HOST RESPONSE TO INFECTION

Cloning of the gene encoding the decorin-binding protein B (DbpB) in *Borrelia burgdorferi sensu lato* and characterisation of the antibody responses to DbpB in Lyme borreliosis

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A genome walking technique was applied to borrelial DNA to clone the gene encoding decorin-binding protein B (DbpB) in *Borrelia garinii* and *B. afzelii*. Sequence analysis showed 62–67% identity of the predicted amino acid sequences of DbpB between the *B. afzelii* and *B. garinii* strains and *B. burgdorferi sensu stricto*. Within subspecies, the sequences were 99–100% identical. The respective recombinant DbpBs (rDbpBs) were produced and tested as antigens in an enzyme-linked immunosorbent assay (ELISA) for Lyme borreliosis (LB). In IgG ELISA, with rDbpBs as antigens, 11 (73%) of 15 adult patients with Lyme arthritis and 9 (64%) of 14 with neuroborreliosis were positive. Of children with Lyme arthritis, 40 (77%) of 52 were positive. All adult and paediatric patients with disseminated LB had high titres of anti-flagellin IgG antibodies. Seropositivity against rDbpB from *B. garinii* predominated, 39 (65%) of 60 of the positive samples reacting with rDbpB from *B. garinii*. In patients with erythema migrans, IgM antibodies to rDbpB were detected in 1 (4%) of 23 and IgG antibodies in 6 (26%) of 23. These results indicate that DbpB may be a useful antigen in the IgG serology for disseminated LB. The high inter-species sequence heterogeneity observed indicates that a combination of the variant DbpBs should be included in the antigen set to cover all the relevant borrelial subspecies in the serodiagnosis of LB.

Introduction

Lyme borreliosis (LB) is the most frequent tick-borne infectious disease in North America and Europe. The disease is caused by spirochaetes belonging to the *Borrelia burgdorferi sensu lato* complex and is characterised by multi-stage skin, joint, neurological and cardiac manifestations [1]. In Europe, in contrast to the USA, all three pathogenic borrelial subspecies, *B. burgdorferi sensu stricto*, *B. afzelii* and *B. garinii*, are known to be aetiological agents of LB [2].

In the absence of erythema migrans (EM), the clinical diagnosis of disseminated LB requires laboratory confirmation. This depends primarily on detection of antibodies specific to *B. burgdorferi*, because direct demonstration of the organisms in clinical specimens by culture or by PCR has a low sensitivity. The serological methods most commonly used for LB are enzyme-linked immunosorbent assay (ELISA) and Western blotting (WB), but these assays are not without limitations. These assays with whole-cell lysates (WCL) or flagellin as antigens are not standardised and their performance in different laboratories is highly variable [3]. To increase specificity, several recombinant borrelial proteins (e.g., OspA, OspB, OspC, OspE, OspF, p22, DbpA, BBK32, BBK50, VlsE, BmpA, FlaA and p41) [4–14] have been studied as antigens in serological assays. Some of these proteins have improved the sensitivity or specificity, or both, but none has proved superior to the current commercial serological assays.
The decorin-binding proteins A and B (DbpA and DbpB) are borrelial surface lipoproteins that have been shown to elicit early antibody responses in experimental murine borreliosis [15–17]. A previous study cloned and sequenced dbpA genes from the three pathogenic subspecies of *B. burgdorferi sensu lato* and investigated the antigenic properties of the variant rDbpAs [9]. These studies showed substantial sequence heterogeneity of DbpA. In the serodiagnosis of LB, with a single rDbpA as antigen, the sensitivity was low, but with all the variant rDbpAs included in the antigen panel, the sensitivity was comparable to that of routine seroassays [9]. At present, the amino acid sequences of DbpB in *B. burgdorferi sensu lato* have not been studied in detail, possibly on account of sequence heterogeneity and difficulties in the cloning of dbpB genes from various borrelial subspecies. The dbpB sequences in isolates from *B. burgdorferi sensu stricto* may be highly conserved [18]. However, except for a dbpB sequence from *B. garinii* available in the GenBank database (AF069263), information about dbpB sequences other than those of *B. burgdorferi sensu stricto* is lacking. Consequently, the antigenic potential of DbpB has not been thoroughly evaluated. The purpose of the present study was to clone and sequence dbpB from the three European pathogenic borrelial species, *B. burgdorferi sensu stricto*, *B. afzelii* and *B. garinii*. The respective recombinant DpbBs (rDbpBs) were then evaluated as antigens in the serodiagnosis of LB.

### Materials and methods

**Bacterial strains**

Finnish borrelial strains were obtained from the National Public Health Institute, Turku, Finland. *B. burgdorferi sensu stricto* strain ia (here referred to as Bbia) was isolated from the cerebrospinal fluid of a Finnish patient with neuroborreliosis (NB) and *B. afzelii* strains A91, 1082 and EM9 and *B. garinii* strains 40, 46 and 50 (referred to, respectively, as BaA91, Ba1082, BaEM9 and Bg40, Bg46 and Bg50) were obtained from skin biopsy samples of Finnish patients with LB. The strains were genotyped by PCR and sequencing, the target DNA being a fragment from the flagellin gene of *B. burgdorferi* [19]. Borreliae were cultivated in Barbour-Stoenner-Kelly (BSK-H) medium (Sigma, USA) at 33°C in air with CO2 5%. *Escherichia coli* host cells for cloning and expression of recombinant proteins were INFrE (Invitrogen, Groningen, The Netherlands) and M15 (Qiagen, Hilden, Germany), respectively.

### DNA purification

Borrelial genomic DNA was purified with a Dneasy Tissue Kit (Qiagen). Purified DNA was used in PCR and cloning experiments. Plasmid DNA was purified with a QIAprep-spin plasmid kit (Qiagen).

### PCR

A PCR-based approach was employed to amplify and sequence the dbpB from *B. burgdorferi sensu stricto* (Bbia). Primers for Bbia dbpB PCR amplification were designed on the basis of published dbpB sequences (Table 1). Several primer pairs were designed and tested to ensure that the entire coding sequence of the dbpB was obtained. Expression primers for strain Bbia, encoding the mature portion of the DbpB protein after cysteine at the site of post-translational acylation, were chosen from the sequence analysed. Approximately 1 ng of template DNA was used in standard PCR conditions: 30 cycles of denaturing at 94°C for 1 min, annealing at 50°C for 1 min and extension with

### Table 1. Primers used for PCR amplification of dbpB

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Primer</th>
<th>Location</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>B. burgdorferi sensu stricto</em></td>
<td>5'-TGC ATA AAA CAA ATT CAC ACT-3'</td>
<td>-208 to -187</td>
<td>B31 (AF069269)</td>
</tr>
<tr>
<td>2</td>
<td>B. burgdorferi sensu stricto</td>
<td>5'-CCG GAT CCA GTG TAT TAG AAA GAA C-3'</td>
<td>64–86</td>
<td>Bbia (AY083920)</td>
</tr>
<tr>
<td>3</td>
<td>B. burgdorferi sensu stricto</td>
<td>5'-AEC ATT TTC GTT ATT TGA TTA TTA TTT TTT TTT TTT TTC-3'</td>
<td>600–639</td>
<td>B31</td>
</tr>
<tr>
<td>4</td>
<td>B. burgdorferi sensu stricto</td>
<td>5'-CCG GTA CCT TTT TAA TAT TTA TTT TTT TTT TTT TTC GC-3'</td>
<td>628–604</td>
<td>Bbia</td>
</tr>
<tr>
<td>5</td>
<td><em>B. garinii</em></td>
<td>5'-CCT TTC TTT TTT TTT TAA GAC C-3'</td>
<td>-341 to -320</td>
<td>Bg40 (AY083917)</td>
</tr>
<tr>
<td>6</td>
<td><em>B. garinii</em></td>
<td>5'-CCG GAT CCA ATT TGG TAA CAG GAG AGG-3'</td>
<td>58–79</td>
<td>Bg40</td>
</tr>
<tr>
<td>7</td>
<td><em>B. garinii</em></td>
<td>5'-GCT TCC TCI GAA ATG GAG CCT TTT ATT C-3'</td>
<td>416–389</td>
<td>Bg40</td>
</tr>
<tr>
<td>8</td>
<td><em>B. garinii</em></td>
<td>5'-CAT TAA ATC AAA CAT AGC CAA GAA GAA TGG AC-3'</td>
<td>348–320</td>
<td>Bg40</td>
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<tr>
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<td><em>B. garinii</em></td>
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<td>109–90</td>
<td>Bg40</td>
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<tr>
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<td>5'-CCG GTA CGG TTT TTT TTA GGC AAT TCT AAT TAC-3'</td>
<td>594–573</td>
<td>Bg40</td>
</tr>
<tr>
<td>11</td>
<td><em>B. garinii</em></td>
<td>5'-CAT GCT ACT AAC AGG CTA AC-3'</td>
<td>744–725</td>
<td>Bg40 (AF441382)</td>
</tr>
<tr>
<td>12</td>
<td><em>B. afzelii</em></td>
<td>5'-CCC CTG GCA AAA TAA AAT TTC-3'</td>
<td>-458 to -439</td>
<td>BaA91 (AY083914)</td>
</tr>
<tr>
<td>13</td>
<td><em>B. afzelii</em></td>
<td>5'-CCG GAT CCA ATT TGG TAA AAG AAA C-3'</td>
<td>58–80</td>
<td>BaA91</td>
</tr>
<tr>
<td>14</td>
<td><em>B. afzelii</em></td>
<td>5'-CTG ATT GAA GCC TCT TTT TTT CTG GC-3'</td>
<td>765–739</td>
<td>BaA91 (AF441383)</td>
</tr>
<tr>
<td>15</td>
<td><em>B. afzelii</em></td>
<td>5'-TGG TGC CAA AAC AAC AGG AAG TAA AG-3'</td>
<td>728–703</td>
<td>BaA91 (AF441383)</td>
</tr>
<tr>
<td>16</td>
<td><em>B. afzelii</em></td>
<td>5'-TAA ACC TCA ATT AAT CTC TTA G-3'</td>
<td>461–440</td>
<td>BaA91</td>
</tr>
<tr>
<td>17</td>
<td><em>B. afzelii</em></td>
<td>5'-CCG GAT CCA TTT TTA TTA TTA TTA G-3'</td>
<td>513–491</td>
<td>BaA91</td>
</tr>
<tr>
<td>18</td>
<td>GenomWalkerKit</td>
<td>5'-GTA CGA CTC ACT ATA GGC G-3'</td>
<td>Adapter primer 1</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>GenomWalkerKit</td>
<td>5'-ACT ATA GGC CTC GGC TGG TTA-3'</td>
<td>Adapter primer 2</td>
<td></td>
</tr>
</tbody>
</table>

Restriction enzyme sites of *BsmHI* and *KpnI* in expression primers are underlined.
AmpliTaqGold DNA Polymerase (Perkin Elmer, USA) at 72°C for 1.5 min.

Genome walking
The genome walking methodology (Universal Genom-Walker Kit, Clontech, USA) was applied to borrelial DNA from B. afzelii and B. garinii. Firstly, borrelial DNA from B. afzelii strain BaA91 and B. garinii strain Bg40 was digested with four different restriction endonucleases (DraI, EcoRV, PvuII and StuI) separately. GenomWalker adapters were ligated to both ends of the cut DNA. Then DNA fragments were amplified by PCR, with downstream primers selected from known dbpA sequences downstream from dbpB [9] and the GenomWalker adaptor primers as upstream primers (Table 1). Two PCR amplifications were performed, the second as a nested PCR, and primers were designed from the sequences obtained (Table 1). The dbpB sequences of the other B. afzelii and B. garinii strains (Ba1082, BaEM9, Bg46 and Bg50) were obtained by PCR, with primers chosen from analysed BaA91 and Bg40 sequences. To eliminate any errors possibly due to the use of Taq polymerase, the two strands of each dbpB were sequenced independently at least twice. These sequences were used to choose expression primers for the BaA91 and Bg40 strains encoding the mature portion of the DbpB protein after cysteine at the site of post-translational acylation.

DNA sequencing
The full-length or partial dbpB DNA strands obtained by PCR were cloned into the pCR 2.1-TOPO vector (Invitrogen) and sequenced at the Core Facility of the Haartman Institute, University of Helsinki, with a DyePrimer (T7, M13Rev) cycle sequencing kit (Applied Biosystems). Sequencing reactions were run and analysed with the automated sequencing apparatus model 373A (Applied Biosystems). DNA and protein sequences were analysed with Lasergene software (DNASTAR, USA).

Cloning and expression of recombinant DbpB
For expression of the rDbpBs, six histidine-tagged protein constructs were generated as described previously [9].

ELISA
For ELISA assays measuring anti-DbpB antibodies, the wells in the microtitration plates were coated with 100 μl (2 μg/ml) of the variant recombinant DbpB proteins overnight. After washing, 100 μl of a diluted serum sample were added to each well and the wells were incubated overnight. Serum samples were diluted 1 in 10 (EM) or 1 in 100 (neuroborreliosis and Lyme arthritis) with bovine serum albumin (BSA) 5 mg/ml in 155 mM NaCl-Tween 20 0.04% buffer (BSA-NaCl-Tween). Mouse plasma samples were diluted 1 in 100 in 1 M PBS-Tween 20 0.01%-gelatine 0.25%. After washing, the wells were incubated with alkaline phosphatase-conjugated rabbit anti-human IgG or IgM (Jackson Immuno Research Laboratories, USA) diluted 1 in 5000 or alkaline phosphatase-conjugated rabbit anti-mouse IgG (Orion, Espoo, Finland) diluted 1 in 1000 in BSA-NaCl-Tween for 2 h. The reactions were visualised with 4-nitrophenylphosphate (Boehringer Mannheim GmbH, Mannheim, Germany) 1 mg/ml in diethanolamine buffer, pH 10.0. After 10–20 min, the optical density (OD) measurements were made with a Multiscan photometer (Thermo Labsystems, Helsinki, Finland) at 405 nm wavelength. The cut-off values for human and mouse samples were determined as the means +3 SD of the respective control samples.

Samples
For ELISA analyses serum samples were collected from 14 adult patients with NB, 15 with Lyme arthritis (LA) and 23 with culture- or PCR-positive EM (B. afzelii n = 17, B. garinii n = 4, and genotyping not feasible n = 2). In the patients with NB and LA, the clinical diagnosis was confirmed in ELISA by demonstrating serum antibodies (and C3F anti-flagellin antibodies in NB patients) against flagellin and B. burgdorferi WCL [20]. The EM patients provided serum samples at diagnosis (acute) and at 1–3 months after treatment (convalescent). Serum samples from patients with syphilis (SY), systemic lupus erythematosus (SLE) and Epstein–Barr virus (EBV) infection, and from patients positive for rheumatoid factor (RF) or anti-streptolysin antibodies (ASO), and from healthy blood donors were used as controls.

In a second set of samples, sera from 52 children with well characterised LA from Germany were analysed [21, 22]. Serum samples from 20 children with other inflammatory joint diseases and samples from 20 healthy blood donors were used as controls.

Plasma samples from mice infected with B. garinii strain A218 were obtained from Matti Viljanen (National Public Health Institute, Turku, Finland). The infection was verified by culturing ear pinnae from each mouse at each time point when groups of five mice were killed. Plasma from individual infected mice was pooled at time points 2, 4, 8 and 16 weeks after infection. Plasma samples from an identical set of sham-infected mice were used as control samples in ELISA.

Nucleotide sequence accession nos
The nucleotide sequences for dbpB were submitted to GenBank under accession nos: BaA91, AY083914; Ba1082, AY083915; BaEM9, AY083916; Bg40, AY083917; Bg46, AY083918; Bg50, AY083919; and
Bbia, AV083920. Published dbpB sequences from B. burgdorferi sensu stricto strains 297 (U75867), LP4 (AF069264), LP5 (AF069261), LP7 (AF069255), NCH-1 (AF069259), FRED (AF069260), HB19 (AF069254), ZST (AF069251), B31 (AF069266) and N40 (AF069252) and B. garinii strain 20047 (AF069263) were obtained from GenBank.

Results
Analysis of dbpB sequences of the Finnish borrelial isolates
The open reading frames of the dbpB sequences analysed consisted of 552–564 nucleotides. The identity of the dbpB sequences between the borrelial subspecies was 78–82%. Within B. garinii strains, dbpB in Bg46 differed by one nucleotide (position 312) from the Bg40 and Bg50 sequences, but this did not change the amino acid composition. Similarly, dbpB in B. afzelii strain Ba1082 differed by one nucleotide (position 416) from BaA91 and BaEM9. The codon difference caused a putative translational change from alanine to valine.

DbpBs of the Finnish borrelial isolates
The deduced mature proteins DbpB BaA91,1082,EM9, DbpBBg40,46,50 and DbpBBbia contained 165, 166 and 167 residues, respectively (Fig. 1). Differences in the amino acid sequences were distributed along the entire sequence. The calculated molecular masses of the predicted mature proteins DbpBBaA91, DbpBBg40 and DbpBBbia (without lipid acylation) were 18.6, 18.1 and 18.2 kDa, respectively. Comparison of the deduced mature amino acid sequences of DbpBBaA91 and DbpBBbia, DbpBBaA91 and DbpBBg40, and DbpBBg40 and DbpBBbia showed 67%, 67% and 62% identity, respectively (Fig. 2). Protein analysis revealed differences in the frequencies of charged, polar and hydrophobic amino acid composition, yielding calculated iso-electric points of 8.61, 9.35 and 9.47 for DbpBBaA91, DbpBBg40 and DbpBBbia, respectively.

Analysis of DbpB of B. burgdorferi sensu lato
The amino acid sequences of DbpBs from the three B. afzelii isolates (BaA91, Ba1082 and BaEM9) were 99–100% identical. In the GenBank database there are no published dbpB sequences of B. afzelii strains. The identical DbpBBg40–DbpBBg46 and DbpBBg50 sequences were compared with the published B. garinii DbpB sequence from strain 20047 (AF069263). There was 62% identity. The sequence from strain 20047 was

Fig. 1. Alignment of predicted mature DbpB amino acid sequences of B. burgdorferi sensu stricto (Bbia, 297 and B31), B. garinii (Bg40) and B. afzelii (BaA91 and Ba1082). Residues differing from Bbia are boxed. The B. garinii strain Bg40 also represents the identical strains Bg46 and Bg50. The B. afzelii strain BaA91 also represents the identical strain BaEM9.

Fig. 2. Identities of deduced amino acid sequences of DbpB among isolates of B. burgdorferi sensu lato. The identities (%) were calculated without the sequence encoding the leader peptide by the Jotun Hein method with Lasergene software. Underlined figures represent identities of DbpB between the Finnish borrelial isolates. B. garinii strain Bg40 also represents the identical strains Bg46 and Bg50. B. afzelii strain BaA91 also represents the identical strain BaEM9.
available for amino acid analysis from position 6 to the end of the DbpB protein. The DbpB_{Bba} sequence was compared with published DbpB sequences of human B. burgdorferi sensu stricto strains 297 (U75867), LP4 (AF069264), LP5 (AF069261), LP7 (AF069255), NCH-1 (AF069259), FRED (AF069260), HB19 (AF069254) and tick isolates B31 (AF069266), ZS7 (AF069251) and N40 (AF069252) (Fig. 2). The sequence identity of the deduced mature amino acids ranged from 99% to 100%. The deduced amino acid sequences of DbpB in strains HB19, FRED, LP5, LP7, NCH-1, B31, N40 and ZS7 were identical and differed by only one amino acid from the Bbia sequence. The amino acid at position 127 was isoleucine in Bbia, but leucine in all the other sequences. In the identical DbpB sequences of strains 297 and LP4, glutamic acid at position 60 was substituted in the other sequences by aspartic acid. The DbpB sequence in B. garinii 20047 was identical with that of most strains of B. burgdorferi sensu stricto.

The DbpBs of all three subspecies had in common a C-terminal decapeptide that contained six or seven positively charged amino acids but no negative charges. Elsewhere in the sequences, there were marked differences in the position of the charged amino acids. The location of the hydrophobic amino acids also differed, e.g., at position 74 of the putative mature sequence, the isoleucine of B. burgdorferi sensu stricto was replaced by charged arginine in B. afzelii. In place of isoleucine at position 81 in B. garinii DbpB, in the other two species there was lysine.

**ELISA**

In IgG ELISA, 9 (64%) of 14 samples from patients with NB were positive. When rDbpB_{BaA91}, rDbpB_{Bg40} and rDbpB_{Bba} were used as antigens, 3 (21%), 7 (50%) and 1 (7%) of 14, respectively, were positive (Fig. 3). Of the 15 samples from patients with LA, 11 (73%) were positive, 1 (7%), 9 (60%) and 6 (40%) of 15 with rDbpB_{BaA91}, rDbpB_{Bg40} and rDbpB_{Bba}, respectively (Fig. 3). Of the nine positive samples from patients with NB, five were positive only with DbpB from B. garinii, two only with DbpB from B. afzelii, one with DbpB from B. afzelii and B. garinii and one with DbpB from B. garinii and B. burgdorferi sensu stricto. Of the 11 positive samples from LA patients, five were positive only with DbpB from B. garinii, one with DbpB from B. afzelii and B. burgdorferi sensu stricto, one only with DbpB from B. garinii and B. burgdorferi sensu stricto and four with DbpB from B. garinii and B. burgdorferi sensu stricto. Of the 20 positive samples, 13 reacted with one antigen only. Of the control samples, one from a patient with SLE was positive with all three DbpBs and a few others were low positives (Fig. 3). The calculated specificity was 98%, 88% and 92% when rDbpB_{BaA91}, rDbpB_{Bg40} and rDbpB_{Bba} were used as antigens, and the total specificity was 93%.

In the 52 paediatric LA patients, 20 (38%), 23 (44%) and 30 (58%) serum samples were positive when rDbpB_{BaA91}, rDbpB_{Bg40} and rDbpB_{Bba}, respectively, were used as antigens (Fig. 4). In total, 40 (77%) of the 52 serum samples were positive in rDbpB IgG ELISA. Of the 40 positive samples, 18 reacted with one antigen only, 15 with two antigens and 7 reacted with all 3 antigens. In the control patients with other joint diseases, 2 (10%) of 20 had low positive reactions in ELISA with rDbpB_{Bba} as antigen. No positive reactions were observed with the sera of healthy blood donors. The calculated overall specificity was 98%.
In IgM and IgG ELISA of samples from 23 early LB patients with culture- or PCR-positive EM, 1 (4%) and 6 (26%), respectively, were positive with the variant DbpB proteins used as antigens (data not shown).

IgG ELISA with mouse plasma

At 2 weeks after inoculation, plasma from mice infected with *B. garinii* already showed highly positive OD405 values with rDbpB from *B. garinii* as an antigen (Fig. 5). At 4 weeks, the immunoreactivity was further increased and remained at high levels at 8 and 16 weeks after infection. When heterologous rDbpBs from *B. afzelii* and *B. burgdorferi sensu stricto* were used as antigens, the immunoreactivity in ELISA at 2 and 4 weeks was low. At 8 weeks the OD405 values were clearly positive, but at lower levels than the OD405 values against the homologous rDbpB from *B. garinii*.

Discussion

The present study describes, for the first time, the sequences of DbpB in *B. afzelii* and *B. garinii* strains, and characterises the antibody responses to the variant DbpB proteins in the serodiagnosis of LB. The results indicate that DbpB may be a useful antigen in IgG serology for disseminated LB. The high heterogeneity observed in the sequences implies that, to cover all the relevant borrelial subspecies in the serodiagnosis of LB, a combination of the variant DbpBs should be included in the antigen set.

As the PCR-based approach for cloning of individual *dbpB* genes was feasible only for *B. burgdorferi sensu stricto*, the genome walking methodology was used to clone *dbpB* genes from *B. afzelii* and *B. garinii* isolates. This method relies on established sequences in close proximity to unknown DNA. The present study took advantage of the *dbpA* sequences from various borrelial subspecies analysed in a previous study [9]. The inter-species sequence identity of the deduced DbpB proteins varied from 62% to 68%. Interestingly, except for *B. garinii* 20047, in which the DbpB sequence was identical with that of most strains of *B.*
bi-cistronic operon and have been shown in vitro to be transcribed together [16]. In experimental murine borreliosis, early and long-lasting immune responses have been reported against both proteins [15–17]. Interestingly, in a previous study, rDbpA proved to be 100% sensitive as an antigen in the serodiagnosis of disseminated LB. In the present study, in contrast, the sensitivity of DbpB was lower. Furthermore, the immune reactivity to rDbpBs seemed to remain quantitatively at a lower level than that to rDbpAs originating from the same pathogenic isolates (data not shown). Given the functional and (secondary) structural similarities between DbpA and DbpB [24], it can be speculated that, during human LB, DbpB may be expressed at lower levels than DbpA. The mechanisms by which B. burgdorferi regulates its gene expression in various environments are insufficiently characterised. However, it has been shown that at least some environmental signals, such as pH and temperature, may influence the levels of expression of several borrelial proteins, including DbpA and DbpB [30–32].

In summary, the high sequence heterogeneity of DbpB implies that further studies are needed to elucidate the roles of the variant DbpBs in the tissue adherence of borreliae. Our hypothesis is that the heterogeneity of the amino acid sequences may have implications for the usefulness of a given antigen in the serodiagnosis of LB. Consistent with previous results for DbpA [9], the present study provides evidence that, in a European epidemiological setting, a combination of recombinant antigens derived from all the pathogenic borrelial species appears to be a promising approach for the development of new serodiagnostic tests with higher sensitivity.

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References


