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Serodiagnosis of Lyme Borreliosis by *Borrelia burgdorferi* Sensu Stricto, *B. garinii*, and *B. afzelii* Western Blots (Immunoblots)

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The performance of Western blots (immunoblots) prepared with eight strains of *Borrelia burgdorferi* representing *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii* genospecies was tested with a panel of sera with various clinical presentations collected from eight geographic regions. European sera were generally more reactive to blots prepared with *B. garinii* or *B. afzelii* strain antigens, in particular *B. garinii* 20047 and *B. afzelii* VS461. North American sera were more reactive with *B. burgdorferi* sensu stricto strains. Our observation of significant differences in the levels of reactivity of some sera on Western blots of certain strains is potentially important for the development and implementation of generic interpretive criteria. Preferential reactivity of sera from patients with nerve and/or palsy symptoms to *B. garinii* strains and with cutaneous disease to *B. afzelii* strains was observed. On the basis of our results, we have concluded that strain 20047 is the best strain to use for the development of a generic Lyme borreliosis Western blot for Europe.

Borrelia burgdorferi, the etiologic agent of Lyme borreliosis, is found in parts of North America, Europe, Asia, and other temperate regions of the world. Clinical illnesses produced by infection with *B. burgdorferi* are diverse and can include arthritic, neurologic, dermatologic, and cardiac manifestations. In North America, arthritis is a predominant symptom, while in Europe neurologic and cutaneous symptoms are more common (27). Evidence suggests that particular disease manifestations are related to the genospecies of the infecting spirochete and that geographic differences in genospecies distribution may account for regional variations in clinical disease patterns (1, 13, 27, 30, 32).

Molecular and serological studies have delineated four genospecies (genogroups) within the species of *B. burgdorferi* sensu lato (8). Genospecies 1, *B. burgdorferi* sensu stricto, is found in North America and Europe. Genospecies 2, *Borrelia garinii*, and genospecies 3, *Borrelia afzelii*, are found in Europe and Asia (2). Genospecies 4, *Borrelia japonica*, the most recently recognized group and one which has yet to be shown to be a human pathogen, is found in Japan (17). Additional isolates have been described which do not appear to belong to any of the genospecies described above, and it is likely that other genospecies will be delineated.

In North America, serologic assays based on a low-passage *B. burgdorferi* sensu stricto strain antigen are currently recommended (9). In Europe, the presence of three major genospecies has raised concern that the potential diversity of antibody responses seen in European sera may not be recognized by serodiagnostic tests with antigen prepared from heterologous isolates, especially North American strain B31 (genospecies 1).

Since Lyme borreliosis can generally be treated once diagnosed and laboratory support for the diagnosis of *B. burgdorferi* infection is largely dependent on serological assays, the definition and use of optimal antigen preparations are critical to the performance and clinical utility of *B. burgdorferi* serological assays. Magnarelli et al. (21) concluded that the use of antigens from local strains was not critical to their enzyme immunoassay's (EIA's) overall sensitivity or specificity, although the use of antigens from particular strains did result in overall higher levels of reactivity. In a related study, Karlsson (14, 16) reported that although one of four patients showed increased reactivity to the autologous infecting strain, whole-cell extracts from different isolates seemed to be of comparable diagnostic value in their EIA. A recent report from Sweden, however, demonstrated the potential significance of strain selection in assay design. In this study, EIAs prepared with a local *B. garinii* strain detected a higher number of seropositive individuals in an area in which borreliosis is endemic compared with that detected by an EIA prepared with B31 antigen. On the other hand, when sera from North American Lyme borreliosis patients were tested, higher numbers of seropositive sera were detected with a B31 antigen-based EIA (7).

In the present study, we investigated this issue by testing a panel of European and North American Lyme borreliosis sera representing a range of clinical states and geographic regions on Western blots (immunoblots) prepared with eight *B. burgdorferi* sensu lato isolates representing the three major genospecies to determine the relative reactivity of the specimens to the various strains. Our primary objective was to determine which strain or strains were the best candidates for use in preparing Western blots for general use in Europe.

(Portions of this work were presented as abstract P117T at the VI International Conference on Lyme Borreliosis, 19 to 22 June 1994, Bologna, Italy.)

MATERIALS AND METHODS

Study samples. Physicians in each of the study countries were asked to submit specimens they believed to be consistent with Lyme borreliosis. Physicians were also asked to provide information about the clinical symptoms of the patients. A total of 69 European and 8 North American specimens were examined in this study.

***Borrelia* culture and antigen preparation.** Eight *Borrelia* strains representing the three major genospecies were examined in this study. The specific isolates studied were *B. burgdorferi* sensu stricto (genospecies 1) B31, 297, and CB from the United States and the IRS isolate from Switzerland; *B. garinii* (genospecies 2) 20047 (France) and PBi (Germany); and *B. afzelii* (genospecies 3) VS461 (Switzerland) and B023 (Germany). *Borrelia* was grown in BSK II medium at

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TABLE 1. Monoclonal antibodies used to calibrate Western blots in this study

Specificity	Antibody	Reference	Result with monoclonal antibody to genospecies[a]:							
			1 (*B. burgdorferi* sensu stricto)				2 (*B. garinii*)		3 (*B. afzelii*)	
			CB	297	IRS	B31	20047	PBi	VS461	B023
p93	181.1	20	P	P	P	P	P	P	N	P
GroEL (p62)	149	19	P	P	P	P	P	P	P	P
Fla (p41)	H9724	3	P	P	P	P	P	P	P	P
BmpA (p39)	H1141	26	SP	SP	P	SP	P[b]	WP	P	P
OspB (p34)	84C	11	SP	SP	N	SP	N	P	P	P
OspA (p31)	H5332	5	SP	SP	SP	SP	SP	N	N	N
p30	NYSP30H1	13a	P	P	P	P	P	P	P	P
OspD (p29)	H1C8	25	N	N	N	N	N	N	N	N
OspC (p23)	4B8F4	23	SP	SP	P	P	SP	P	P	N
p21 (p22)	CB625	10	P	P	P	P	P	P	WP	WP
p18	NYSP18$_a$H1	13a	P	P	P	P	P	P	P	P

[a] N, negative; P, positive; SP, strong positive.
[b] BmpA and monoclonal antibody NYSP39H1, another anti-p39 monoclonal antibody (28), both detect p39 reactivity as a doublet on strain 20047.

32°C. Bacteria were harvested by centrifugation at 4°C and washed three times with phosphate-buffered saline (PBS [pH 7.2]). The final pellet was resuspended in PBS and frozen in aliquots at −70°C. Protein determinations were performed with the Pierce bicinchoninic acid (BCA) protein assay. All eight strains were assayed together on a single run for maximum standardization of protein loading on the gels.

SDS-PAGE and Western blotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by standard procedures (18). Briefly, each antigen was electrophoresed on 12% polyacrylamide gels (16 by 20 cm [Bio-Rad Protean II apparatus]) or, for the monoclonal antibody study, on minigels (8 by 7.2 cm [Bio-Rad Miniprotean II]). The separated proteins were electroblotted onto nitrocellulose with a Hoeffer TE-70 Transphor unit (29). Transfer uniformity and efficiency were verified by Ponceau S staining of each blot. Blots were blocked with nonfat dry milk in 0.05% Tween 20 in PBS (PBST) and cut into 3-mm strips. Antigen strips were incubated with 1:100 dilutions of patient sera for 1 h, washed with PBST three times, incubated with goat anti-human immunoglobulin G (IgG)–alkaline phosphatase (MRL Diagnostics) for 30 min, and washed again three times with PBST, and the antibody reactivity was visualized by incubation with 5-bromo-4-chloro-3-indolylphosphate toluidinium–nitroblue tetrazolium substrate (MRL Diagnostics). Reactive bands observed on each strip were scored independently by two readers.

Monoclonal antibodies. A panel of nine monoclonal antibodies, including antibodies specific for p22 (referred to as p21 throughout text), OspC (p23), OspD (p29), OspA (p31), OspB (p34), BmpA (p39), Fla (p41), GroEL (p62), and p93, was kindly provided by Marty Schriefer of the Centers for Disease Control. These nine antibodies were combined into a cocktail and reacted with Western blot strips. Monoclonal antibodies specific for p18 and p30 proteins were kindly provided by Karim E. Hechemy from the New York State Department of Health. Refer to Table 1 for details about the monoclonal antibodies used.

RESULTS

Reactivity of *Borrelia* strains with the monoclonal antibody panel. The reactivity of the eight *Borrelia* strains with the monoclonal antibody cocktail is shown in Fig. 1. Included in Fig. 1 is strain PG1, which is a strain we recently isolated from a Lyme borreliosis patient from the United States. This strain reacts similarly to strain CB and will not be discussed further. In addition, the strains were reacted with monoclonal antibodies to p18 (NYSP18$_a$H1) and p30 (NYSP30H1) (results not shown in Fig. 1). The reactivity of the various monoclonal antibodies to the strains is summarized in Table 1. All strains showed similar reactivity to the p18, p21, p30, p41, GroEL (p62), and p93 antibodies; however, VS461 and B023 showed weak reactivity to p21. VS461 did not show any reactivity to the p93 monoclonal antibody; however, the presence of p93 was clearly demonstrated when VS461 (as well as all of the other seven strains) was reacted with sera from a German Lyme borreliosis patient (data not shown). The OspC (p23) monoclonal antibody reacted to all strains except B023. Reactivity to the OspB (p34) monoclonal antibody was not detected in strains 20047 or IRS, while reactivity to the OspA (p31) monoclonal antibody was not detected in PBi, VS461, or B023. No reactivity to the OspD monoclonal antibody was detected in any of the strains examined.

Serologic reactivity of *B. burgdorferi* strains with Lyme borreliosis sera. Specimens were reacted with Western blot strips prepared from each of the eight *Borrelia* strains, and IgG reactivity to protein bands corresponding to p15, p18, p21, p23 (OspC), p28, p31 (OspA), p34 (OspB), p39, p41, p66, p75, and p93 was scored. As a result of the small volume of many of the specimens available to us, only IgG reactivity was evaluated in the present study. The strains exhibiting the two highest band scores for each specimen were designated as "preferentially reactive" for that specimen. Table 2 summarizes the number of specimens within each geographic group which were preferentially reactive for each strain. Most sera demonstrated preferential reactivity to multiple strains.

The European sera were generally more reactive to *B. garinii* and *B. afzelii* strains than to *B. burgdorferi* sensu stricto strains. Two exceptions were the similar reactivity of strains CB, PBi, 20047, and VS461 with the Belgian serum panel and the similar reactivity of strains IRS, PBi, and 20047 for the Spanish serum panel. The preferential reactivity results obtained with the Belgian panel dramatically demonstrated that while one *B. burgdorferi* sensu stricto strain (CB) was equivalent in performance to the *B. garinii* and *B. afzelii* strains, the other three *B. burgdorferi* sensu stricto strains scored very low. A similar trend was seen in the German and Swiss panels. The North American panel was most reactive with North American *B. burgdorferi* sensu stricto 297 and CB and European *B. burgdorferi* sensu stricto IRS. Of the eight strains studied, *B. afzelii* VS461 and *B. garinii* 20047 and PBi showed the most band reactivity on the European serum panels. When all of the European specimens were grouped together and the average number of bands detected by each strain and genospecies were calculated, the same three strains (VS461, 20047, and PBi) demonstrated the highest levels of reactivity with the European specimens. These three strains detected an average of 5.8 bands (range, 5.7 to 6.0), compared with 4.6 bands (range, 4.0 to 5.2) detected by blots prepared with the other five strains.

An example of the different reactivities of sera to different *Borrelia* strains is shown in Fig. 1B. The U.S. specimen intensely reacted to the p18 and p23 (OspC) bands of genospecies 1 strain CB (a U.S. isolate) but did not react to either of these bands on the 20047 strips. In a similar manner, the

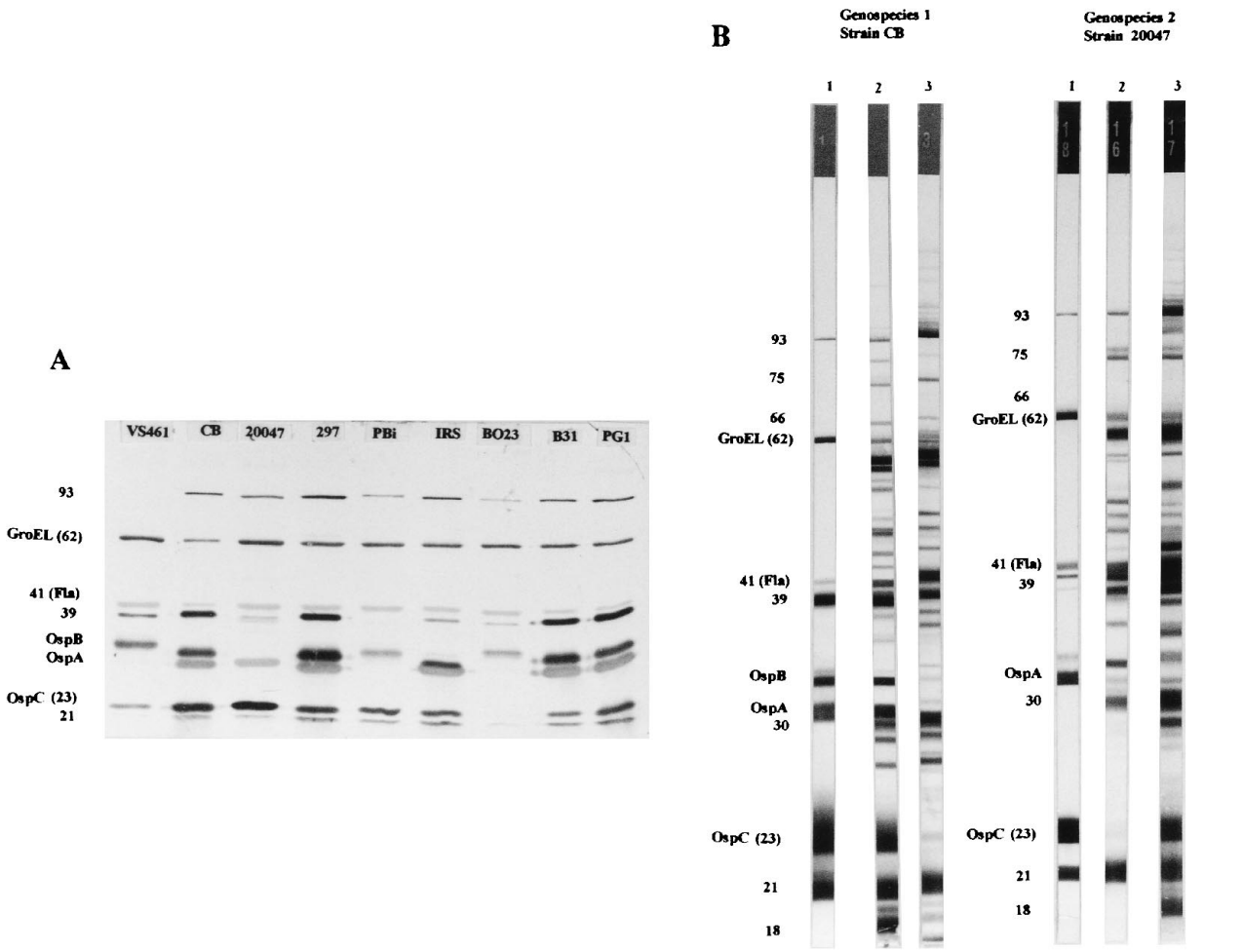

FIG. 1. Lyme borreliosis IgG Western blots of *Borrelia* strains. (A) Reactivity of study strains to a cocktail of the indicated monoclonal antibodies. (B) Different responses of U.S. and German Lyme borreliosis patient sera to different *Borrelia* strains. Lanes: 1, monoclonal antibody panel; 2, U.S. Lyme borreliosis patient sera; 3, German Lyme borreliosis patient sera.

German sera reacted intensely to the p18 and p23 (OspC) bands of the 20047 strain but only weakly to the p18 and p23 (OspC) bands of the CB strain. Both serum samples reacted strongly to the p21 band of each strain. In the case of these particular sera, the absence of the p18 or p23 bands on either strip would not affect the positive interpretation, since the sera are very reactive to multiple bands. In other cases, however, the lack of reactivity to these bands, especially OspC, could be very significant for interpretation.

To date, no consensus has been reached on Western blot criteria for interpretation of European specimens as "positive" or "negative." We therefore, interpreted the reactivity observed for each of the *Borrelia* strains by using a criterion established in our clinical laboratory for North American specimens tested with *B. burgdorferi* sensu stricto B31. By this criterion, a positive result required any four of the following bands: p18, p21, p23 (OspC), p27, p31 (OspA), p34 (OspB), p39, p41, p66, p75, or p93. The results of this analysis were similar to those found by the analysis of preferential reactivity (Table 3). The strains which would result in the assignment of the most positives (i.e., the strains with the highest sensitivity) were *B. garinii* 20047 and PBi, followed by *B. afzelii* VS461, and *B. burgdorferi* sensu stricto CB and IRS.

Five of 69 (7.2%) European Lyme borreliosis specimens would have changed between a positive or negative interpretation, depending on the strain used to prepare the Western blot. One of the eight North American specimens tested would also have changed interpretation. When the data from both groups are combined, the interpretation of 6 of 77 (7.8%) would change, depending on the strain used for analysis.

Western blot reactivity of clinically defined sera. When specimens were grouped by reported clinical status and their preferential reactivity to the various strains was evaluated, the nerve and/or palsy group was found to be most reactive with *B. garinii* 20047 and PBi, followed by *B. afzelii* VS461 and *B. burgdorferi* sensu stricto CB (Table 3). The asymptomatic group demonstrated similar reactivity to strains 20047 and VS461. The arthritis group was preferentially reactive to strains 20047 and VS461, while the erythema migrans group was preferentially reactive to strain IRS.

In addition to the examination of preferential reactivity, the average number of bands detected for each strain and genospecies for each clinical group was determined (Table 4). The results shown in Tables 3 and 4 demonstrated that overall *B. garinii* 20047 and *B. afzelii* VS461 were the most reactive of the strains investigated. It was interesting to note that when the average number of bands detected by all strains was totaled, the highest average number of bands was associated with the

TABLE 2. Preferential reactivity observed by region on Western blot (IgG)

Specimen (*n*)	Preferential reactivity (no. of serum samples/group) with genospecies[a]:							
	1 (*B. burgdorferi* sensu stricto)				2 (*B. garinii*)		3 (*B. afzelii*)	
	B31	CB	297	IRS	PBi	20047	VS461	BO23
Swiss (6)	1	2	2	2	4	5	4	2
Italian (7)	0	2	0	1	1	2	4	2
German (9)	0	2	0	0	4	4	8	4
Belgian (15)	1	10	2	2	11	12	10	4
French (15)	2	5	5	4	6	7	6	6
Spanish (12)	4	4	4	9	5	7	6	2
Swedish (5)	0	2	2	1	3	3	3	3
North American (8)	3	5	6	5	3	2	2	4
Total (77)	11	32	21	24	37	42	43	27

[a] Values indicate the number of serum samples per group showing preferential reactivity to each strain. Most sera demonstrated maximum reactivity to multiple strains.

two carditis specimens. The second highest number of bands was detected for the arthritis group.

Analysis of the sensitivity of the various strains in detecting positive specimens among the different clinical groups indicated that for the erythema migrans group, *B. garinii* 20047 and *B. burgdorferi* sensu stricto IRS each detected 60% (6 of 10) of the specimens as positive compared with only 20 to 40% detected as positive by the other strains. For the nerve and/or palsy group, the *B. garinii* strains were the most sensitive, followed by *B. afzelii* VS461 and B023 and *B. burgdorferi* sensu stricto CB (Table 5).

DISCUSSION

The recognition of multiple genospecies of *B. burgdorferi* differing in their molecular and antigenic properties has led to concern about the detection of *B. burgdorferi* infections by assays with heterologous antigens (4). In the present study, we evaluated the relative effectiveness of various *B. burgdorferi* strains representing the three major genospecies for the detection of Lyme borreliosis in European specimens.

Studies of Lyme borreliosis Western blots have been hampered by problems of band identification and nomenclature. Resolution of the uncertainty of particular band location and equivalence must be achieved in order for new criteria to be evaluated by investigators at different geographic locations with different antigens and gel systems. The use of calibrating panels of monoclonal antibodies such as that provided by the Centers for Disease Control will help alleviate this problem. In the present study, in addition to the monoclonal antibodies included in the Centers for Disease Control panel, we were able to use monoclonal antibodies provided by K. Hechemy to identify p18 and p30 proteins. A manuscript characterizing the $NYSP18_aH1$ (for p18) and NYSP30H1 (for p30) monoclonal antibodies is in preparation (13a). The p18 protein has been a particular problem, since some investigators have referred to it as p21 and some have referred to it as p18. Figure 1A clearly shows three distinct bands corresponding to the p18, p21, and p23 proteins. Each of these proteins has been identified by a specific monoclonal antibody. The location of the p30 band, which has also often been difficult to determine, can be identified with the NYSP30H1 antibody. Assignment of a consistent nomenclature to the bands is critical if meaningful interpretive criteria for these bands, such as the Centers for Disease Control-Association of State and Territorial Public Health Laboratory Directors recommendations (9), are to be developed, refined, and accurately applied.

Since there are no established criteria for interpretation of European Lyme borreliosis Western blots, the number of bands detected with each strain was used to determine the strain or strains which demonstrated preferential reactivity. We found that European sera were generally more reactive with *B. garinii* 20047 and *B. afzelii* VS461, while North American sera were more reactive with *B. burgdorferi* sensu stricto 297, CB, and IRS. The results with the Belgian and Spanish panels, however, in which *B. burgdorferi* sensu stricto CB and IRS, respectively, scored as well as the *B. garinii* and *B. afzelii* strains, indicate that the preferential reactivity to genospecies 2 and 3 is not absolute and that regional variations in the reactivity to the genospecies strains may occur. Since arthritic symptoms have commonly been associated with *B. burgdorferi* sensu stricto strains and nerve and/or palsy symptoms have been associated with the *B. garinii* genospecies, the results observed with the Belgian panel may partly be explained by the makeup of the Belgian panel, in which 36% (5 of 14) of the specimens were in the arthritis group and 9 of the remaining 14 specimens were in the palsy group. In the case of the Spanish panel, only 2 of 12 specimens were from patients with arthritic symptoms. Although the Belgian sera did react to strains representing all three genospecies, the striking preferential reactivity to the *B. garinii* and *B. afzelii* strains and one specific strain of *B. burgdorferi* sensu stricto underscores the need to carefully validate the use of *B. burgdorferi* sensu stricto strains for testing European sera. While a genospecies 1-based assay may be as effective as a *B. garinii*- or a *B. afzelii*-based assay for some regions, in others a *B. garinii*- or *B. afzelii*-based assay may be more effective.

While the overall level of reactivity is of interest, once the threshold value for an enzyme-linked immunosorbent assay (ELISA)-positive result or a certain number of designated bands on a Western blot-positive result is reached, additional reactivity (evaluated either by optical density or bands) is irrelevant for the interpretation and does not necessarily add to the clinical utility of the assay. Analysis of our results by looking at the number of positive or negative results obtained with each strain is complicated by the lack of established criteria for

TABLE 3. Preferential reactivity on IgG Western blots observed within clinically defined panels

Group (*n*)	Preferential reactivity (no. of serum samples/group) with genospecies[a]:							
	1 (*B. burgdorferi* sensu stricto)				2 (*B. garinii*)		3 (*B. afzelii*)	
	B31	CB	297	IRS	PBi	20047	VS461	BO23
Dermatologic (3)	1	0	1	2	1	1	2	2
Arthritis (14)	1	5	2	5	5	6	6	1
Asymptomatic (8)	1	1	0	1	4	5	6	4
Carditis (2)	0	1	0	0	1	2	2	1
Erythema migrans (10)	2	1	3	5	3	2	3	3
Neuroborreliosis (4)	0	1	0	0	2	3	2	1
Nerve and/or palsy (23)	1	11	5	3	13	14	11	7
Miscellaneous (5)	0	3	1	1	3	2	3	1
Total (69)	6	23	12	17	32	35	35	20

[a] Values indicate the number of serum samples per group showing preferential reactivity to each strain. Most sera demonstrated maximum reactivity to multiple strains.

TABLE 4. Average number of bands detected for strains in various clinical groups

Western blot antigen by genospecies	Isolate	Average no. of bands for group (n = samples)[a]							
		Dermatologic (3)	Arthritis (14)	Asymptomatic (8)	Carditis (2)	Erythema migrans (10)	Neuroborreliosis (5)	Nerve and/or palsy (14)	Miscellaneous (5)
B. burgdorferi sensu stricto	B31	3.3	5.9	5.0	4.5	3.0	3.8	3.3	5.2
	CB	4.0	6.6	5.9	7.5	2.9	3.8	4.8	7.4
	297	4.0	6.5	5.0	7.0	2.8	3.3	3.9	6.4
	IRS	5.3	6.2	5.4	6.0	3.9	3.3	4.0	5.8
Average		4.2	6.3	5.3	6.3	3.2	3.5	4.0	6.2
B. garinii	PBi	4.3	6.9	7.5	8.0	3.6	5.8	4.9	7.2
	20047	4.7	7.4	7.1	8.5	4.1	5.8	5.0	6.2
Average		4.5	7.1	7.3	8.3	3.9	5.8	5.0	6.7
B. afzelii	VS461	6.0	7.9	8.4	9.0	3.5	4.8	4.9	7.0
	B023	5.0	6.4	7.0	7.5	2.8	4.8	3.9	5.8
Average		5.5	7.1	7.7	8.3	3.2	4.8	4.4	6.4
Average all strains		4.6	6.7	6.4	7.2	3.3	4.4	4.3	6.3

[a] Note that the following bands were scored: p15, p18, p21, p23 (OspC), p28, p31/p32 (OspA), p34 (OspB), p39, p41, p66, p75, and p93.

the interpretation of European Lyme borreliosis Western blots. Although some criteria have been proposed (15, 33), more study is needed to determine if generic criteria could be used for blots prepared with antigens from different genospecies or even different strains within a genospecies. Our results showing different levels of reactivity with different strains suggest that development, optimization, and validation of Western blot criteria must be done for each specific strain.

Cognizant of its limitations, we applied the Western blot criteria established for North American strains in use in our laboratory at the time of this study to assign a positive or negative interpretation to our Western blot results. The results of this analysis were similar to the preferential reactivity results, with *B. garinii* strains showing the most positive results, followed by the *B. afzelii* and *B. burgdorferi* sensu stricto strains. Our finding that 7.2% of the specimens would have been interpreted differently depending on the strain used is in general agreement with that seen with the ELISA studies showing small differences in performance (7, 14, 21). Although the 7.2% number is small, it does show that the strain choice is significant (a positive to negative result) in some cases. The degree of impact of strain choice on a particular area will depend on the prevalence of particular strains in the test area. The results of Bunikis et al. (7), for whom an enzyme immunoassay prepared with the local *B. garinii* strain detected more seropositive individuals than an assay with *B. burgdorferi* sensu stricto B31, support this result. While strain differences are probably not critical for most ELISAs or other screening assays, strain differences are likely to be more important for the Lyme borreliosis Western blot assays in which reactivity to discrete proteins is scored and the specific profile of reactivity (the number and the identity of the specific bands) is critical to the interpretation of results.

In practical terms, any Western blot used in Europe will be challenged by a variety of clinical specimens from a wide geographic region. Analysis of the overall performance of the strains was assessed by evaluating each strain's performance with the complete panel of European specimens. When strains were evaluated either by the criterion of being preferentially reactive to the European specimens or by determination of the strains detecting the highest number of bands, *B. garinii* 20047 and *B. afzelii* VS461 demonstrated the best overall performances (Tables 2 and 3). On the basis of these two analyses, either VS461 or 20047 would be a candidate for use in a generic European Western blot. A difference between the performance of the two strains, however, was observed when interpretative criteria were applied to the blot results. In this case, strain 20047 detected five more positive specimens than strain VS461. It should be recognized that the criteria applied were developed for North American Lyme borreliosis sera tested with *B. burgdorferi* sensu stricto B31, and it is possible that alternative criteria would lead to different results.

Several studies have reported an association between clinical symptoms and preferential reactivity to particular genospecies. Our results showing the nerve and/or palsy groups were most reactive with the two *B. garinii* strains 20047 and PBi support the preferential reactivity of patients with nerve and/or palsy clinical histories with *B. garinii* strains reported by others (1, 6,

TABLE 5. Number of IgG Western blot positive results obtained with strains in clinical groups

Group (n)	No. of positive results with genospecies[a]:							
	1 (*B. burgdorferi* sensu stricto)				2 (*B. garinii*)		3 (*B. afzelii*)	
	B31	CB	297	IRS	PBi	20047	VS461	BO23
Dermatologic (3)	1	1	1	2	2	3	2	2
Arthritis (14)	8	10	10	9	10	10	10	9
Asymptomatic (8)	6	7	6	7	7	6	6	6
Carditis (2)	1	2	2	1	2	2	2	2
Erythema migrans (10)	3	3	2	6	4	6	3	2
Neuroborreliosis (4)	2	2	1	2	3	3	2	3
Nerve and/or palsy (23)	6	11	9	9	15	14	13	11
Miscellaneous (5)	3	4	3	3	3	4	4	2
Total (69)	30	40	34	39	46	48	42	37

[a] Note that the positive criterion used was the presence of any four of the following bands: p18, p21, p23 (OspC), p28, p31/p32 (OspA), p34 (OspB), p39, p41, p66, p75, or p93.

13, 30, 32). The strong reactivity of patients reported to have arthritic symptoms to *B. garinii* 20047, *B. afzelii* VS461, and *B. burgdorferi* CB is interesting in light of the association of arthritis and genospecies 1 strains (27). Although arthritis is regarded as a more common clinical symptom in North America, the arthritis group represented the second largest clinical group among the European specimens in the present study. Assous et al. (1) recently reported a preferential reactivity of specimens from patients with arthritis to *B. burgdorferi* sensu stricto strains compared with *B. garinii* and *B. afzelii* strains. Our results for the arthritis group showed similar reactivity to *B. garinii* and *B. afzelii* strains and to two of the four *B. burgdorferi* sensu stricto strains. The reactivity of the European arthritis group specimens to *B. garinii* and *B. afzelii* strains in addition to the *B. burgdorferi* sensu stricto strains might be explained by either the unreported presence of other clinical symptoms in addition to arthritis, by current or past infection with multiple *Borrelia* strains, or by cross-reactivity between the 20047, VS461, and CB strains as a result of the strong immune response of these specimens (12, 31, 32). The association of cutaneous manifestations of Lyme borreliosis with *B. afzelii* strains reported by others (1, 30, 32) was supported in this study, in which *B. afzelii* VS461 detected the highest average number of bands for this patient group.

B. burgdorferi genospecies are distributed throughout Europe, and the prevalence of particular genospecies varies in different regions. In some areas, all three genospecies may be present, while in other areas one or two species may predominate (2, 22, 24). We did find that some *Borrelia* strains were more reactive in certain regional panels: for example, *B. garinii* for the Belgian panel and a European genospecies 1 strain (IRS) for the Spanish panel. This may reflect a true preferential reactivity to strains in these areas, or it may reflect the particular clinical makeup of the panels submitted by investigators from each region.

Accurate serodiagnosis is critical for detection of *B. burgdorferi* infection and to support clinical diagnosis. The study we have presented has shown that the choice of particular *B. burgdorferi* strains for preparation of Lyme borreliosis Western blots does affect the level of reactivity detected with specific European sera. Variable levels of reactivity between different strains may have consequences for the application of generic interpretative criteria for Lyme borreliosis Western blot seropositivity, and therefore criteria must be carefully validated for performance with each assay antigen. In addition, assays must be validated with specimens which represent the intended geographic test target area, since the prevalence of *Borrelia* strains is not uniform throughout Europe. In our study, we found that while the performances of *B. garinii* 20047 and *B. afzelii* VS461 were approximately equivalent in several analyses with the panel of European sera tested, *B. garinii* 20047 was more sensitive in discriminating positive specimens. In no cases would the use of *B. burgdorferi* sensu stricto strains offer performance advantages over the use of *B. garinii* or *B. afzelii* strains for testing the European sera, and in several cases, significantly lower levels of reactivity were observed. The next phase of this work will address the optimization of interpretative criteria for a *B. garinii* Western blot for the serodiagnosis of Lyme borreliosis in Europe.

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