythema migrans (EM) were tested for IgM and IgG antibodies. In tests for IgM antibody, seropositivity of $\geq 25\%$ was recorded when ELISAs had separate preparations of protein (p) 37, p41-G, outer-surface protein (Osp) C, OspE, OspF or VlsE antigens. Sera reacted most frequently (80% positive) with VlsE antigen in analyses for IgG antibodies. When results of both class-specific assays were considered for VlsE, OspC or OspF, 90% of the EM cases were serologically confirmed. Results of specificity testing with a further 59 sera from persons who had syphilis, louse-borne relapsing fever, oral infections, rheumatoid arthritis or human granulocytic ehrlichiosis and 28 normal sera indicated no false positive reactions when VlsE antigen was used in tests for IgM antibody. One of the 11 louse-borne relapsing fever sera cross-reacted with VlsE antigen in tests for IgG antibodies. Minor cross-reactivity also occurred when p37, OspC, OspE or OspF antigens were used. Overall, VlsE was the most suitable antigen for laboratory diagnosis of Lyme borreliosis during the early weeks of *B. burgdorferi* infection because of its high sensitivity and specificity.

Introduction

Lyme borreliosis is reported in widely separated regions of North America and Eurasia where *Ixodes pacificus*, *I. persulcatus*, *I. ricinus* or *I. scapularis* ticks occur. In the USA, Lyme borreliosis is caused by *Borrelia burgdorferi sensu stricto* and is the most frequently reported vector-borne infection [1]. In some areas of Europe, strains of this bacterium co-exist with *B. garinii* and *B. afzelii* [2–4] in nature. These closely related organisms are associated with distinct clinical manifestations of disease [2, 3].

Diagnosis of Lyme borreliosis is based on clinical and

laboratory findings. Erythema migrans (EM) is a useful clinical feature for diagnosis. However, in the absence of EM the clinical picture can be unclear and, consequently, clinicians rely on antibody detection analyses to determine the cause of illness. A two-step process, which usually includes enzyme-linked immunosorbent assays (ELISAs) followed by Western blot analysis, is used in the USA [5] and Europe. Difficulties associated with the sensitivities and specificities of these assays, inter-laboratory discrepancies due to non-standardised tests, complications due to co-migration of proteins of similar molecular masses in blots, and higher costs of performing immunoblot analyses are important limitations. Moreover, the use of whole-cell, sonicated antigens of *B. burgdorferi* in ELISAs can yield false positive results [6, 7] due to cross-reactivity of antibodies to treponemes or other bacteria.

Human granulocytic ehrlichiosis (HGE) occurs in tick-

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infested areas where Lyme borreliosis is reported. When EM is absent, clinical manifestations of HGE, such as headache, fever and fatigue, can be confused with those of Lyme borreliosis and other diseases. Laboratory analyses to identify disorders associated with abnormal blood cell counts (i.e., thrombocytopenia or leukopenia) and to determine concentrations of serum hepatic transaminase can help separate HGE from Lyme borreliosis, but detection of antibodies to specific recombinant antigens also can be used to diagnose HGE infections.

In efforts to improve the utility of immunodiagnostic tests for Lyme borreliosis, numerous recombinant antigens of *B. burgdorferi* have been produced and evaluated [8–13]. ELISAs with outer-surface protein (Osp) C, OspF, protein (p) 35 and p37 yield highly specific results but may exhibit low sensitivity [9, 12]. Recent studies have demonstrated that the surface-exposed lipoprotein VlsE [14] may serve as an effective antigen for immunodiagnosis of Lyme borreliosis. During experimental infection of mice, rapid recombination in the central cassette region of the *vlsE* gene results in substantial sequence changes with six variable regions (VRs 1–6) but not within invariant regions (IRs 1–6) [14]. Surprisingly, VlsE was found to be highly antigenic despite these sequence changes. Incorporation of full-length, recombinant VlsE or a synthetic peptide corresponding to IR6 in polyvalent or class-specific ELISAs resulted in a high degree of sensitivity and specificity in analyses of sera from North American and European Lyme borreliosis patients or from experimentally infected animals [10, 11, 13]. Western blot methods confirmed ELISA results [11]. The purpose of the present study was to test a full-length recombinant VlsE protein in class-specific ELISAs with a panel of North American human sera, with a focus on samples obtained during early Lyme borreliosis, and to compare these results with those obtained by ELISAs utilising other recombinant proteins or whole-cell *B. burgdorferi* antigens.

Materials and methods

Study groups

Serum samples, representing eight study groups, were obtained from archived collections at the Connecticut Agricultural Experiment Station. The first group consisted of 20 serum samples from 20 patients who had physician-diagnosed EM and whose sera contained antibodies against whole-cell *B. burgdorferi sensu stricto* (strain 2591), as determined previously by polyvalent and class-specific ELISAs [9]. All persons resided in tick-infested areas of Connecticut, USA. Blood specimens were obtained 1–6 weeks after onset of illness and before the initiation of antibiotic therapy. Group two contained 44 serum samples from 44 Connecticut patients who had been diagnosed with HGE, had no detectable antibodies to *Anaplasma*

phagocytophila (formerly *Ehrlichia phagocytophila* [15]) but had immunoglobulins that reacted with whole-cell *B. burgdorferi*. The third group included 11 sera from 11 HGE patients whose infections were confirmed by positive cultures, DNA analysis, Western blot analysis with whole-cell antigens of the NCH-1 strain, or by ELISAs that utilised a highly specific p44 HGE recombinant antigen. The HGE patient sera in this group showed no reactivity with *B. burgdorferi* whole-cell antigens by an ELISA [9]. All HGE patients in the second and third groups had thrombocytopenia or leukopenia; serum samples were obtained 3–5 weeks after onset of illness. To further assess specificities, human sera were also selected from frozen collections representing the following study groups: syphilis (24 serum samples), louse-borne relapsing fever (11), acute necrotising ulcerative gingivitis or periodontitis (6), and rheumatoid arthritis (7). Clinical findings, sources of sera and serological reactivities for the specimens in these four groups have been reported previously [6, 9]. Syphilitic sera had high antibody titres (≥ 1024) to *Treponema pallidum* in a standardised fluorescent-treponemal antibody-absorption test [16]. Louse-borne relapsing fever sera were from Addis Ababa, Ethiopia; diagnoses were based on clinical manifestations, positive blood smears for *B. recurrentis*, or the presence of homologous antibodies in immobilisation or microscopic agglutination tests [6]. The final group consisted of 28 serum samples from healthy subjects (i.e., normal controls) who lived in urban or suburban areas of Connecticut where tick-associated diseases are uncommon and who had no histories of Lyme borreliosis or HGE infections. All sera were stored at -60°C before analysis at the Connecticut Agricultural Experiment Station and were promptly re-frozen after analysis to prevent loss of antibodies associated with the freezing and thawing processes. Informed consent was obtained from patients, and research protocols were approved by Yale University and the State of Connecticut Health Department.

Test antigens

A recombinant form of VlsE (VlsE1-His), consisting of an N-terminal polyhistidine tag followed by VlsE1 without the native signal sequence, was produced and purified as described previously [11]. For comparative analyses, whole-cell *B. burgdorferi* strain 2591 from Connecticut, USA, and the recombinant antigens p22, p35, p37, p39, p41-G, OspA, OspB, OspC, OspE and OspF were tested separately in class-specific ELISAs as reported previously [9]. These recombinant antigens were cloned, expressed and purified as glutathione S-transferase (GST) fusion proteins in *Escherichia coli*. The newly acquired p35 (47-kDa fibronectin-binding protein) GST fusion recombinant antigen was prepared as described previously [17]. Affinity-purified GST was used as a negative control.

ELISA

IgM and IgG antibodies were detected by solid-phase, non-competitive class-specific ELISAs [9]. Briefly, the wells of flat-bottomed, polystyrene plates (Nunc A/S, Roskilde, Denmark) were coated with individual antigen preparations as described previously [9]; the optimal concentrations of p35 and VlsE1-His antigens were 5 µg/ml and 1 µg/ml, respectively, as determined by checkerboard titrations (data not shown). Wells incubated with phosphate-buffered saline (PBS) without antigen were also used for each serum sample as a negative control. All serum samples were initially diluted in PBSS (pH 7.2) to 1 in 160, 1 in 320 and 1 in 640. If positive, sera were tested at higher dilutions as needed to establish end-point concentrations. In tests for IgM antibody, a 1 in 8000 dilution of affinity-purified horseradish peroxidase-labelled goat anti-human antibody (µ-chain specific) produced by Kirkegaard & Perry Laboratories (Gaithersburg, MD, USA) was used. In analyses for IgG antibodies, the conjugate was a 1 in 10 000 dilution of γ-chain specific, affinity-purified horseradish peroxidase-labelled goat anti-human IgG antibodies (Kirkegaard & Perry Laboratories). Details of blocking, incubation, washing procedures, the substrate used, and the measurement of absorbance values have been reported elsewhere [9, 18, 19].

Net absorbance values are differences in optical density (OD) readings with or without antigen for each serum dilution. Reactivity cut-off values for each serum dilution were established by statistical analyses as the mean plus 3 SD of the net absorbance values obtained after testing a panel of sera from healthy subjects. In general, the cut-off values obtained at a serum dilution of 1 in 640 were used for defining reactivity at higher serum dilutions. The titration end-point for each serum dilution was defined as the highest dilution at which the net OD was greater than the cut-off values. The cut-off values for whole-cell or all recombinant antigens except p35 and VlsE were reported previously

[9, 17]. To determine cut-off values for each assay with p35 and VlsE antigens, respective panels of 24 and 44 human sera (obtained from persons who had no history of tick-borne infections and who lived in urban or suburban areas in Connecticut) were used. These sera had been analysed earlier [9] in studies of Lyme borreliosis or HGE and had been verified as being negative for antibodies to *B. burgdorferi* by indirect fluorescent antibody or immunoblotting methods. In the ELISA with p35 antigen, net OD values of 0.22, 0.13, and 0.08 were considered positive for serum dilutions of 1 in 160, 1 in 320 and 1 in ≥640 for IgM antibodies. In tests for IgG antibodies, 0.43, 0.25 and 0.17 were positive cut-off values. With VlsE antigen, net OD values of 0.07 and 0.04 were considered positive for serum dilutions of 1 in 160 and 1 in ≥320 for IgM antibodies, whereas a net OD value of 0.03 was the cut-off value for sera diluted to 1 in ≥160 in IgG antibody tests. Each plate contained positive and negative serum controls, GST-only controls where appropriate, and additional wells for assessing reactivity of the conjugates with individual antigens. These controls were consistently negative. Selected positive test sera were re-analysed on different days to determine reproducibility of antibody titration end-points.

Statistical analyses

A statistical software programme (Sigmastat; SPSS, Chicago, IL, USA) was used to calculate z statistics to determine significant differences in proportions of positive results. The Yates correction was applied in all tests.

Results

IgM antibodies

In all, 151 serum samples, representing eight study groups, were tested for IgM antibodies to whole-cell *B. burgdorferi* and 11 recombinant antigens (Table 1).

Table 1. Reactivity of IgM antibody in human sera to whole-cell and recombinant antigens of *B. burgdorferi sensu stricto* in ELISAs

Study group	Total sera tested	Number of sera reactive with												
		whole cells								outer-surface proteins				
			p22	p35	p37	p39	p41-G	VlsE	A	B	C	E	F	
EM (LB)	20	20	4	1	9	4	11	8	3	0	11	7	5	
HGE and LB	44	33	23	1	20	19	23	7	1	2	23	6	9	
HGE	11	0	3	1	4	0	2	1	2	0	1	0	1	
Syphilis	24*	20	2	0	0	3	4	0	0	0	0	0	4	
LBRF	11	5	ND	0	3	1	4	0	ND	ND	2	0	1	
Oral infections	6*	0	0	0	0	0	0	0	0	0	1	0	1	
RA	7*	2	1	0	1	2	0	0	0	0	0	1	0	
Normal	28	0	5	1	0	3	1	0	0	0	1	0	0	

EM, erythema migrans; HGE, human granulocytic ehrlichiosis; LB, Lyme borreliosis serologically confirmed; Syphilis, secondary or latent; LBRE, louse-borne relapsing fever; RA, rheumatoid arthritis; Normal, healthy subjects with no history of tick-borne infections; ND, not done (antigens unavailable).

*All serum group-antigen combinations listed were analysed independently as a part of this study. Some sera reacted with more than one antigen. In some instances, re-analysis of certain serum-antigen combinations resulted in data that were identical to previously published results [9].

Seroprevalence of $\geq 25\%$ was used as an arbitrary guideline for identifying recombinant antigens that may be useful in Lyme borreliosis diagnosis. Analysis of IgM antibodies for patients with EM indicated seroprevalences of $\geq 25\%$ when the following recombinant antigens were used: OspC (55%), p41-G (55%), p37 (45%), VlsE (40%), OspE (35%) and OspF (25%). Statistical examination of the IgM results obtained for the pairings of VlsE and OspF ($z = 0.675$, $p = 0.500$) and VlsE and OspC ($z = 0.633$, $p = 0.527$) showed that these seropositivity rates were not significantly different. ELISA results for sera from persons diagnosed with HGE and Lyme borreliosis but lacking antibodies to *A. phagocytophila* had a similar pattern of reactivity. In this group, the highest proportions of sera with IgM antibody were recorded for OspC (52%), p41-G (52%), p22 (52%), p37 (46%) and p39 (43%). In contrast, IgM antibodies were detected in relatively few sera from patients diagnosed with HGE only (group 3), with patient sera having a seroprevalence $> 25\%$ for only two antigens (p37 and p22). These results were generally considered to be possible false positives because there were no reactions when sera were tested with whole-cell *B. burgdorferi*. However, one serum sample in this group reacted with recombinant p22, p41-G, OspF and VlsE antigens, suggesting that the patient may have had Lyme borreliosis before or at the time the blood sample was obtained.

False positive reactions were recorded for sera from patients with conditions unrelated to tick-borne infections when p22, p37, p39, p41G, OspC, OspE or OspF were used as antigens (Table 1). In addition, eight normal sera reacted to one or more of the following antigens: p22, p35, p39, p41-G or OspC. Assays with VlsE, OspA and OspB antigens were negative when sera from healthy subjects or patients who had syphilis, oral infections and rheumatoid arthritis were tested.

IgG antibodies

In analyses of IgG antibodies (Table 2), positive sera from persons who had EM reacted most frequently with VlsE antigen (80%). Seroprevalence was also relatively high when ELISAs contained p39 (45%),

OspC (40%), OspF (40%) or p41-G (35%) antigens. However, the proportion of samples reactive with VlsE was significantly greater ($z = 2.259$, $p = 0.024$) than percent seropositivities recorded for OspC or OspF. In the second study group (HGE and Lyme borreliosis), VlsE, OspC and OspF also exhibited the highest IgG seroprevalence, i.e., 41%, 34% and 43%, respectively. The proportion of sera with IgG antibodies against VlsE was more than two-fold greater than the number that had detectable IgM antibody to VlsE. In contrast, relatively few sera from clinically diagnosed and serologically confirmed HGE patients had detectable IgG antibodies to all *B. burgdorferi* antigens; a seroprevalence of 36% was recorded for OspF and p22.

False positive IgG antibody reactions were recorded when sera in various control groups were tested (Table 2). There were reactions with p22, p35, p37, p39, p41-G, OspC and OspF antigens when sera from patients who had syphilis, louse-borne relapsing fever, oral infections or rheumatoid arthritis were analysed. Furthermore, 14 normal sera reacted with p22, p39, p41-G, OspC and OspF. Only one serum sample from the non-Lyme borreliosis patients (i.e., louse-borne relapsing fever) reacted weakly (titre of 320) when VlsE antigen was used in assays for IgG antibodies. Thus, tests with VlsE antigen had higher specificity than those with whole-cell *B. burgdorferi* or other recombinant antigens except OspB and OspE. However, the tests utilising the latter two antigens had low sensitivities.

Comparison of recombinant antigen reactivities

In analyses of 20 EM patient sera, 16 (80%) contained IgM or IgG antibodies to the VlsE antigen. This seroprevalence rate exceeded those calculated for OspC (65%) and OspF (50%). Values for VlsE and OspF were not significantly different ($z = 1.657$, $p = 0.097$). When the IgM or IgG results of the three most reactive recombinant proteins (VlsE, OspC and OspF) were combined, 18 (90%) of the sera from patients with EM were positive. Of these, 12 (67%) contained both IgM and IgG antibodies to one or more of these antigens (Table 3). When 76 sera from non-Lyme borreliosis or HGE patients and healthy subjects were tested, the

Table 2. Reactivity of IgG antibodies in human sera to whole-cell and recombinant antigens of *B. burgdorferi sensu stricto* in ELISAs

Study group	Total sera tested	Number of sera reactive with												
		whole cells								outer-surface proteins				
			p22	p35	p37	p39	p41-G	VlsE	A	B	C	E	F	
EM (LB)	20	20	4	3	3	9	7	16	0	0	8	4	8	
HGE and LB	44	26	6	0	4	5	7	18	0	0	15	0	19	
HGE	11	0	4	0	2	0	1	0	1	0	0	0	4	
Syphilis	24*	20	2	8	0	4	3	0	0	0	0	0	4	
LBRF	11	2	ND	0	2	1	1	1	ND	ND	1	0	1	
Oral infections	6*	0	0	0	1	2	0	0	0	0	1	0	0	
RA	7*	1	2	1	0	6	0	0	0	0	0	0	0	
Normal	28*	0	3	0	0	4	1	0	0	0	4	0	2	

*See Table 1 footnotes.

Table 3. Seroprevalences of IgM and IgG antibodies for 20 sera obtained from EM patients and tested by ELISAs with individual recombinant antigens

Antigen group*	Number (%) seropositive		
	IgM	IgG	IgM and IgG
OspC	11 (55)	8 (40)	6 (30)
OspF	5 (25)	8 (40)	3 (15)
VlsE	8 (40)	16 (80)	8 (40)
OspC or OspF	14 (70)	10 (50)	9 (45)
OspC and OspF	1 (5)	3 (15)	0 (0)
OspC or VlsE	9 (45)	8 (40)	3 (15)
OspC and VlsE	5 (25)	8 (40)	3 (15)
OspF or VlsE	7 (35)	10 (50)	5 (25)
OspF and VlsE	3 (15)	7 (35)	1 (5)
OspC or OspF or VlsE	15 (75)	14 (70)	12 (60)
OspC, OspF and VlsE	1 (5)	3 (15)	0 (0)

*All antigens were tested separately.

proportions of false positive reactions for all three recombinant proteins were 13% and 18% for IgM and IgG antibodies, respectively. Nearly all these reactions were due to reactivity with either OspC or OspF. There were also OspC, OspF and VlsE reactivities with a serum sample from one HGE patient where past or present Lyme borreliosis was suspected, as noted above.

Analysis of antibody titres

Antibody concentrations were determined for the sera from EM patients with each of the antigens examined in this study (Table 4). The geometric means and ranges of antibody titres were highly variable among samples and were greatly dependent on the antigens used. In analyses for IgM antibody, maximal antibody titres of ≥ 5120 were recorded for certain sera in assays utilising whole-cell, p22, p41-G, VlsE and OspC antigens. The geometric means for ELISA results with these antigens were also elevated. Maximal titration end-points for IgG antibodies were recorded in tests in which whole-cell, p39, p41-G and VlsE antigens were

used in ELISAs; results for the VlsE antigen indicated the highest geometric mean titre (2061).

Because the assays with VlsE antigen appeared to be sensitive and specific, duplicate ELISAs with this reagent were conducted on separate days to determine if antibody titration end-points were reproducible. Seven sera from EM patients with a wide range of antibody titres (320–40 960) were selected for analysis. Titres for IgM antibodies varied by two-fold for six samples and by four-fold for one sample in duplicate tests. Reproducibility trials for IgG antibody concentrations indicated the same value for two samples, a two-fold variation for two samples and a four-fold difference for three sera. Results for an additional five normal sera remained negative in duplicate trials for both class-specific assays.

Discussion

In an attempt to increase the accuracy of Lyme borreliosis diagnosis, the sensitivities and specificities of 11 different *B. burgdorferi* recombinant antigens were examined by ELISA. Taking both the IgM and IgG antibody results into account, the VlsE antigen was most consistent in laboratory confirmation of Lyme borreliosis, compared with the other antigens tested. These results parallel those reported elsewhere showing high sensitivity and specificity of ELISAs with either full-length recombinant VlsE [11] or a peptide corresponding to IR6 of VlsE as antigen [14]. The seropositivity rate for EM patient sera in the present study was greater for IgG antibodies (80%) than for IgM antibody (40%) when VlsE antigen was used. The other antigens in the present study also showed variability in reactivity when testing for IgM and IgG antibodies, which is consistent with the dynamic nature of immune responses during the early stage of Lyme borreliosis infection. The sensitivity of ELISAs increases with time after onset of illness [10, 11] as antibody concentrations rise. Therefore, when initial

Table 4. Ranges and geometric means of antibody titres for sera obtained from patients who had EM and tested by ELISA with whole-cell or recombinant antigens of *B. burgdorferi sensu stricto*

Antigen	Ranges of antibody titres		Geometric mean titres*	
	IgM	IgG	IgM	IgG
Whole cells	160–40 960	160–20 480	1808	1576
p22	160–10 240	640**	1280	640
p35	640†	320–640	640	508
p37	160–1280	160–320	436	202
p39	160–1280	640–10 240	538	1493
p41-G	320–20 480	320–5120	2257	951
VlsE	160–5120	320–20 480	987	2061
OspA	640†	0	640	0
OspB	0	0	0	0
OspC	320–40 960	160–2560	3093	453
OspE	160–640	160–640	363	453
OspF	640–1280	320–2560	735	830

*Geometric means for positive results only.

†The same antibody concentration for one or more serum samples.

tests for IgM antibody are negative, efforts should be made to detect IgG antibodies.

False positive reactions in ELISAs with whole-cell *B. burgdorferi* antigens are well documented [6, 7, 10, 12, 16, 19]. Heat-shock, flagellin, and several other proteins of this spirochaete share high degrees of homology with proteins from other bacteria, including treponemes, *E. coli* and *A. phagocytophila*. Consequently, the resulting specificity problems have complicated the interpretation of ELISA results when whole-cell antigens are used. Current guidelines recommend the application of Western blot analysis with whole-cell antigens to determine if antibodies detected by ELISAs are specific for Lyme borreliosis infections. The inclusion of immunoblotting methods has improved overall laboratory diagnosis. Unfortunately, blot results can sometimes be inconclusive, particularly during early weeks of infection when relatively few bands or weak reactions are observed. Moreover, co-migration of proteins with similar molecular masses can confuse interpretation of Western immunoblot results when IgG antibody concentrations are elevated and clusters of multiple bands are visible.

Different patterns of reactivities to recombinant antigens were noted in tests for specificity. The use of highly specific recombinant antigens, such as p41-G, OspC and OspF, increases the specificity of ELISAs, as indicated in this and previous work [9]. However, the sensitivities of assays with these antigens can be low. In the current study, ELISAs with the VlsE antigen were highly sensitive and specific for Lyme borreliosis when testing for IgG antibodies. Although less specific than the VlsE antigen, antibody reactivities with p41-G, OspC and OspF, in conjunction with seroreactivity to VlsE, may also be useful diagnostically for identifying past or current *B. burgdorferi* infections. Furthermore, one serum sample from a patient clinically diagnosed with HGE and having no EM [20] exhibited a positive IgM antibody reaction to VlsE. In tick-infested areas of North America, Lyme borreliosis and HGE often co-exist where *I. pacificus* and *I. scapularis* abound [21, 22]. Co-infections in man have been verified by culture methods [23] and suggested by the co-existence of antibodies to *B. burgdorferi* and *A. phagocytophila* in the same host [20, 24, 25]. The HGE patient in the present study had not been diagnosed previously with Lyme borreliosis, but there was reactivity of the patient's serum with p22, p41-G, OspF and VlsE antigens. With antibody presence to VlsE, in particular, we suspect that this reaction was specific for *B. burgdorferi* and that this individual had been exposed to different agents that cause Lyme borreliosis and HGE. However, it is unclear whether the patient had concurrent infections or if exposure to disease organisms occurred separately. Similar antibody reactions have been reported before when HGE was diagnosed, but sera contained antibodies to *B. burgdorferi* rather than to the HGE agent [20]. The use of VlsE antigen in

ELISAs may help clarify seroprevalence of past or current *B. burgdorferi* infections in HGE patients and may facilitate the separation of natural *B. burgdorferi* infections from OspA vaccine-induced seroreactivity [10, 11]. Finally, one serum sample from a patient who had louse-borne relapsing fever cross-reacted with VlsE antigen in tests for IgG antibodies. However, this disease is rare in the USA and Europe and can be distinguished clinically from Lyme borreliosis. This cross-reactivity may be due to sequence similarity between variable large proteins expressed by relapsing fever organisms and VlsE [13].

In Europe, *B. burgdorferi*, *B. garinii* and *B. afzelii* cause human infections [2–4]. Earlier studies indicated that a synthetic peptide based on the VlsE IR6 of *B. garinii* (strain Ip 90) has an epitope that frequently reacts with serum antibodies from Lyme borreliosis patients in the USA and Europe [10, 13]. It is possible that the invariable domains of the VlsE molecule may serve as a probe for universal serodiagnosis of human Lyme borreliosis. However, a recent study [26] showed that a 51 amino acid peptide corresponding to the C-terminal, invariable domain of VlsE is highly immunogenic but does not react consistently with sera from European Lyme borreliosis patients. The low reactivity of sera from some patients may be due to heterogeneity in the C-terminal sequence of VlsE [26]. Further analysis of VlsE heterogeneity and reactivity with human Lyme borreliosis sera from broad geographical regions is needed.

Although an overall seroprevalence of 80% was obtained with VlsE in this study, IgM antibody was not detected in some serum samples representing early *B. burgdorferi* infections regardless of which recombinant antigens were used. Humoral responses vary in infected persons, and IgM antibody may remain at very low, undetectable concentrations. Analyses of sequential serum samples can help to improve laboratory diagnosis, as concentrations of IgG antibodies rise with expansion in immune responses. While VlsE appears to be an excellent candidate as a single antigen in ELISA to test more specifically for Lyme borreliosis antibodies, a mixture of immunodominant antigens – such as VlsE with OspC or OspF – may alternatively enhance the sensitivity of class-specific or polyvalent ELISAs used in initial screening of sera before immunoblotting. To improve assay efficiency and reduce potential interference problems and loss of sensitivity associated with excessive amounts of GST in microplate wells, genes encoding VlsE and selected additional recombinant antigens could be fused to form a composite antigen [27] rather than mixing separate recombinant antigens in cocktail form.

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