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NOTES

Comprehensive Analysis of the Factor H Binding Capabilities of *Borrelia* Species Associated with Lyme Disease: Delineation of Two Distinct Classes of Factor H Binding Proteins

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Some Lyme disease spirochete isolates can bind complement regulatory protein factor H (fH), a process that may allow evasion of complement-mediated killing. Here we demonstrate significant differences in the fH binding capabilities of species of the *Borrelia burgdorferi* sensu lato complex. The percentages of *B. burgdorferi*, *B. afzelii*, and *B. garinii* bacteria that bound fH in either enzyme-linked immunosorbent assays or affinity ligand binding immunoblot assays were 100, 83, and 29%, respectively. The fH binding protein profiles were examined and found to exhibit variability among isolates and to form two distinct classes. Differences in fH binding ability may contribute to the differences in pathogenesis and clinical course observed upon infection with different species of the *B. burgdorferi* sensu lato complex.

The *Borrelia burgdorferi* sensu lato complex is composed of closely related species, including those associated with the chronic infection Lyme disease (*B. burgdorferi*, *B. afzelii*, and *B. garinii*). Lyme disease spirochetes use several mechanisms for immune evasion (14, 16, 21, 24). Factor H (fH) binding to the cell surface with subsequent cleavage of C3b has been demonstrated (2), indicating that fH binding is of biological relevance and potentially important in vivo. OspE is one of several fH binding proteins (FHBPs) produced by Lyme disease spirochetes (7). In *B. burgdorferi* B31MI, the OspE paralogs BBL39 and BBN38 (also referred to as ErpA and ErpP, respectively) have both been demonstrated to bind fH (3, 17) in a conformation-dependent manner (17). The OspE paralogs are the only two FHBPs identified at the sequence level. The potential for OspE to interact with fH in vivo is supported by strong evidence that it is a surface protein (4, 6, 13) and expressed by spirochetes in both the tick and mammalian environments (1, 5, 16, 18, 19, 21).

Data published to date indicate that the fH binding phenotype is not universal among Lyme disease spirochete isolates and that the phenotype may correlate with individual species of the *B. burgdorferi* sensu lato complex (2, 11, 12, 23). The ability or inability to bind fH and cleave C3b could be an important determinant that influences the different pathogenic properties of *B. burgdorferi* sensu lato complex species. However, since only a limited number of isolates of each species have been analyzed to date, a conclusive correlation between fH binding and individual species has not been established. The goals of this study were to conduct a comprehensive analysis of the fH binding capabilities of *B. burgdorferi* sensu lato complex species to determine if a correlation between specific *Borrelia* species and fH binding exists.

In this study, fH binding to a group of 69 diverse Lyme disease spirochete isolates was assessed. Of these, 59 were tested with an enzyme-linked immunosorbent assay (ELISA) format (17). Briefly, cells were immobilized in microtiter plate wells and incubated (4°C, 15 h) with human fH (hfH; 10 ng μl^{-1}; Calbiochem), goat anti-fH serum was added (Calbiochem; 1:800; 4 h, 4°C), and binding was detected by incubation (1 h, 4°C) with horseradish peroxidase-conjugated rabbit anti-goat immunoglobulin G (1:40,000; Calbiochem). All assays were conducted in triplicate. The percentages of *B. burgdorferi*, *B. afzelii*, and *B. garinii* isolates that bound hfH by this approach were 100% (22 of 22), 46% (5 of 11), and 31% (5 of 16), respectively (Fig. 1; Table 1). Regarding other species of the *B. burgdorferi* sensu lato complex, one or more isolates of *B. valaisiana*, *B. japonica*, *B. turdi*, and *B. tanukii* bound fH while *B. andersonii*, *B. bissettii*, and *B. miyamotoi* isolates did not. Recombinant BBL39, an OspE paralog of isolate B31MI and a demonstrated FHBP (3, 7, 10, 17), served as the positive control and bound fH at a high level. When hfH was omitted from the ELISAs (Fig. 1), fH-*Borrelia* complexes were still detected with some isolates. This likely results from binding of endogenous fH present in the goat anti-fH sera used in the binding assay. To verify this, a 1-μl aliquot of the goat anti-fH antiserum was fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), immunoblotted, and screened with goat anti-hfH sera. An immunoreactive band of ~150 kDa, consistent with the size of fH, was detected, dem-

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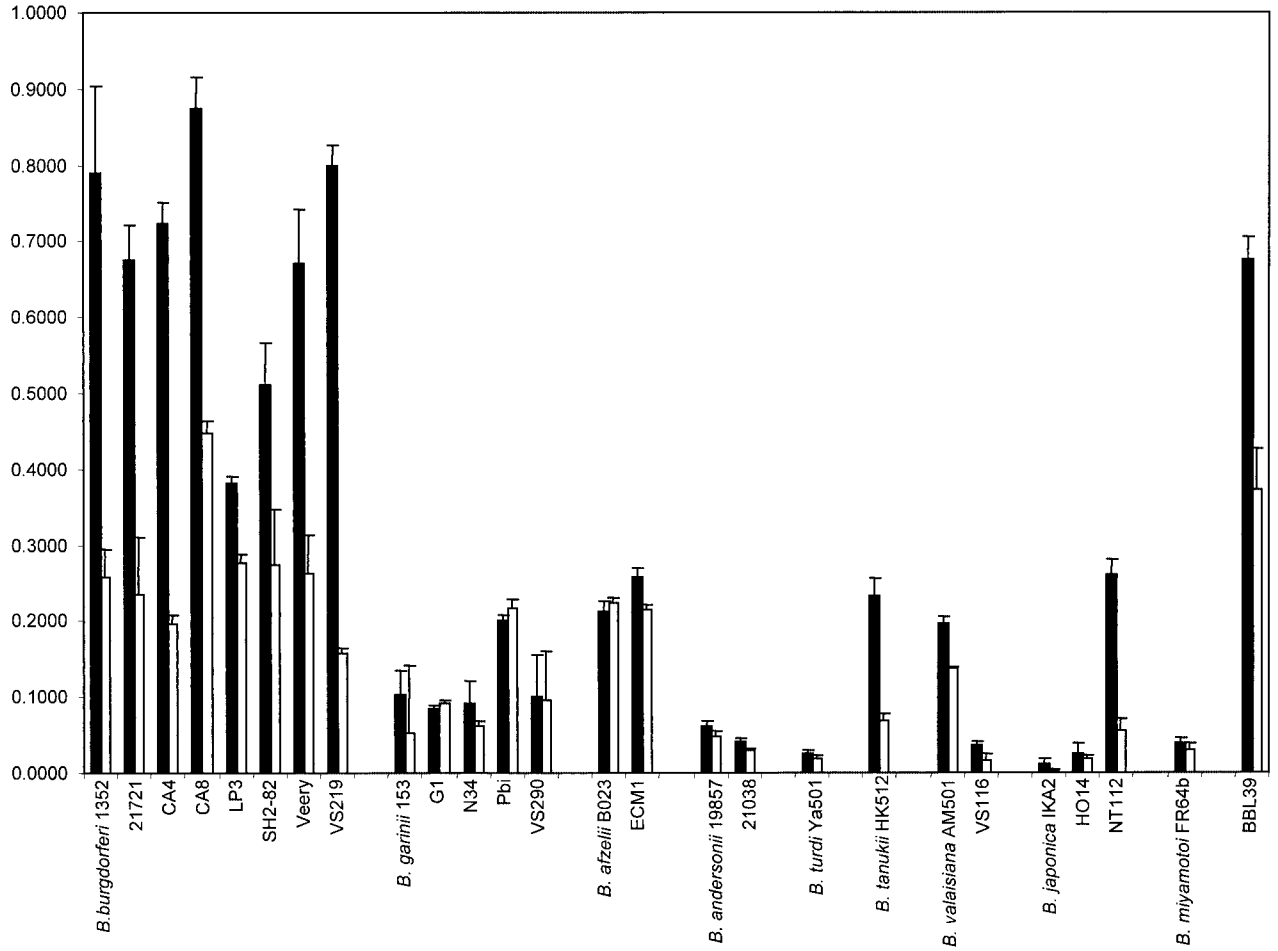

FIG. 1. Whole-cell ELISA analysis of fH binding by *B. burgdorferi* sensu lato complex isolates. All of the methods used are briefly described in the text and presented in detail in reference 17. Bacteria were harvested, gently washed with PBS, and immobilized in triplicate in the wells of microtiter plates. hfH was added to one set of wells (black bars) and omitted from a second set (open bars). Goat anti-hfH serum was added, and antibody binding was detected as described in the text and expressed as A_{450} values. Recombinant BBL39 (OspE paralog of *B. burgdorferi* B31MI) served as a positive control, and bovine serum albumin served as a negative control. The low degree of fH binding to bovine serum albumin represents the baseline. The baseline value was subtracted to obtain the data presented.

onstrating that fH is present in the antiserum and recognized by the anti-human fH antibodies (data not shown). However, a possible alternative interpretation of the ELISA data presented above is that the anti-hfH serum cross-reacted with the cells in an fH-independent fashion. This issue has been addressed in a separate study in which hfH binding directly to borreliae was demonstrated with an hfH monoclonal antibody (17). Lastly, Alitalo and colleagues unequivocally demonstrated the direct binding of hfH to some *Borrelia* isolates with radiolabeled fH (2).

FHBP profiles of each isolate were assessed with an affinity ligand binding immunoblot assay as previously described (17). Briefly, cell lysates were fractionated by SDS-PAGE, immunoblotted, incubated (2 h, 4°C) with or without hfH (10 ng μl^{-1}), and screened with goat anti-fH sera (1:800; with a horseradish peroxidase-conjugated rabbit anti-goat immunoglobulin G secondary antibody at 1:40,000). Two to six FHBPs were detected in all isolates that bound fH, and differential binding along species lines was evident (Fig. 2). By this approach, the percentages of *B. burgdorferi*, *B. afzelii*, and *B. garinii* isolates that bound hfH were 100, 83, and 25%. Note that with a shorter exposure of the film, the 27-kDa FHBP could clearly be resolved as two proteins. These ~27-kDa FHBPs appear to be the dominant FHBPs; however, it is unclear if this is due to a higher expression level of these proteins or a greater affinity for hfH.

To compare the expression profiles and fH binding properties of members of the OspE protein family in diverse isolates, an identical immunoblot was screened with anti-OspE sera (Fig. 2) generated as previously described (17). Recombinant OspE derived from the BBL39 locus of *B. burgdorferi* B31MI as part of a separate study (17) was used to produce the antisera. Immunoblot analyses (16) with various recombinant proteins and lysates of *B. burgdorferi* B31MI confirmed the specificity of the antisera. Extensive variation in the OspE expression patterns was observed, with each isolate expressing zero to four OspE paralogs. Sixty-three percent of the isolates tested produce at least one OspE paralog. Many *B. garinii* isolates did not produce OspE-related proteins, and in these isolates, *ospE*-related sequences were not detected by Southern hybridization

TABLE 1. *B. burgdorferi* sensu lato isolates used in this study

Species and isolate designation	Biological source	Geographic origin	ELISA result[a]	Molecular mass(es) (kDa) of fH binding protein(s)	Detection of OspE proteins by immunoblotting[d]
B. burgdorferi					
B31M1	*Ixodes scapularis*	New York	+++	19, 26.5, 27	+
272	Human skin	United States	+++	22, 24, 26.5, 27	+
1352	*Amblyomma americanum*	Texas	+++	22, 26	+
20004	*I. ricinus*	France	ND[c]	27, 27.5	ND
21343	Mouse skin	Texas	+++	20, 26.5, 27	+
21721	*I. scapularis*	Wisconsin	+++	22, 27	+
25015	*I. scapularis*	New York	+++	23, 30, 31	+
27985	*I. scapularis*	Connecticut	+++	19, 22, 27, 28	+
297CH	Human CSF[b]	Connecticut	+++	18, 27.5	+
CA2-87	*I. pacificus*	California	+++	20, 26.5	+
CA3	*I. pacificus*	California	+++	20, 26.5	+
CA4	*I. pacificus*	California	+++	21, 22, 26.5, 27	−
CA8	*I. pacificus*	California	+++	16, 20, 27, 27.5, 30	+
CA12	*I. pacificus*	California	ND	19, 27.5	+
HB19	Human blood	California	+++	23, 26.7	−
JD1	*I. scapularis*	Massachusetts	+++	21, 26.5	ND
LP3	Human skin	Connecticut	+++	19, 24, 26	+
LP4	Human skin	Connecticut	+++	19, 22, 23, 28	+
LP5	Human skin	Connecticut	+++	19, 26.5, 27	+
LP7	Human skin	Connecticut	ND	20, 21	+
N40CH	*I. scapularis*	New York	+++	22, 27	+
NY13-87	Human skin	New York	+	19, 27	±
SH2-82	*I. scapularis*	New York	+++	18, 23, 29	+
Veery	Veery bird	Connecticut	+++	19, 26.5, 27	+
VS219	*I. ricinus*	Germany	+++	22, 26, 26.5, 27	+
B. garinii					
153	*I. ricinus*	France	−	—[e]	−
20047	*I. ricinus*	France	−	—	−
AO1	Human CSF	The Netherlands	−	—	−
B-4/91	Human skin	Norway	−	22, 26.5	−
B-5/92	Human skin	Norway	+	22, 26.5	+
FRG	*I. ricinus*	Germany	−	—	ND
G1	Human CSF	Germany	−	ND	ND
G2	Human CSF	Germany	ND	—	−
G25	*I. ricinus*	Sweden	−	—	ND
IP90	*I. persulcatus*	Russia	−	—	−
N34	*I. ricinus*	Germany	−	—	−
Pbi	*I. ricinus*	Germany	+	20.5, 26	ND
Pbo	*I. ricinus*	Germany	+	21.5, 22, 26	+
Pbr	*I. ricinus*	Germany	−	—	ND
PTrob	Human CSF	Slovenia	ND	—	−
PHoe	Human CSF	Germany	ND	—	−
PBaeII	Human CSF	Germany	ND	—	−
VSBP	*I. ricinus*	Switzerland	+	20, 21, 28	+
VS102	*I. ricinus*	Switzerland	ND	—	ND
VS290		Switzerland	−	—	−
VS492		Switzerland	+	—	−
B. afzelii					
BO23	Human skin	Germany	+	22	−
ECM1	Human skin	Sweden	+	22, 27	−
HT10	*I. persulcatus*	Japan	+	19, 22, 27	+
R-IP3	*I. persulcatus*	Russia	−	—	−
R-IP21	*I. persulcatus*	Russia	−	22, 27	−
IPF	*I. persulcatus*	Japan	ND	18, 27	−
J1	*I. persulcatus*	Japan	+	19, 26.5, 33	−
Pgau	Human skin	Germany	+	22, 26.5	ND
PKo	Human skin	Switzerland	−	20, 27	+
UMO1	Human skin	Sweden	−	21, 27	+
UO1	Human skin	Sweden	−	—	−
VS461	Tick	Switzerland	−	22, 26.5	ND
B. andersonii					
19857	Rabbit kidney	New York	−	—	+
21038	*I. dentatus*	New York	−	—	+

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TABLE 1—*Continued*

Species and isolate designation	Biological source	Geographic origin	ELISA result[a]	Molecular mass(es) (kDa) of fH binding proteins	Detection of OspE proteins by immunoblotting
B. turdi					
Ya501	*I. turdus*	Japan	−	19, 20, 26.5	+
B. tunukii					
HK512	*I. tanukii*	Japan	+	19, 21, 21.5, 25, 26.5, 27	+
B. valaisiana					
AM501	*I. columnae*	Japan	+	—	ND
VS116	*I. ricinus*	Switzerland	−	—	+
B. bissettii DN127	*I. pacificus*	California	ND	—	−
B. japonica					
IKA2	*I. ovatus*	Japan	−	—	−
HO14	*I. ovatus*	Japan	−	—	+
NT112	*I. ovatus*	Japan	+	20, 26	+
B. miyamotoi FR64b	*Apodemus argenteus* (blood)		−	—	+

[a] For ELISAs, A_{450} readings were scored as follows: +, 0.15 to 0.3; +++, >0.3; −, <0.15.
[b] CSF, cerebrospinal fluid.
[c] ND, not done.
[d] +, OspE proteins detected; ±, weak detection; −, no OspE proteins detected.
[e] —, none detected.

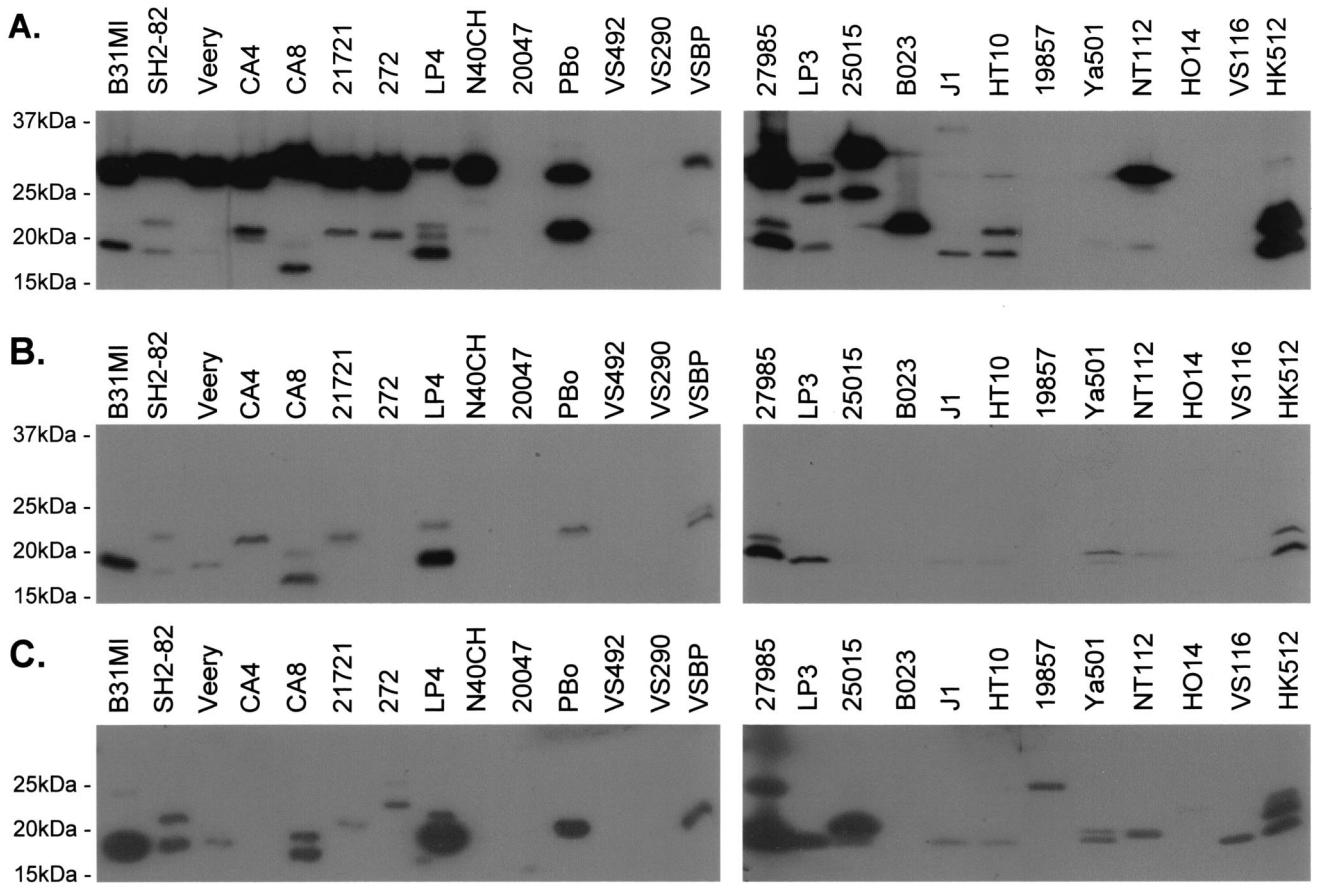

FIG. 2. Affinity ligand binding immunoblot analysis of fH binding of *B. burgdorferi* sensu lato complex isolates. Cell lysates were generated, fractionated by SDS-PAGE (15% gels), and immunoblotted to generate several identical immunoblots. All of the procedures used are briefly described in the text and in detail in reference 17. The immunoblots in panel A were incubated with hfH and screened with goat anti-hfH serum. HfH was not added to the immunoblots in panel B. The blots in panel C were screened with mouse anti-OspE sera. The isolates analyzed are indicated above the lanes. Note that blots A and B were exposed to the same piece of film for exactly the same amount of time. Note that detection of some fH binding proteins required longer exposure of the film.

(data not shown). This variability of OspE profiles among isolates is consistent with studies that demonstrated that OspE is part of a highly variable protein family (15, 21, 22). Comparison of the OspE immunoblot with the immunoblots used in the affinity ligand binding assays indicated that not all OspE paralogs can bind fH. Examples include the OspE paralogs of *B. valaisiana* VS116 and *B. turdi* Ya501 and one of the three produced by *B. burgdorferi* LP4 (Fig. 2). In a separate study, we demonstrated that conformational or structural determinants are important in fH binding (17). Future sequence analyses of OspE proteins that can or cannot bind fH may allow the identification of the sequence and structural determinants that convey fH binding.

As in the ELISA analyses, an hfH-negative control was included in the affinity ligand binding immunoblot assays. In this control, the only available fH would be the endogenous fH present in the goat anti-hfH sera. Goat fH bound readily to the OspE paralogs but not to other FHBPs (Fig. 2). Hence, this control yielded important information about the specificity of the fH binding properties of individual FHBPs. On the basis of antigenic relatedness to OspE and the differential binding of goat fH and hfH, two classes of FHBPs were delineated in this study. Class I FHBPs are related to OspE and bind both hfH and goat fH. Class II proteins are not related to OspE and bind only hfH. The identity of the class II FHBPs remains to be determined and is the subject of ongoing analyses. Regarding the fH-negative control, in an earlier study (20), the presence of high levels of endogenous fH in the goat anti-fH sera was apparently overlooked. This affected the interpretation offered in that report regarding the fH binding specificity of individual *Borrelia* proteins. For example, it was concluded that the recombinant OspE paralogs BBL39 and BBN38 (referred to as ErpA and ErpP in that report) bind fH from all of the mammals tested. However, the presence of endogenous goat fH in the goat-anti fH sera used to measure fH binding makes it impossible to reach conclusions about binding specificity.

It has been suggested that differences in serum sensitivity among *B. burgdorferi* sensu lato isolates (12, 23) may fall along species lines and reflect differences in fH binding capability (3, 7, 10). However, the numbers of isolates analyzed in earlier studies were limited, and as a result, the data were insufficient to correlate the fH binding phenotype with individual species (2, 12, 23). In addition, the abilities of less-studied species of the *B. burgdorferi* sensu lato complex to bind fH have not been investigated. This study, which builds upon work done by other groups (2, 3, 8–12), is the first comprehensive assessment of the correlation between individual species and fH binding. The fH binding phenotype was determined to be universal among *B. burgdorferi* isolates (100%), widespread among *B. afzelii* isolates (83%), and uncommon among *B. garinii* isolates (31%). These percentages correlate exceptionally well with the percentage of isolates of each species reported to be serum resistant (12, 23). The panel of isolates used here, which are now well characterized with regard to their fH binding abilities, can now be exploited to test hypotheses regarding the influence of fH on *Borrelia* serum sensitivity and pathogenesis. For example, the tropism of *B. garinii* for the central nervous system may reflect the inability of this species to bind fH. Residence within the central nervous system may provide some protection from complement attack. In contrast, *B. burgdorferi*, because of its fH binding capability, may be able to efficiently disseminate throughout the body. While fH binding by Lyme disease spirochetes is likely to be important in human disease, fH binding may also be important in spirochetal population maintenance in nature. The ability to circumvent complement-mediated killing would facilitate the maintenance of spirochetes in their mammalian hosts and ensure the completion of their enzootic cycle. In summary, on the basis of the data present here and our present understanding of the interaction of Lyme disease spirochetes with fH, we hypothesize that fH binding is an important pathogenic mechanism and an important determinant in the tropism of different Lyme disease spirochete species for specific anatomical niches.

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