Heterogeneity of BmpA (P39) among European Isolates of 
*Borrelia burgdorferi* Sensu Lato and Influence of 
Interspecies Variability on Serodiagnosis

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The molecular and antigenic variabilities of BmpA (P39) among European isolates of *Borrelia burgdorferi* were analyzed. The *bmpA* sequences of 12 isolates representing all three species of *B. burgdorferi* sensu lato pathogenic for humans were amplified by PCR, cloned, and sequenced. The BmpA protein of *Borrelia garinii* is heterogeneous, with an amino acid sequence identity ranging from 91 to 97%, whereas the BmpA proteins of *Borrelia afzelii* and *B. burgdorferi* sensu stricto strains appear to be highly conserved (>95% intraspecies identity). The interspecies identities ranged from 86 to 92%. Cluster analysis of BmpA reflected the subdivision of *B. burgdorferi* sensu lato isolates into the three species as well as a considerable heterogeneity among *B. garinii* strains. The BmpA protein of each species of *B. burgdorferi* sensu lato was recombinantly expressed in *Escherichia coli*, purified, and used to generate monoclonal antibodies. Seven BmpA-specific antibodies were identified; six of them recognized conserved epitopes of all three species, whereas one was specific for BmpA of *B. afzelii* and *B. garinii*. A monoclonal antibody (H1141) recommended by the Centers for Disease Control and Prevention for use in the standardization of immunoblots showed strong reactivity with BmpA of *B. burgdorferi* sensu stricto but no or only weak reactivity with BmpA of *B. garinii* and *B. afzelii*, respectively. Sera from 86 European patients with Lyme borreliosis in different stages and 73 controls were tested in immunoglobulin G (IgG) and IgM immunoblots with the recombinant BmpA proteins of the three species, revealing specificities of 98.6 to 100%. IgM antibodies against recombinant BmpA were only rarely detected (1.1 to 8.1%). With the BmpA proteins of *B. afzelii* and *B. garinii*, sensitivities for the IgG test (sera from stages I to III) were 36.0 and 34.9%, respectively, in contrast to 13.9% with BmpA of *B. burgdorferi* sensu stricto. Therefore, we recommend that recombinant BmpA of *B. afzelii* or *B. garinii* should be used solely, or in addition to *B. burgdorferi* sensu stricto BmpA, in serodiagnostic tests for Lyme borreliosis in Europe.

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In Europe, *Borrelia burgdorferi* sensu lato, the etiologic agent of Lyme borreliosis, comprises three species pathogenic for humans (4). Many antigens of *B. burgdorferi* sensu lato have been described to be heterogeneous between the three species *B. burgdorferi* sensu stricto, *Borrelia afzelii*, and *Borrelia garinii*. The molecular variability was especially demonstrated for the plasmid-encoded antigens OspA (43, 47), OspC (19, 41, 48), and OspD (23), whereas chromosomally encoded antigens like p83/100 (32) and flagellin (19) are more conserved. Among different strains of *B. garinii* this antigenic variability of defined antigens is more pronounced, e.g., OspA types 3 to 8 (44). Several studies have shown differences in immunoblot reactivity patterns depending on the strain, serotype, or species used as the antigen (8, 16, 24, 26, 49, 50). Proper identification of reactive bands and thus comparison of the reactivities of defined borrelial proteins is difficult in immunoblots with ultrasonicated antigens (conventional immunoblotting). Recently, we showed differences in reactivity among homologous proteins derived from representatives of the three species pathogenic for humans using recombinant proteins for the proper identification of reactive proteins (45). Such differences were shown for OspA, OspC, p83/100, and truncated flagellin in the immunoblot (45) as well as by other investigators with enzyme-linked immunosorbent assay (ELISA) for truncated flagellin (6) and for OspC with conventional immunoblots (24).

Simpson et al. (38) described a specific and sensitive antigen for the serodiagnosis of Lyme disease in U.S. patients. Several studies (all done with *B. burgdorferi* sensu stricto antigen) confirmed the high value of P39 as a serodiagnostic antigen (9–11, 16, 22, 27, 35). Recently, the gene locus for P39 was identified in *B. burgdorferi* sensu stricto strain Sh-2-82 by Simpson and coworkers (37). The chromosomally encoded *bmpA* gene is located in a *bmp* gene cluster consisting of *bmpA* to *bmpD* (31). The *bmpA* gene is arranged tandemly with *bmpB* (37) downstream of the putative monocistronic transcribed *bmpD* or *bmpC* (31). The deduced translation products of all four *bmp* reading frames are of similar size and contain putative cleavage sites for signal peptidase II, and thus, their locations might be within the borrelial membrane, as shown for BmpA (40). Only BmpA (P39) of the *bmp* gene cluster is described as an important immunogenic protein for the serodiagnosis of Lyme borreliosis. So far, no data on the in vivo expression and immunogenicity of *bmpB*, *bmpC*, or *bmpD* are available. The molecular heterogeneity of BmpA (P39) among the three *B. burgdorferi* sensu lato species pathogenic for humans and its possible effects on the serodiagnosis of Lyme borreliosis in Europe have not been investigated yet.

Thus, we analyzed the molecular and immunological heterogeneities of BmpA proteins among *B. burgdorferi* sensu lato isolates. We found considerable heterogeneity among BmpA proteins and evaluated the influence of this heterogeneity on
serodiagnosis. Seven BmpA-specific monoclonal antibodies (MABS) were produced with recombinantly expressed BmpA. They were tested for their reactivities with BmpA of \textit{B. burgdorferi} sensu lato (OspA types 1 to 7). Recombinant BmpA was highly specific but a weak marker for immunoglobulin M (IgM), and the BmpA proteins of \textit{B. afzelii} and \textit{B. garinii} were more than twice as sensitive as \textit{B. burgdorferi} sensu stricto BmpA at detecting IgG antibodies.

(Prop. of this work was presented at the VII International Congress on Lyme Borreliosis, San Francisco, Calif., 16 to 21 June 1996.)

**MATERIALS AND METHODS**

**Patient sera.** Sera from the following groups of patients with Lyme borreliosis \textit{(n = 86)} and controls \textit{(n = 73)} were investigated. Sera from unselected, untreated patients \textit{(n = 30)} with erythema migrans (EM) were obtained from a dermatologist during a former study of therapy for Lyme borreliosis \textit{(42)}. The median time of onset of the neurological symptoms before the serum samples were obtained was 4 weeks (range, 2 to 18 weeks). The group with late Lyme borreliosis \textit{(n = 26)} comprised 16 patients with acrodynatitis chronica atrophicans (ACA) diagnosed by a dermatologist and 10 patients with Lyme arthritis \textit{(43)}. Possible differential diagnoses had been excluded. Sera from 52 healthy blood donors, sera from 10 patients with syphilis in stage II or III, and 11 serum specimens with rheumatoid factor levels of \textit{$\geq$}45 IU/ml served as controls. The actual healthy blood donors had no history of frequent tick bites, erythemas, neurological symptoms, or joint disorders.

**Borrele sp. strains and cultivation.** The \textit{B. burgdorferi} strains used in this study (Table 1) were grown in modified Kelly medium at 33°C for 4 to 5 days as described previously \textit{(44, 47, 48)}.

**PCR, molecular cloning, and sequencing.** If not mentioned otherwise, standard techniques of molecular cloning were performed as described elsewhere \textit{(2)}.

**Table 1. Oligonucleotides used for PCR amplification, cloning, and sequencing of bmpA and bmpB**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequence \textit{5’-3’} \textsuperscript{a}</th>
<th>Primer direction</th>
<th>Target site position \textsuperscript{b}</th>
<th>Sites for restriction enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>bmpA-A1</td>
<td>ggggtacccggttcctctcTATGACATTAATATTTTGTAGTGGTAA</td>
<td>Forward</td>
<td>1–24</td>
<td>KpnI, BamHI</td>
</tr>
<tr>
<td>bmpA-A3</td>
<td>aaccaacctgttacctTCTTCTAGTTGGGAAA</td>
<td>Forward</td>
<td>43–62</td>
<td>NcoI, KpnI</td>
</tr>
<tr>
<td>bmpA-B1</td>
<td>gtttgcgagcaccagctttTATAATTTCTATTAAAG</td>
<td>Reverse</td>
<td>1020–1003</td>
<td>SalI, HindIII</td>
</tr>
<tr>
<td>bmpA-B2</td>
<td>aaagtgaccaagaagttatgttattgtattgtattgtgATATAAAATTTTTAAG</td>
<td>Reverse \textsuperscript{c}</td>
<td>1017–997</td>
<td>SalI, HindIII</td>
</tr>
<tr>
<td>bmpA-C</td>
<td>TTATTATCTTGTATTGGGAAA</td>
<td>Forward</td>
<td>216–235</td>
<td></td>
</tr>
<tr>
<td>bmpA-D</td>
<td>GAAATGATTGTTTTTTAAAT</td>
<td>Reverse</td>
<td>834–915</td>
<td></td>
</tr>
<tr>
<td>bmpA-E</td>
<td>CTCAGGACGAGTAAAATTAA</td>
<td>Reverse</td>
<td>235–216</td>
<td></td>
</tr>
<tr>
<td>bmpA-F</td>
<td>ATTGAAAAAATTAATTTTCT</td>
<td>Forward</td>
<td>815–834</td>
<td></td>
</tr>
<tr>
<td>bmpB-A1</td>
<td>ggggtacccggttcctctcTATGACATTAATATTTTGTAGTGGTAA</td>
<td>Forward</td>
<td>1–21</td>
<td>KpnI, BamHI</td>
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<tr>
<td>bmpB-B1</td>
<td>gtttgcgagcaccagctttTATAATTTCTATTAAAG</td>
<td>Forward</td>
<td>1026–1009</td>
<td>SalI, PstI</td>
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<tr>
<td>bmpB-C</td>
<td>CCATCTTATAATCCTATT</td>
<td>Reverse</td>
<td>891–873</td>
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</tr>
<tr>
<td>bmpB-D</td>
<td>CCAGAAAAATTTGAAAGAATTTT</td>
<td>Forward</td>
<td>175–197</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Unpaired nucleotides are indicated by lowercase letters.

\textsuperscript{b} Position numbering refers to that in the open reading frames.

\textsuperscript{c} The primer encodes an additional six histidine residues.
produce an in-frame construct of bmpA lacking its own 3′ signal sequence but containing the Escherichia coli ompA signal sequence of the secretory pASK40 expression vector. The PCR amplification of the truncated bmpA gene was carried out with primer pair bmpA-A3 and bmpA-D. The overall bmpA coding region was amplified by PCR with primers bmpB-A1 and bmpB-B1.

For cloning of bmpA and bmpB, PCR fragments were either cloned directly into pCR II (Invitrogen, Leek, The Netherlands) or cut with a restriction enzyme (Boehringer Mannheim GmbH, Mannheim, Germany), purified by agarose gel electrophoresis (EMC NuSieve GTG agarose; Biozym, Hess, Oldendorf, Germany), and eluted (QIAquick Gel Extraction Kit; Qiagen, Hilden, Germany). About 60 fmol of the expression unit was ligated with 6 fmol of an appropriately prepared pUC18 (BamHI, PvuI) or pASK40 (KpnI, HindIII) expression vector by standard procedures. Competent E. coli JM83 cells were electroporated (Gene Pulser II; Bio-Rad, Munich, Germany) with a 10% aliquot of the ligation mixture (ethanol precipitated). Transformants were selected after restriction analysis and subsequent DNA sequencing was performed on an ABI 377 DNA Sequencer (ABI Prism dye terminator cycle sequencing; Perkin-Elmer Applied Biosystems, Weiterstadt, Germany).

Results

BmpA and BmpB sequence heterogeneity. The coding regions (positions 43 to 1020) of bmpA were amplified from 2 B. burgdorferi sensu lato strains listed in Table 1. Only bmpA of B. garinii PBr, TN, and PKi failed to give a full-length PCR product and were amplified as shorter products lacking approximately 180 to 230 bp at the 3′ terminus.

The deduced amino acid sequences showed a high degree of identity (86.4 to 99.7%) to those of the B. burgdorferi sensu stricto strain Sh-2-82. Alignment studies at the amino acid level (amino acid positions 15 to 257; numbering for strain Sh-2-82) showed highly conserved BmpA within B. burgdorferi sensu stricto (98.7 to 99.7% identity) and B. afzelii strains (98.5 to 99.4% identity), whereas B. garinii BmpA appeared to be more heterogeneous (91.4 to 97.5% identity). The interspecies identities between the three B. burgdorferi species pathogenic for humans ranged from 86.4 to 91.6%. Cluster analysis of the BmpA sequences (amino acid positions 15 to 257; numbering for strain Sh-2-82) showed three main clusters representing the three species and a higher variability within B. garinii BmpA compared to that within the BmpA proteins of B. afzelii and B. garinii (Fig. 1). The average identity of the BmpA sequences compared to that of the BmpB sequence is 57.7%. The theoretically determined isoelectric points of BmpAs (varying from pl 4.96 to pl 5.98) were confirmed by isoelectric focusing with the rBmpAs of strains B31, PKo, and PBr (data not shown).

The three amplified bmpB sequences of strains B31, PKo, and PBr were compared to the previously published sequence of strain Sh-2-82. The identities of the deduced amino acid sequences ranged from 97.9% between the two B. burgdorferi sensu stricto strains (B31 and Sh-2-82) to 83.8 and 85.6% compared to the BmpB sequence of B. garinii PBr and the BmpB sequence of B. afzelii PKo, respectively.

N-terminal sequence of rBmpA. For internal quality assurance and to test the integrity of the secreted and purified rBmpA, the material was subjected to N-terminal sequencing. As expected, the rBmpA of strain B31 revealed a homogeneous N terminus harboring the seven additional amino acid residues derived from the OmpA signal sequence linker peptide of pASK40 (ANSSSV-[F15-I339]6×H-BmpA). Recombinant BmpA of strain PKo showed the expected N terminus (about 40%) as well as an N-terminal prolonged variant (60%) with an additional six amino acid residues (underlined). ATP AQAAAANSV-[F15-I339]6×H-BmpA. rBmpA of strain PBr showed N-terminal truncated forms missing 4 (approximately 50%) or 11 amino acid residues resulting in SVP-[F15-I339]6×H-BmpA or [S19-I339]6×H-BmpA of strain PBr.

Isolation, characterization, and reactivities of Mabs. After screening by ELISA with rBmpA as the antigen as well as by conventional immunoblotting with B. burgdorferi total cell ly-
sates (data not shown), seven different BmpA-specific MAbs (MAbs L39B1, L39B2, L39B3, L39B5, L39B7, L39B8, and L39B10) could be identified. All MAbs belong to the IgG1 subclass. One MAb (MAb L39B8) was reactive with B. afzelii (strains PKo and PGau; OspA type 2) and B. garinii (strains PBr, PBi, W12, TN, and T25; OspA types 3 to 7, respectively) but not with B. burgdorferi sensu stricto (strains PKa2 and B31; OspA type 1) by using conventional immunoblots. All other MAbs were reactive with all three species of B. burgdorferi sensu lato. None of the antibodies was reactive with whole-cell lysates of relapsing fever borreliae (Borrelia recurrentis, Borrelia duttonii, Borrelia parkeri, Borrelia hermsii, and Borrelia turicatae) or Treponema pallidum, Treponema phagedenis, Treponema denticola, Leptospira grippotyphosa, Listeria monocytogenes, Legionella micdadei, or Salmonella typhimurium (data not shown).

All MAbs were also tested for their reactivities with rBmpA of strains B31, PKo, and PBi. MAb L39B8 recognized only the rBmpA proteins of B. garinii PBi and B. afzelii PKo but not the rBmpA of B. burgdorferi sensu stricto strain B31 (Fig. 2, lane D). All other MAbs were reactive with recombinant BmpAs from all three species (Fig. 2, lane C; MAb L39B5). A BmpA-specific monoclonal antibody (MAb H1141) recommended by the Centers for Disease Control and Prevention for the calibration of immunoblots in the United States showed weak reactivity with rBmpA of B. afzelii PKo, no reaction to rBmpA of B. garinii PBi, but a clear reaction with rBmpA of B. burgdorferi sensu stricto strain B31 (Fig. 2, lane E). The same results were obtained with MAb H1141, but by using B. burgdorferi sensu lato whole-cell lysates in conventional immunoblots (data not shown).

Serological testing of rBmpA. A panel of serum specimens from 86 patients and 73 controls was tested for the strain-specific reactivities of the sera with the rBmpAs of strains PKo, B31, and PBi in immunoblots and with a mixture of the three rBmpAs.

To determine the cutoff criteria for positive results, control serum specimens from 73 healthy control persons were examined. A five-grade criterion evaluation (strongly positive, positive, weakly positive but definitely positive, detectable “shading,” and no reaction) was performed. Only 1 of the 73 control serum samples reacted weakly positive by IgG testing with rBmpA of strain B31. None of the other serum specimens were reactive (Table 3), thus giving specificities of 100% for rBmpA of PKo, PBi, or the rBmpA mixture and 98.6% for rBmpA of

<p>| Table 3. Reactivity of 73 control serum samples with rBmpA proteins from various B. burgdorferi sensu lato strains |
|------------------|-------------------|-------------------|-------------------|</p>
<table>
<thead>
<tr>
<th>Strain</th>
<th>Species</th>
<th>Protein</th>
<th>No. of serum specimens (specifity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B31</td>
<td>B. burgdorferi sensu stricto</td>
<td>rBmpA</td>
<td>(98.6) (100)</td>
</tr>
<tr>
<td>PKo</td>
<td>B. afzelii</td>
<td>rBmpA</td>
<td>(100) (100)</td>
</tr>
<tr>
<td>PBi</td>
<td>B. garinii</td>
<td>rBmpA</td>
<td>(100) (98.6)</td>
</tr>
<tr>
<td>Mixture</td>
<td>rBmpA</td>
<td>(100) (100)</td>
<td></td>
</tr>
</tbody>
</table>

a. Sera were from 52 healthy blood donors, 10 syphilis-positive patients, and 11 rheumatoid factor-positive patients.

b. The mixture included proteins from all three species.

FIG. 1. Cluster analysis of the deduced BmpA protein sequences (amino acid positions 15 to 257; numbering for strain Sh-2-82) of various B. burgdorferi sensu lato strains and BmpB of B. afzelii PKo as the outgroup. The scale bar represents the average identity (in percent) for the branching points. Numbers in parentheses indicate OspA types.

FIG. 2. Immunoblot strips with rBmpA of strains of the three B. burgdorferi sensu lato species pathogenic for humans. Lanes: A, transfer control (colloidal gold stain); B, purification control of BmpA with polyclonal anti-E. coli antiserum (K69); C to E, MAbs L39B5, L39B8, and H1141, respectively; F, reference serum IgG; G to L, selection of IgG-positive sera from patients with Lyme borreliosis.
strain B31. Similar results were obtained in the IgM tests (Table 3). IgM antibodies were detected in only 1.1 to 8.1% of the Lyme borreliosis patients. In contrast, IgG sensitivities for the three rBmpAs and the rBmpA mixture were significantly higher (Table 4). Recombinant BmpA of PKo or PBl and the rBmpA mixture reacted more than twice as often (34.9 to 36.0% overall sensitivity) in the IgG immunoblot than rBmpA of B31 (13.9%) alone. IgG antibodies were detected by the rBmpAs of strains PKo and PBl in 65 to 69% of the sera from patients with stage III of the disease (AT plus ACA). 33 to 40% of the sera from patients with stage II (NB), and only 6.6% of the sera from patients with the early (EM) stage (Table 4). In contrast, rBmpA of B. burgdorferi sensu stricto strain B31 was recognized by none of the sera from patients with stage I, only 10% of the sera from patients with stage II, and 34.6% of the sera from patients with stage III, resulting in this low overall sensitivity. The rBmpA mixture used in this study showed IgG sensitivities similar to that of rBmpA of strain PKo or strain PBl alone (Table 4). Results for a selection of various IgG-positive patient sera are presented in Fig. 2, lanes F to L.

**DISCUSSION**

Many studies have indicated that strain heterogeneity has a considerable influence on the serodiagnosis of Lyme borreliosis, especially in Europe (16, 24, 45, 50). Therefore, we investigated by molecular and immunological methods BmpA (P39) from various B. burgdorferi sensu lato strains and evaluated the seroreactivity of the respective recombinantly expressed antigens with sera from Lyme borreliosis patients.

We found considerable heterogeneity among BmpAs derived from strains belonging to different species of B. burgdorferi sensu lato. This was also true for B. garinii strains, whereas BmpA was highly conserved among B. afzelii and B. burgdorferi sensu stricto strains. The topology of the BmpA cluster analysis is similar to that of the plasmid-encoded OspA (43) and reflects heterogeneity among the three species as well as the intraspecies heterogeneity found solely among different strains of B. garinii.

Since BmpA is heterogeneous and variable regions are evenly distributed (only the region between amino acid positions 164 and 184 is highly conserved), this heterogeneity might influence serodiagnosis. Therefore, BmpAs from three different B. burgdorferi species were expressed recombinantly in E. coli. The expression of recombinant protein in a soluble form was most effective when an E. coli secretory system with the E. coli OmpA signal sequence instead of the BmpA signal sequence was used. Variations within the N terminus (linker peptide) of the expression products might be explained by an inaccurate endoproteolytic cleavage of the E. coli signal peptide during secretion. Unfortunately, no data on the expression and purification procedure of recombinant P39 used by other investigators are available in the literature (11, 27, 34). Thus, the presence of the BmpA signal sequence within the recombinant protein and its possible influence on the sensitivity of serodiagnosis remain unclear.

The BmpA molecule has epitopes conserved among the three species, as shown by MAbs L39B1, L39B2, L39B3, L39B5, L39B7, and L39B10. These MAbs were specific for three species, as shown by MAbs L39B1, L39B2, L39B3, L39B5, L39B7, and L39B10. These MAbs were specific for B. burgdorferi sensu lato antigens. Whereas attempts to develop a borrelian vaccine solely with outer surface proteins appear to be successful (12, 29, 33), the importance of P39 for a protective immune response is still controversial (15, 18, 36). The potential bactericidal capability of the MAbs presented here must be evaluated in future experiments.

MAb H1141 recommended by the Centers for Disease Control and Prevention (7) for the calibration of immunoblots showed a preferential reactivity with BmpA of B. burgdorferi sensu stricto, weak reactivity with B. afzelii, but no reactivity with B. garinii, irrespective of the nature of BmpA (whole-cell lysate in conventional or single rBmpA in recombinant immunoblots). On the other hand, MAb L39B8 reacted with B. afzelii and B. garinii but failed to react with B. burgdorferi sensu stricto. Since in Europe all three species, B. burgdorferi sensu stricto, B. afzelii, and B. garinii, have been isolated from patients (3, 47), we recommend the use of BmpA-specific MAbs recognizing conserved epitopes for the calibration of immunoblots.

The serological specificity of rBmpA is in good accordance with the results of other studies. Only a low specificity was described by Dressler et al. (9), who used conventional immunoblotting. Others reported specificities that ranged from 94 to 100% (1, 11, 16, 22, 25, 27, 34). Data for sensitivities of the P39 IgM test are controversial, depending on the assay system.
used, and may also be influenced by the manifestation of the disease.

In our hands rBmpA was not a useful antigen for IgM antibody detection. Results from laboratories in the United States varied considerably, with the assays having from low (1, 9) to high (10, 22) sensitivities. Those studies used conventional blots with B. burgdorferi sensu stricto as the antigen, and it is not clear whether the band at 39 kDa identified in those blots truly represents BmpA. Very recent immunoprint data presented by Padula et al. (28) are in agreement with a general low IgM reactivity of this protein.

The diagnostic sensitivity of the rBmpA IgG test in this study is in accordance with most data from other investigators (1, 9–11, 22, 34). rBmpA and nBmpA in the conventional blot appear to be equally sensitive. Only Oksi and coworkers (27) found a very low sensitivity (14.6% for a B. burgdorferi sensu stricto-derived P39 ELISA), even though the sera investigated were defined to be from patients with late-stage disease. Since these patients were from Northern Europe (Finland), it appears reasonable that the lack of sensitivity is at least partially caused by the use of recombinant B. burgdorferi sensu stricto P39 as a single antigen. Our results indicate that rBmpAs from strains PKo and PBI are considerably more sensitive than rBmpA of B. burgdorferi sensu stricto alone for the serodiagnosis of Lyme borreliosis in European patients. Thus, a significant increase in sensitivity can be achieved by using rBmpA of B. afzelii and B. garinii, in addition to or instead of B. burgdorferi sensu stricto BmpA. None of the sera tested in this study showed an exclusive reaction with rBmpA of B. burgdorferi sensu stricto but lacked reactivity with rBmpA of the other two species. However, it cannot be excluded that infection with B. burgdorferi sensu stricto may lead to antibodies solely reactive with B. burgdorferi sensu stricto BmpA.

In our hands recombinant BmpA is as sensitive as nBmpA for the detection of IgG antibodies in patients with stage I and stage II manifestations. There are different explanations for a higher sensitivity of the blot with the whole-cell lysate late in the course of the disease. Late in the course of the disease a very broad panel of proteins is recognized by the sera. Thus, especially the use of conventional blots does not exclude the recognition of other overlapping borrelial proteins with similar apparent molecular masses by SDS-PAGE. Recently, a flagellum-associated protein, FlaA, with an estimated size of approximately 38 kDa has been described (14). In addition, not all members of the bmp gene cluster have been examined for transcription and translation in vivo (31), and it has not been examined whether they are immunogenic. Since bmpB, bmpC, and bmpD are similar in size, their putative translation products might appear as one protein band in conventional immunoblots if they are expressed by the borreliae and recognized by patient sera. A reason for the lower sensitivity of rBmpA compared to that of nBmpA might also be the expression of rBmpA without its own signal sequence.

For the serodiagnosis of Lyme borreliosis in Europe, we recommend the use of rBmpA derived from all three species and the use of BmpA-specific MAb recognizing conserved epitopes for the standardization of immunoblots.

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